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Research Article

Determination of Six Eugenol Residues in Aquatic Products by Gas Chromatography-Orbitrap Mass Spectrometry

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Eugenol compounds are widely used in the circulation and transportation of fresh aquatic products because of their good anesthetic effects. However, some studies have shown that eugenol compounds are potential carcinogens. Therefore, in order to ensure the edible safety of aquatic products, eugenol compounds in aquatic products need to be screened quickly. A method for determination of six eugenol residues in aquatic products was established by multiplug filtration cleanup (*m*-PFC), combined with gas chromatography-Orbitrap mass spectrometry (Orbitrap GC-MS). Samples were ultrasonically extracted with acetonitrile, and the extracts were frozen at −18°C for 1 h, then purified with the *m*-PFC column, and detected by Orbitrap GC-MS in full scan mode. The results showed the linear relationships for six eugenols were good in the range of 0.001–0.1 μg/mL, and the correlation coefficients (R^2) were above 0.9950. The limits of detection (LODs) were 2–10 μg/kg, and the limits of quantitation (LOQs) were 5–20 μg/kg. The average recoveries at the spiked levels of 5–200 μg/kg were in the range of 76.4%–105.1%, with relative standard deviations (RSDs) of 1.2%–7.5%. Eighty aquatic products were detected by this method, of which only eugenol was detected in 12 samples, and eugenol and isoeugenol were detected in two samples at the same time. The other eugenol compounds were not detected in any sample. The detection rate of positive samples was 17.5%. The method is simple, accurate, and suitable for the rapid screening of eugenol compounds in aquatic products.

1. Introduction

Eugenols are phenylpropanoid compounds, mainly including eugenol, isoeugenol, methyl eugenol, methyl isoeugenol, eugenol acetate, and acetyl isoeugenol [1]. Eugenol compounds have a good anesthetic effect on fish, which can keep them dormant by reducing the metabolic rate, thereby reducing injury and death caused by the stress reaction and the deterioration of environment during storage and transportation. At the same time, they have the advantages of low cost, good effect, and short residual period, so they are widely used in the living transportation of aquatic products [2, 3]. However, the safety of eugenols as the fishing anesthetic is controversial in the world. It had been reported that eugenol is a potential carcinogen, and it can cause damage to human liver [4]. International Agency for Research on Cancer (IARC) listed it as the third category of carcinogens [5]. Some countries imposed restrictions on the use of eugenol compounds. Japan stipulates that the maximum residue limit of eugenol is 0.05 mg/kg in aquatic products. New Zealand established a residue limit of 0.1 mg/kg, which was later revoked. The United States and Canada prohibit the use of eugenol compounds in aquatic products [6]. In China, eugenol, isoeugenol, and methyl eugenol are allowed to be used in food as spices, but not in raw and fresh meat and fresh aquatic products. However, there is no clear provision on the use of eugenol compounds as fishing anesthetics. According to domestic reports in China, eugenol compounds have been detected in aquatic products, such as fish, shrimps, and crabs. It shows that eugenol compounds are being illegally used in aquatic products in China. Therefore, the establishment of detection methods for eugenol compounds in aquatic products can provide strong technical support and guarantee for China to supervise

eugenol compounds residues in fresh aquatic products and formulate relevant standards.

In the current research, the preprocessing methods of eugenol residues include derivative [7], solid-phase extraction [8], and QuEChERS [5]. The main detection methods include liquid chromatography (LC) [9], liquid chromatography-tandem mass spectrometry (LC-MS/MS) [6], gas chromatography-mass spectrometry (GC-MS) [10], and gas chromatography-tandem mass spectrometry (GC-MS/MS) [11]. Sun et al. [12] established a new method for the determination of eugenols in aquatic products by dispersive solid-phase extraction with graphitized carbon black as absorbent coupled with ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Ni et al. [13] determined the residues of four eugenol anesthetics in aquatic products by HLB solid-phase column combined with UHPLC-MS/MS. Ke et al. [14] determined the residues of eugenol, methyl eugenol, and isoeugenol in fish by phenyl solid-phase column combined with GC-MS/MS. The SPE involves activation, leaching, and elution. QuEChERS requires vortex and centrifugation. These two methods are not suitable for batch processing of a large number of samples. Multiplug filtration cleanup (*m*-PFC) is a new and rapid method for sample pretreatment based on the QuEChERS method. As shown in Figure 1, this method places purification packing in the syringe. The extraction solution is pushed through the packing layer containing multiwalled carbon nanotubes (MWCNTs), primary secondary amine (PSA), and $MgSO_4$. The filler adsorbs the interfering substances in the matrix, such as pigments, lipids, some sugars, sterols, and organic acids. However, it does not adsorb the target compounds. By realizing one-step purification, the purification time is greatly shortened, and the pretreatment efficiency can be greatly improved. At present, *m*-PFC column has been used to detect pesticides [15–20], antibiotics [21], and veterinary drugs [22] in foods. Aquatic products are rich in proteins and fats and contain pigments, organic acids, fatty acids, and other impurities. Ordinary mass spectrometry analysis is prone to interference and false positive. Gas chromatography-Orbitrap mass spectrometry (Orbitrap GC-MS) is a new technology for mass spectrometry emerged in recent years, which has high resolution, high quality accuracy, and high sensitivity. So, it can accurately determine the target compounds in complex matrix. At present, Orbitrap GC-MS has been used in environment analysis [23, 24], pharmaceutical research [25], and food analysis [26–28]. So far, there are no reports on the determination of eugenol compounds in aquatic products by *m*-PFC column combined with Orbitrap GC-MS.

In order to screen eugenol residues in aquatic products accurately and quickly, the sample was prepared by *m*-PFC column with MWCNTs as purification packing and detected by Orbitrap GC-MS in full scan mode through retention time and accurate molecular mass. In this study, it was the first time that *m*-PFC column combined with Orbitrap GC-MS was applied to detect six eugenol residues in aquatic products. This method can not only achieve rapid pretreatment of samples but also has a strong ability to resist matrix interference and achieve accurate detection, which provides an effective technical support for the supervision of eugenol compounds in aquatic products.

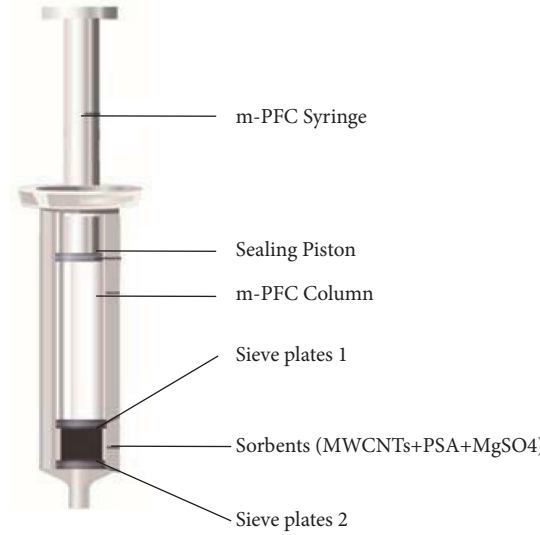

Figure 1: Structure diagram of *m*-PFC column.

2. Materials and Methods

2.1. Reagent. Eugenol, methyl eugenol, isoeugenol, methyl isoeugenol, eugenol acetate, and acetyl isoeugenol were used as standards, with a purity more than 98%; they were purchased from Shanghai Anpu Experimental Technology Co., Ltd. Acetonitrile, methanol, acetone, ethyl acetate, *n*-hexane, and ether were all in HPLC grade and were purchased from Merck, Germany. Anhydrous sodium sulfate was used as the analytical reagent, purchased from Tianjin Guangfu Science and Technology Development Co., Ltd. QuEChERS pipe 1 (2 mL) included 150 mg $MgSO_4$, 50 mg PSA, and 50 mg C_{18}, and QuEChERS pipe 2 (2 mL) included 150 mg $MgSO_4$, 50 mg PSA, 50 mg C_{18}, and 50 mg GCB, purchased from Thermo, USA. The *m*-PFC column included 150 mg $MgSO_4$, 15 mg PSA, and 15 mg MWCNTs, purchased from Beijing Lvmian Technology Co., Ltd.

2.2. Materials. All the aquatic products used in the experiment were purchased from the local supermarket.

2.3. Preparation of Sample. 2.0 g crushed samples were weighed, and then, 1 g anhydrous sodium sulfate was added to the samples. Then, the samples were stirred and mixed, and 5 ml acetonitrile was added. Next, the sample was vortexed for 1 min and extracted by ultrasound for 10 min. After centrifugation at 9500 rpm for 3 min, the extract was transferred to another 50 mL centrifuge tube, and the sample was repeatedly extracted with 5 mL acetonitrile. Last, all the extracts were combined and frozen for 1 h at −18°C. As shown in Figure 2, 2 mL extracts were added to the *m*-PFC column, which was connected with a 0.22 μm organic filter membrane placed on top of the sample bottle. The injection

Figure 2: Purification procedure diagram of *m*-PFC column.

rod was slowly pushed (1–1.5 s/drop) to get the filtrate, which was used for detection by Orbitrap GC-MS.

2.4. Preparation of Standard Solution

2.4.1. Preparation of Mixed Standard Solution. Six eugenol standards were accurately weighed and diluted with acetonitrile to prepare a mixed standard solution containing 1000 μg/mL and 10 μg/mL.

2.4.2. Preparation of Matrix-Matched Standard Solution. 2.0 g blank sample was accurately weighed and pretreated according to Section 2.3, to obtain a blank matrix solution. The mixed standard solution (10 μg/mL) was diluted into a series of standard working solutions of 0.001 μg/mL, 0.002 μg/mL, 0.003 μg/mL, 0.004 μg/mL, 0.01 μg/mL, 0.02 μg/mL, 0.05 μg/mL, and 0.1 μg/mL with blank matrix solution.

2.5. Orbitrap GC-MS Analytical Conditions. The chromatography assay was performed on Orbitrap GC-MS system equipped with an auto sample manager (Thermo Scientific, Bremen, Germany). The chromatographic separation was performed on Agilent DB-5MS column, 30 m × 0.25 mm × 0.25 μm. The injection port temperature was set to 230°C, and the injection mode was splitless. The helium (purity ≥ 99.999%) was used as carrier gas. The column flow was set to 1.0 mL/min. The temperature of the column was set as follows: maintain the initial temperature at 100°C for 1 min, first, increase the temperature to 200°C at a rate of 6°C/min; increase the temperature to 300°C at a rate of 25°C/min; finally, hold for 5 min. The injection volume was 1 μL. The detector was electrostatic field Orbitrap high resolution mass spectrometry. The Orbitrap resolving power was set at 60,000 full width at half maxima (FWHM) at 200 m/*z*. The dates were collected in the range of 50–550 m/*z* by full scan mode. Ion source was electron ionization (EI), and the electron energy was 70 eV. The ion source temperature was set at 230°C, and the MS transfer line temperature was set at 300°C. The mass spectrometry parameters are shown in Table 1.

3. Results and Discussion

3.1. Establishment of Database. The standard solution (0.2 μg/mL) of eugenol compounds was injected into Orbitrap GC-MS, and the data were collected in full scan mode to obtain the retention time for the target compounds, the exact molecular mass, and the chemical formula of the fragment ions. Figure 3 shows the full-scan mass spectrogram of six eugenol compounds. Three fragment ions with high response were selected as quantitative and qualitative ions for each compound. Then, the retention time for each compound and ion information (exact molecular mass, chemical formula) were imported into the database. According to the established database, the rapid qualitative and quantitative determination of eugenol compounds can be realized. Compared with GC-MS/MS, Orbitrap GC-MS does not need to optimize ion pairs and collision voltages and can establish the database in full scan mode. So, the method is simpler to establish.

3.2. Optimization of Extraction Solvent. Eugenol compounds are weak in polarity, slightly soluble in water, and easily soluble in some organic solvents. In this study, the extraction effects of six kinds of solvents (acetonitrile, acetone, methanol, ethyl acetate, *n*-hexane, and ether) for eugenol compounds (the spiked level of 200 μg/kg) were compared (Figure 4). The results showed that when acetonitrile and methanol were used as extraction solvents, the recoveries of six eugenol compounds were 81.4%–95.7% and 82.7%–94.1%, respectively, and the RSDs were in the range of 3.2%–6.8% and 3.7%–7.1%, respectively. When extracting with *n*-hexane, the recovery rate of each compound was poor, ranging from 29.7% to 75.8%, and RSD was 2.5% to 6.6%. When extracting with ethyl acetate, acetone, and ether, the recovery rates of eugenol and isoeugenol were all over 75%, while the recovery rates of the other four compounds were poor, ranging from 35.6% to 68.7%. In addition, the solution color after extraction with methanol, acetone, ethyl acetate, and ether was darker than the solution color after extraction with acetonitrile and *n*-hexane. Therefore, considering the recovery rate and the subsequent purification, acetonitrile was selected as the extraction solvent for subsequent experiments.

3.3. Optimization of Extraction Method. In this study, negative prawns were selected to investigate the extraction effects of oscillation extraction, ultrasonic extraction, and homogeneous extraction for six eugenol compounds at the spiked level of 200 μg/kg (in Figure 5). The result showed that the recoveries of oscillation extraction were in the range of 69.5%–82.7%, and the RSDs were in the range of 3.4%–6.6%. The recoveries of ultrasonic extraction and homogeneous extraction were in the range of 84.7%–96.6% and 82.4%–94.9%, respectively, and the RSDs were in the range of 2.6%–7.3% and 2.0%–6.4%, respectively, which were

Table 1: The retention time and MS parameters of six eugenols.

Compound	CAS registry number	Retention time (min)	Quantitative ion (m/z)	Qualitative ion 1 (m/z)	Qualitative ion 2 (m/z)
Eugenol	97-53-0	7.94	164.08318	131.04914	149.05971
Methyl eugenol	93-15-2	8.76	178.09883	147.08044	107.04914
Isoeugenol	97-54-1	9.70	164.08318	131.04914	149.05971
Methyl isoeugenol	93-16-3	10.55	178.09883	163.07536	107.04914
Eugenol acetate	93-28-7	11.10	164.08318	131.04914	149.05971
Acetyl isoeugenol	93-29-8	12.77	164.08318	149.05971	131.04914

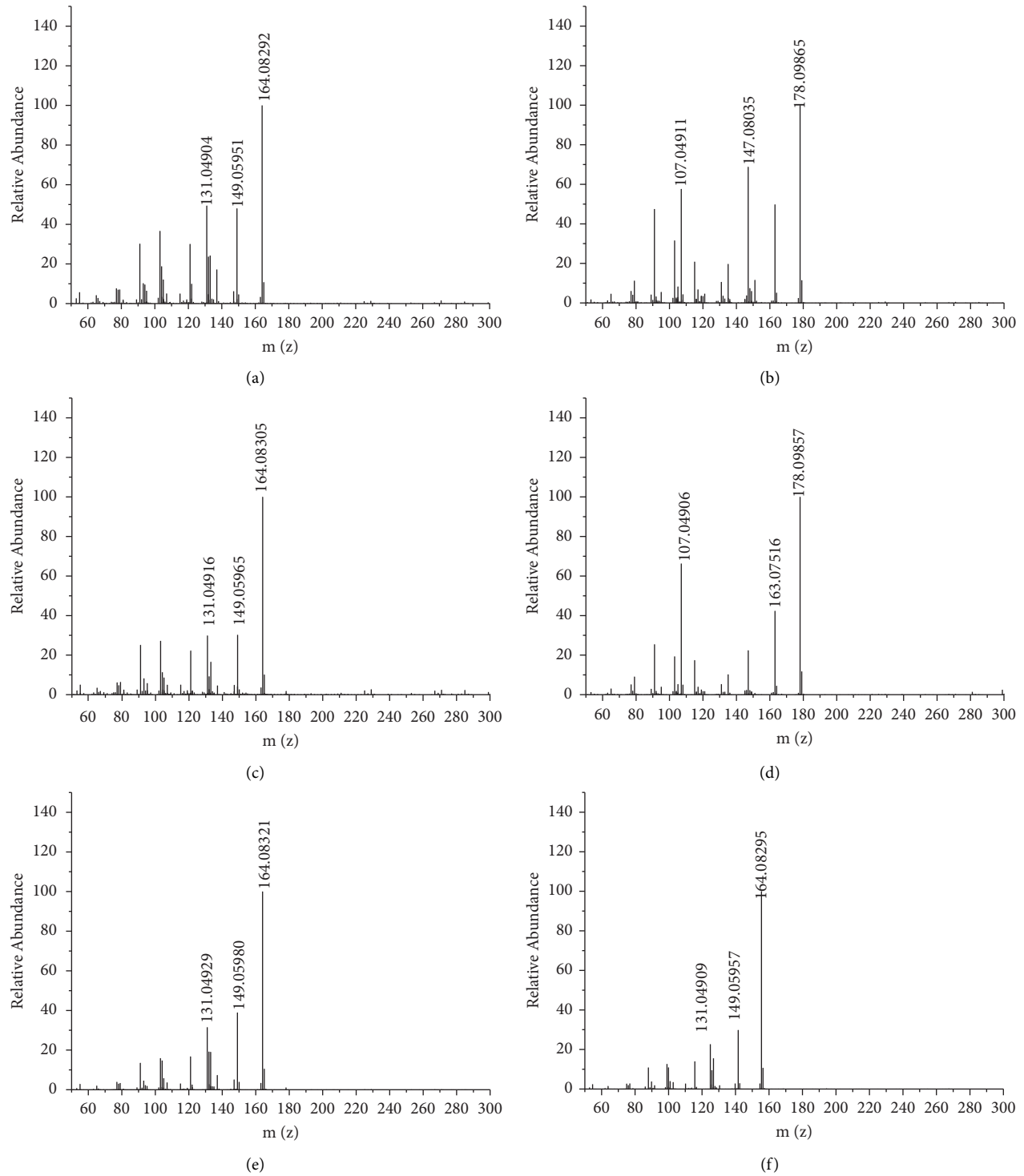

Figure 3: Mass spectrogram of six eugenols. (a) Eugenol. (b) Methyleugenol. (c) Isoeugenol. (d) Methyl isoeugenol. (e) Eugenol acetate. (f) Acetyl isoeugenol.

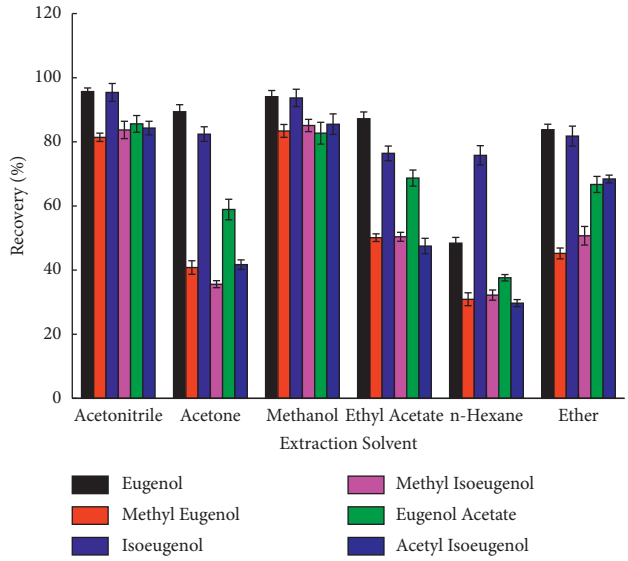

FIGURE 4: Selection of the extraction solvents. The error bars represent the standard deviation of measurements for each compound ($n = 6$).

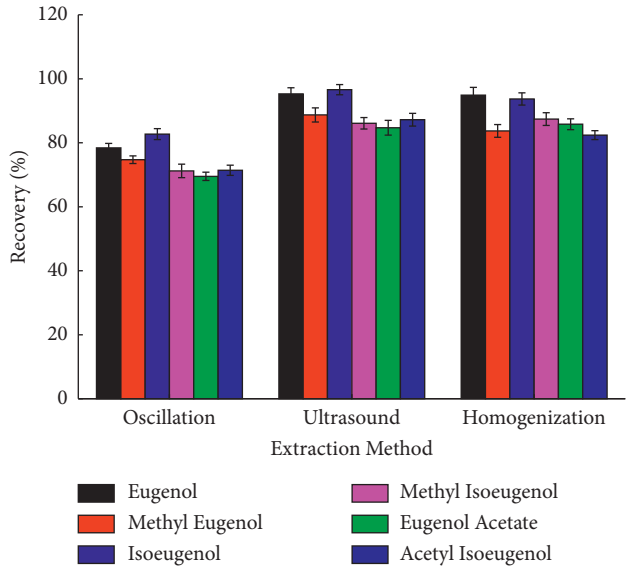

FIGURE 5: Selection of extraction methods. The error bars represent the standard deviation of measurements for each compound ($n = 6$).

better than oscillation extraction. During ultrasonic extraction, ultrasonic wave produced the high-speed and strong cavitation effect on the samples, and the sample cells were broken, which could release the target compounds better and faster, thus realizing the full extraction of the target compounds. During homogenization extraction, the samples could be completely broken by the homogenizer and fully homogenized with the extraction solvent, so that the target compounds could be well extracted. These two methods can extract the target compounds well, but the homogenized cutter head needs to be cleaned after use, and the number of samples treated by homogenization extraction is small. The ultrasonic instrument is not only easy to maintain but also can deal with a large number of samples at one time, which is suitable for batch processing of samples. Considering the recovery rate and pretreatment efficiency, the ultrasonic extraction method was selected in subsequent experiments.

3.4. Optimization of Purification Conditions. The matrix of aquatic products is complex, which is rich in proteins, fats, organic acids, pigments, and other substances. During the pretreatment, these impurities would be extracted with the target substances, which not only interfere the determination but also pollute the instrument, so it is necessary to purify the extracts effectively. Before the purification, the extracts were frozen for 1 h at −18°C to make some proteins and fats condensed and precipitated. However, the impurities could not be completely removed, which needed further purification. The matrix mixed standard solution (0.1 μg/mL) was prepared with the extracts of negative prawns to investigate the purification effects of QuEChERS pipes, *m*-PFC column, and *n*-hexane saturated with acetonitrile (Table 2).

After purification by QuEChERS pipe 1 and QuEChERS pipe 2, the recoveries of six eugenols were 87.2%–102.4% and 77.0%–90.5%, respectively. After purification by *m*-PFC column and *n*-hexane saturated with acetonitrile, the recoveries of six eugenols were 93.8%–103.7% and 93.2%–102.5%, respectively. PSA is a kind of weak anion exchange packing, which can adsorb polar compounds through hydrogen bonds and remove polar substances, such as pigments, organic acids, and sugars. However, the purification ability is limited, and the interfering substances in the matrix cannot be effectively removed [29]. Because of the high specific surface area, GCB can effectively adsorb impurities such as pigments, sterols, and fats. However, because of the hexagonal structure on its surface, GCB has a strong adsorption effect on molecules containing planar aromatic rings. Eugenol compounds contain benzene rings, so their recovery rates were lower when GCB was used for purification. MWCNTs are a kind of nanometer hollow material with higher specific surface area and more excellent mechanical properties and chemical stability than traditional adsorption materials. It can adsorb interfering substances, such as pigments, fatty acids, organic acids, and fats, in samples through electrostatic interaction and π-π bond interaction among MWCNTs [30, 31]. It can also reduce the adsorption of substances with planar structure. Therefore, the recovery rate of eugenol compounds after purification by *m*-PFC column was higher than QuEChERS pipe 2. In addition, compared with QuEChERS, because the *m*-PFC column adopts injector design, the purification does not require vortex oscillation and centrifugation. So, the operation is simple and the purification process takes less than 2 min. *N*-hexane saturated with acetonitrile is a kind of effective organic reagent for purification, but it not only pollutes the environment but also is harmful to health. The purification process of *m*-PFC column does not consume any organic reagents, which is convenient to operate and

Table 2: Effects of different purification methods on the recoveries of six eugenols ($n = 6$).

Compound	Recovery (RSD) (%)			
	QuEChERS 1 (PSA + C_{18})	QuEChERS 2 (PSA + C_{18} + GCB)	*m*-PFC column (PSA + MWCNTs)	*N*-hexane saturated with acetonitrile
Eugenol	87.2 (3.4)	88.4 (2.4)	99.1 (3.2)	98.7 (2.9)
Methyl eugenol	102.4 (2.9)	90.5 (1.8)	103.7 (2.4)	102.5 (2.2)
Isoeugenol	92.8 (3.9)	78.2 (2.3)	93.8 (1.8)	93.2 (3.0)
Methyl isoeugenol	99.6 (3.2)	77.0 (3.0)	99.3 (2.5)	98.6 (2.8)
Eugenyl acetate	97.4 (2.6)	90.0 (2.7)	101.5 (3.4)	100.2 (2.7)
Acetyl isoeugenol	93.7 (3.7)	82.1 (3.1)	101.1 (1.7)	99.8 (2.4)

suitable for batch processing of a large number of samples. Therefore, *m*-PFC column was selected for purification in subsequent experiments.

3.5. Matrix Effect. Negative pomfrets, prawns, and crabs were selected to obtain the corresponding blank matrix solutions according to Section 2.3. The matrix effects of six eugenol compounds in the three substrates were investigated through comparing 0.1 μg/mL matrix standard solutions and 0.1 μg/mL acetonitrile standard solutions. The relative intensity of matrix effect (ME) = (peak area of matrix standard solution/peak area of solvent standard solution) × 100%. The results showed that six eugenol compounds showed different matrix enhancement effects in pomfrets, prawns, and crabs, with the ME ranging from 106% to 152%. It was found that the matrix effects of six eugenol compounds were different in the same matrix. For example, the matrix effects of six eugenol compounds were in the range of 111.6%–148.1% in the prawns, and the ME of isoeugenol was the strongest. In addition, the matrix effects of the same compound were also different in different substrates. For example, the matrix effects of eugenol in pomfrets, prawns, and crabs were 116.7%, 131.3%, and 151.2%, respectively. This result is basically consistent with the research result of Yu et al. [32], which may be caused by the differences of proteins, fats, pigments, and organic acids in different aquatic products. However, when Xiaoqin Yu used *n*-hexane saturated with acetonitrile to purify samples, the ME of eugenol, methyl eugenol, and isoeugenol was in the range of 148%–211%, which was much greater than that of *m*-PFC column. This showed that *m*-PFC column can effectively remove the interfering substances, obviously reduce the matrix effect, ensure a good recovery rate, and make the quantification more accurate. Considering the existence of matrix effect, the corresponding matrix standard solutions were prepared for quantitative analysis.

3.6. Method Validation

3.6.1. Linearity and Sensitivity. A series of matrix mixed standard working solutions (0.001–0.1 μg/mL) were prepared. The standard curves were drawn between the peak area (Y) and the injection mass concentration (X, μg/mL) of each compound. The limit of detection (LOD) was defined as the lowest addition resulting in a signal-to-noise ratio of 3 : 1. The limit of quantification (LOQ) was defined as the lowest addition resulting in a signal-to-noise ratio of 10 : 1 (Table 3). The correlation coefficients (R^2) for each compound were all above 0.9950. The LODs and the LOQs were in the range of 2–10 μg/kg and 5–20 μg/kg, respectively, which were lower than those of the references (the LODs and the LOQs of six eugenols were all 0.01 mg/kg and 0.02 mg/kg, respectively) [32, 33].

3.6.2. Accuracy and Precision of Method. To test accuracy and precision of the developed method, recovery experiments were performed for each matrix (pomfret, prawn, and crab) in sextuplicate at three spiked levels (1 × LOQ, 2 × LOQ, and 10 × LOQ). The accuracy was estimated by recoveries (%) and the precision was evaluated by RSDs (%) of the spiked samples (Table 4). The results showed that the mean recoveries of six compounds were in the range of 76.4%–105.1% at three levels. The average RSDs were 1.2%–7.5%. The results met the requirements of experimental analysis.

3.7. The Advantages of the Method. To compare this experimental method with BJS 201908 [33] method, the recovery rates of six eugenol compounds were investigated at the spiked level of 100 μg/kg in prawns. The results showed that the recoveries of two methods were in the range of 75.6%–106.1% and 76.7%–104.3%, respectively, without significant difference. However, the LODs and LOQs of this method are lower than BJS 201908. In addition, all six eugenol compounds are liquid at room temperature, with low boiling point and strong volatility. Many steps for purification and nitrogen blowing concentration can easily cause loss of eugenol compounds. In this study, the extracts were directly purified by *m*-PFC column after being frozen and degreased. The purification did not need organic reagents, vortex vibration, and elution. The purified solution did not need to be concentrated and could be directly analyzed by the instrument. Compared with QuEChERS and SPE, the purification by *m*-PFC column is simple and fast, which can avoid the loss of eugenol compounds and ensure a higher recovery rate. In addition, the study used Orbitrap GC-MS to collect data in full scan mode. Compared with ordinary mass spectrometry, Orbitrap GC-MS has the advantages of high resolution, sensitivity, and quality accuracy, which can reduce interference, avoid false positives, and achieve accurate determination in complex matrix. On the basis of ensuring the sensitivity, accuracy, and precision of the

Table 3: Linearity, LODs, and LOQs of six eugenols.

Compound	Linear range (μg/mL)	Substrate	Linear equation	R^2	LOD (μg/kg)	LOQ (μg/kg)
Eugenol	0.003–0.1	Pomfret	$Y = 6.456 \times 10^7 X + 5.571 \times 10^4$	0.9991	5	15
		Prawn	$Y = 7.460 \times 10^7 X + 6.102 \times 10^4$	0.9993		
		Crab	$Y = 8.906 \times 10^7 X + 4.201 \times 10^4$	0.9987		
Methyl eugenol	0.002–0.1	Pomfret	$Y = 6.058 \times 10^7 X + 1.545 \times 10^3$	0.9984	3	10
		Prawn	$Y = 5.042 \times 10^7 X + 3.711 \times 10^3$	0.9981		
		Crab	$Y = 7.011 \times 10^7 X + 6.143 \times 10^3$	0.9990		
Isoeugenol	0.004–0.1	Pomfret	$Y = 4.141 \times 10^7 X + 3.957 \times 10^4$	0.9981	10	20
		Prawn	$Y = 4.969 \times 10^7 X + 1.745 \times 10^4$	0.9974		
		Crab	$Y = 5.590 \times 10^7 X + 3.002 \times 10^4$	0.9987		
Methyl isoeugenol	0.001–0.1	Pomfret	$Y = 1.183 \times 10^8 X + 9.997 \times 10^3$	0.9976	2	5
		Prawn	$Y = 1.689 \times 10^8 X + 1.151 \times 10^3$	0.9991		
		Crab	$Y = 1.301 \times 10^8 X + 3.147 \times 10^3$	0.9977		
Eugenyl acetate	0.001–0.1	Pomfret	$Y = 1.489 \times 10^8 X + 6.276 \times 10^3$	0.9998	2	5
		Prawn	$Y = 2.322 \times 10^8 X + 4.307 \times 10^3$	0.9980		
		Crab	$Y = 1.786 \times 10^8 X + 4.112 \times 10^3$	0.9983		
Acetyl isoeugenol	0.001–0.1	Pomfret	$Y = 1.727 \times 10^8 X + 4.174 \times 10^3$	0.9989	2	5
		Prawn	$Y = 2.172 \times 10^8 X + 3.014 \times 10^3$	0.9986		
		Crab	$Y = 1.986 \times 10^8 X + 5.751 \times 10^3$	0.9971		

Table 4: The recoveries of six eugenols at three spiked levels ($n = 6$).

Compound	Spiked level (μg/kg)	Recovery (RSD) (%)		
		Pomfret	Prawn	Crab
Eugenol	15	101.2 (4.1)	95.2 (5.5)	100.2 (5.2)
	30	98.7 (4.8)	99.7 (3.8)	102.7 (7.2)
	150	105.1 (7.5)	103.4 (3.3)	101.8 (6.6)
Methyl eugenol	10	89.4 (5.3)	83.1 (6.3)	86.3 (4.9)
	20	81.5 (3.3)	88.9 (3.7)	84.3 (7.1)
	100	81.9 (2.5)	84.4 (7.4)	85.1 (5.8)
Isoeugenol	20	100.4 (6.7)	96.6 (4.8)	98.7 (4.9)
	40	93.5 (3.4)	93.8 (5.5)	92.6 (2.1)
	200	91.3 (3.5)	96.5 (2.8)	95.2 (4.6)
Methyl isoeugenol	5	84.6 (5.2)	86.1 (5.4)	83.0 (5.7)
	10	79.7 (1.2)	80.6 (3.1)	81.2 (1.9)
	50	88.4 (4.1)	82.9 (4.4)	84.6 (2.3)
Eugenyl acetate	5	81.7 (5.5)	82.8 (6.8)	85.8 (2.6)
	10	87.2 (2.8)	84.7 (4.6)	78.4 (4.9)
	50	86.7 (4.6)	82.8 (2.9)	83.5 (6.3)
Acetyl isoeugenol	5	82.6 (1.5)	84.6 (7.2)	76.4 (2.7)
	10	77.9 (6.2)	80.8 (5.2)	84.8 (1.8)
	50	83.4 (2.8)	81.4 (4.5)	81.3 (1.9)

Table 5: Detection value of eugenol compounds in positive samples.

No.	Sample	Contents (μg/kg)	
		Eugenol	Isoeugenol
1	Grouper 1	44.2	102.5
2	Grouper 2	105.1	80.3
3	Grouper 3	331.4	—
4	Grouper 4	98.3	—
5	Perch 1	189.7	—
6	Perch 2	225.2	—
7	Perch 3	75.4	—
8	Pomfret 1	136.4	—
9	Pomfret 2	209.1	—

Table 5: Continued.

No.	Sample	Contents (μg/kg)	
		Eugenol	Isoeugenol
10	Pomfret 3	36.4	—
11	Turbot 1	48.1	—
12	Turbot 2	111.9	—
13	Tilapia 1	84.8	—
14	Tilapia 2	17.0	—

"—" indicates undetected.

method, the usability and analysis efficiency of the method are greatly improved.

3.8. Testing of Actual Samples. Eighty samples (50 fish, 15 shrimps, and 15 crabs) were detected by the established method, and the results are shown in Table 5. Eugenol was detected in 14 samples with concentrations ranging from 17.0 to 331.4 μg/kg. Isoeugenol was detected only in two samples at concentrations of 80.3 μg/kg and 102.5 μg/kg, respectively. The other eugenol compounds were not detected in any sample. The incidence of anesthetic residue in all the samples was 17.5%. The positive samples included 12 seawater fish (grouper, perch, pomfret, and turbot) and two freshwater fish (tilapia), and six eugenol compounds were not detected in shrimps and crabs. The frequencies of eugenol compounds were higher in seawater fish than freshwater fish. According to the test results, eugenol compounds have been widely used in fisheries as anesthetics in China, and the detection and supervision of eugenol compounds in aquatic products should be strengthened.

4. Conclusions

The method for determination of six eugenol compounds in aquatic products was established by *m*-PFC column combined with Orbitrap GC-MS and applied to detect actual samples. The samples were ultrasonically extracted with acetonitrile and purified by *m*-PFC column, which was easy to operate. The compounds could be identified accurately by Orbitrap GC-MS through the retention time and precise molecular mass, which could avoid false positive samples. The method provides a new technical support for rapid and accurate determination of six eugenol compounds in aquatic products.

Data Availability

The data used to support this study are available within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Yunxia Huang and Qiang Li contributed equally to this work.

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