

## Trifluralin in aquatic products: QuEChERS and Gas chromatography-tandem mass spectrometry for trace amount detection

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**Abstract:** In the present study, an analytical method was proposed for detecting trifluralin in aquatic products at trace concentrations. The method employed QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) and gas chromatography coupled with tandem mass spectrometry (GC-MS/MS) as the sample preparation and measurement, respectively. The effect of the aqueous phase volume used in the QuEChERS was demonstrated, and the ratio of 10:10 (mL) between water and acetonitrile phase was used for 5 g of sample. Besides, dSPE using C18 and primary-secondary amine (PSA) was applied to remove the potential interferences from the food matrices, indicating no remarkable analyte loss. The linear range was built up from 0.50  $\mu\text{g L}^{-1}$  to 3.0  $\mu\text{g L}^{-1}$  ( $R^2 = 0.9993$ ). Other criteria, i.e., repeatability ( $\text{RSD}_r = 0.86\text{-}1.96\%$ ), reproducibility ( $\text{RSD}_R = 1.09\text{-}2.01\%$ ), and recovery (over 90%), were in accordance with Appendix F of AOAC (2016) for method performance. Although no trifluralin was detected for the commercial samples (fish, shrimp, and breaded shrimp), the spiked samples performed favorable recoveries and precision.

**Key words:** trifluralin, aquatic products, QuEChERS, dSPE, GC-MS/MS

### 1. Introduction

Trifluralin is a commonly used herbicide in agriculture. It is a pre-emergent herbicide, which means it is applied to the soil before weed seeds have germinated. Trifluralin works by inhibiting plant cell division, preventing the growth of roots and shoots, and controlling many annual grasses and broadleaf weeds. Trifluralin is often used with other herbicides to provide broad-spectrum weed control. It is used in

various crops, including corn, soybeans, cotton, and vegetables.<sup>1-3</sup> Trifluralin can be toxic to humans and animals if it is ingested, inhaled, or comes into contact with the skin or eyes. The level of toxicity depends on the dose and duration of exposure, as well as individual factors such as age, health status, and sensitivity to the chemical. Ingesting trifluralin can cause gastrointestinal symptoms such as nausea, vomiting, and diarrhea. Long-term exposure to high levels of the chemical may also affect the liver,

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kidneys, or other organs.<sup>4,5</sup>

Trifluralin is classified as a possible human carcinogen by the United States Environmental Protection Agency (EPA) based on studies in laboratory animals. However, the risk of cancer from exposure to trifluralin in humans is not well established. Specifically, the EPA classified trifluralin as a Group C (possible human) carcinogen based on an increased incidence of liver tumors in mice and thyroid tumors in rats in chronic feeding studies. However, the EPA also noted that the relevance of these findings to humans is uncertain and that there is limited human epidemiological data on the carcinogenicity of trifluralin. The allowable limits of trifluralin in food products can vary depending on the specific food item and the country in which it is being produced or imported. For example, in the United States, the Environmental Protection Agency (EPA) has established tolerances for trifluralin in many different food commodities, ranging from 0.01 parts per million (ppm) in milk to 15 ppm in sugarcane.<sup>1</sup>

In the European Union (EU), the allowable limits for trifluralin in food are established under Regulation (EC) No 396/2005,<sup>6</sup> with specific limits set for each food item or group. In the European Union, Maximum Residue Levels (MRLs) have been established for trifluralin in various food products, including cereals, fruits, vegetables, and nuts. The MRLs for trifluralin in the EU range from 0.01 ppm to 0.05 ppm, depending on the food product. For instance, the limit for trifluralin in cereals and cereal products is set at 0.02 ppm, while the limit for root vegetables is set at 0.05 ppm.<sup>7</sup>

There are several methods for the detection and quantification of trifluralin in various samples, including food and environmental matrices. Gas Chromatography (GC) is a widely used analytical technique for the detection and quantification of trifluralin. In this method, the sample is extracted with a suitable solvent, and the extract is analyzed by GC with a suitable detector, e.g., a mass spectrometer (MS) or a tandem mass spectrometer (MS/MS).<sup>8,9</sup> Liquid Chromatography (LC) is another widely used analytical technique for the detection and quantification of trifluralin. In this method, the sample is extracted with a suitable solvent, and the extract is analyzed by LC with detector

MS.<sup>10-12</sup>

Although trifluralin is widely recognized for its effectiveness as a plant protection drug. However, its potential to replace malachite green in combating aquatic fungal diseases in fish and shrimp has attracted considerable attention.<sup>13</sup> Experimental studies have demonstrated its ability to effectively treat larval fungal diseases and its utilization as a treatment method in shrimp farming.<sup>14</sup> In aquaculture, trifluralin was initially introduced in shrimp seed production to prevent and treat fungal diseases in shrimp larvae, with recommended dosage levels of approximately 0.05 mg L<sup>-1</sup> for disease prevention and 0.1 mg L<sup>-1</sup> for disease treatment. After that, trifluralin is extensively used in water treatment to control and eliminate various parasitic pathogens in fish farming ponds, particularly in the case of fingerling catfish production. As a result, some fish and shrimp farmers have resorted to using trifluralin for seedling treatment, thereby posing a potential risk of residual trifluralin accumulation in these animals as they mature.<sup>15</sup>

Given the aforementioned concerns, it is crucial to effectively monitor and control trifluralin levels in shrimp and fish. Consequently, the main objective of the present study is to validate an analytical approach utilizing gas chromatography-tandem mass spectrometry (GC-MS/MS) for accurate determination of trace amounts of trifluralin in shrimp and fish samples. Specifically, the QuEChERS technique has been thoroughly investigated and implemented during the sample preparation process to streamline analysis time while ensuring method efficiency. Furthermore, the incorporation of trifluralin D14 as an internal standard throughout the entire sample processing procedure enhances the accuracy and reliability of the results obtained. The present study can contribute to ensuring the safety and quality of seafood products, as it addresses the potential adverse effects of trifluralin on aquatic organisms, thereby safeguarding public health.

## 2. Experimental

### 2.1. Chemicals and equipment

Trifluralin (97.5 %, Sigma-Aldrich, Germany),

trifluralin D14 (96.2 %, Sigma-Aldrich, Germany), acetonitrile (analytical grade, Merck, Germany), n-hexane (analytical grade, Merck, Germany), acetic acid (analytical grade, Merck, Germany), sodium chloride (analytical grade, Merck, Germany), QuEChERS Mix Extraction (CHROMABOND, 6000 mg magnesium sulfate anhydrous and 1500 mg sodium acetate anhydrous), Bondesil-C18 (40  $\mu\text{m}$ , Agilent), ethylenediamine-N-propyl, polymerically bonded (PSA, 40  $\mu\text{m}$ , Agilent), graphitized carbon black (GCB, 40  $\mu\text{m}$ , Agilent). PTFE membrane, diam. 50 mm, pore size 0.2  $\mu\text{m}$  (Merck, Germany) was used for sample filtration.

## 2.2. QuEChERS as the sample preparation

In the present study, fish, shrimp, and breaded shrimp samples were collected (5 samples for each type)

from supermarkets in Ho Chi Minh City (Vietnam). The fish samples were rinsed with deionized water (DIW) and removed from their heads, guts, and skin. The shrimp samples were also rinsed with DIW and removed from their shells. The pre-treated fish and shrimp samples along with the breaded samples were homogenized by a blender, then stored in a zip-lock bag at  $-18\text{ }^{\circ}\text{C}$  prior to analysis.

QuEChERS was used for sample preparation (Fig. 1). Generally, there are three main steps in a typical QuEChERS procedure, i.e., extraction, clean-up, and concentration. In the present study, the extraction step was followed by AOAC 2007.01<sup>16</sup> with some modifications obtained from the investigation and optimization. Then, the sample clean-up was carried out by dispersive solid phase extraction (dSPE), which involved the addition of certain sorbent(s) to

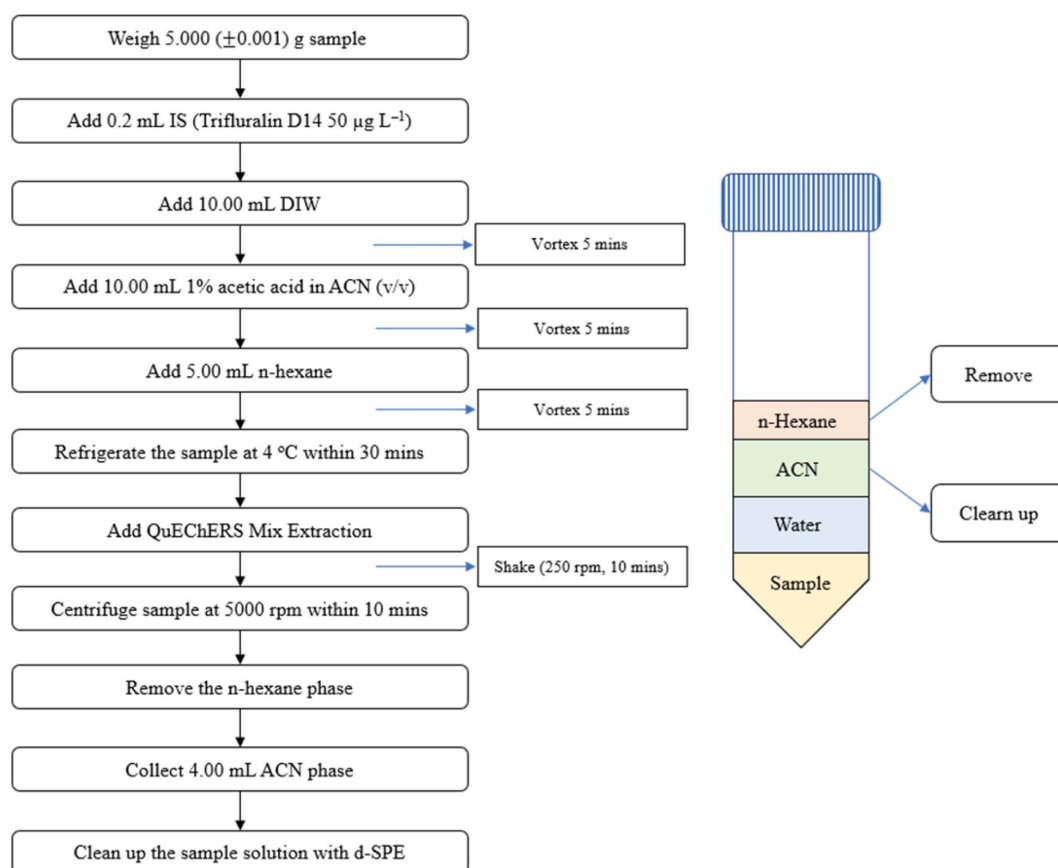


Fig. 1. The sample preparation procedure using the QuEChERS method for the determination of trifluralin in seafood samples and processed seafood products.

remove the potential interferences from the matrix components. Finally, the analyte was concentrated by evaporating the solvent (usually the solvent volume is higher in this stage) under a gentle nitrogen gas stream before dissolving the residue by a smaller solvent volume (the two solvents before the evaporation and for residue dissolution could be different). As can be seen from *Fig. 1*, after centrifugation, the ACN layer at the top was subjected to dSPE by transferring a 4 mL sample extract solution into a reaction tube containing the dSPE mixture, followed by vortexing for 2 minutes and centrifugation for 5 minutes at 5000 rpm. Subsequently, 2 mL of the cleaned extract solution was transferred into a 10 mL glass tube using a micropipette, and the solution was dried by 99.999% N<sub>2</sub> gas. The residue was then dissolved in 1 mL of n-hexane, filtered through a 0.2 µm PTFE filter membrane, and collected in a 1.5 mL vial.

The spiked samples were used for recovery tests, i.e., 0.1 mL of 50 µg L<sup>-1</sup> trifluralin was spiked in 5 g of shrimp samples. In the present study, the water phase was initially used to leach the analyte from the matrix, followed by other steps. Therefore, different water volumes, i.e., 2 mL, 5 mL, 10 mL, and 15 mL, were attempted to discover to most favorable in terms of the highest recovery (while the sample weight and volume of ACN phase were kept constant for all experiments).

For food matrices, clean-up is necessary to remove the potential interferences. In the present study, different sorbents were tried to investigate their efficiency, i.e., MgSO<sub>4</sub>:C<sub>18</sub>:PSA (600:200:200 mg), MgSO<sub>4</sub>:C<sub>18</sub> (600:200 mg), and MgSO<sub>4</sub>:PSA (600:200 mg).

### 2.3. The operating parameter of GC-MS/MS instrument

The Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS, Thermo Fisher Scientific) was used in the present study. A non-polar fused silica capillary column TG-5MS (30 m × 0.25 mm), 0.25 µm film thickness (Thermo Fischer Scientific, USA) was used. Helium was used as the carrier gas at a flow rate of 1.2 mL min<sup>-1</sup>. The injection volume was 1.0 µL. The oven temperature was programmed as: (i) 80 °C for 1 min; (ii) rate of 30 °C min<sup>-1</sup> from 80 °C to 220 °C

(hold for 1 min); (iii) rate of 30 °C min<sup>-1</sup> from 220 °C to 280 °C (hold for 4 min). The temperature of the injector, ion source, and MS interface was set at 250 °C, 280 °C, and 230 °C, respectively. The MS scan mode was SRM with electron impact ionization at 70 eV. The collision gas was nitrogen (1.5 mL min<sup>-1</sup>) and helium (2.25 mL min<sup>-1</sup>). The quantification ions of trifluralin were m/z of 306 and 264 (collision energy of 10 eV), and confirming ions were m/z of 306 and 206 (collision energy of 15 eV). Meanwhile, the trifluralin D1 had its quantification ions m/z of 315 and 267 (collision energy of 8 eV) while the confirming ions m/z of 315 and 209 (collision energy of 10 eV). NIST 2.2 library collection was used for the qualification.

### 2.4. Statistical analysis

All the investigations were conducted in triplicate (n = 3), except for the intra- and inter-day precision (n = 6), to ensure the repeatability among runs. The data and charts were processed by Microsoft Office Excel 2016, then expressed as a mean value ± standard deviation (SD) for sample analysis. A significance level of 0.05 was applied.

The analytical method for trifluralin in aquatic products using QuEChERS and GC-MS/MS was validated according to Appendix F of AOAC (2016).<sup>17</sup> The method performance includes the estimation of detection and quantification limits (LOD and LOQ), calibration curve (linear relationship: standard solution concentrations vs. their respective chromatographic peak areas normalized by the internal isotope standard), repeatability/intra-day precision, reproducibility/inter-day precision, recovery tests (spiked shrimp samples at LOQ, 2LOQ, and 3LOQ), and Shewhart quality control chart.

The validated method was applied to determine the trifluralin in real samples, including fish, shrimp, and breaded shrimp.

## 3. Results and Discussion

### 3.1. Effects of water phase volume on the QuEChERS

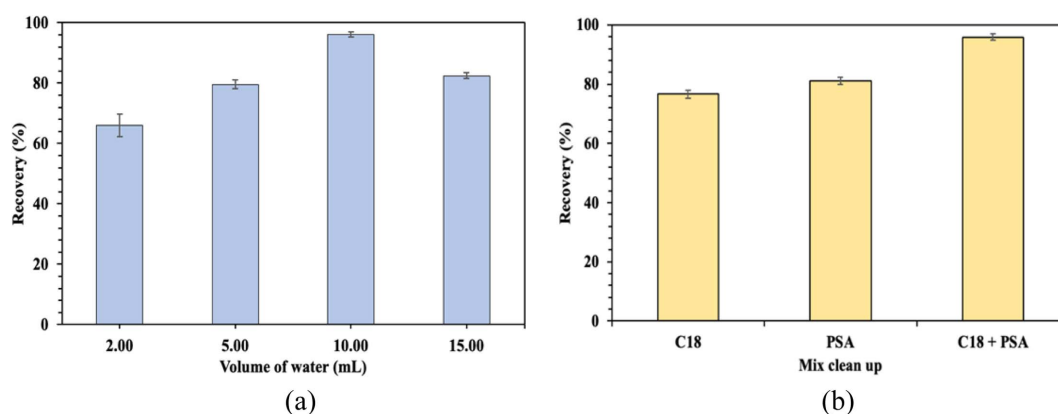
The QuEChERS has been a widely used sample

preparation technique for the analysis of pesticides and contaminants in food and environmental samples. It involves a combination of different steps, i.e., extraction, clean-up, and concentration, to provide a simple, quick, and cost-effective approach. There are commonly three versions of the salt mixture used to create a buffer medium to control the pH during the QuEChERS. The first version, published by Anastasiades et al., uses ACN as the extraction solvent without a buffer solution.<sup>18</sup> The second version, according to AOAC 2007.01, uses ACN with a salt mixture of  $\text{MgSO}_4\text{-CH}_3\text{COONa}$  to create an acetate buffer system ( $\text{pH} = 4.5$ ).<sup>16</sup> The third version, according to CSN EN 15662, uses ACN with a salt mixture of  $\text{MgSO}_4$ , NaCl, trisodium citrate dihydrate, and disodium hydrogen citrate sesquihydrate to form a citrate buffer system ( $\text{pH} = 5.0\text{-}5.5$ ).<sup>19</sup> Trifluralin is a weak acid with a  $\text{pK}_a$  value of approximately 5.3.<sup>20</sup> In order to optimize the extraction efficiency of trifluralin, it is necessary to ensure that the compound dominantly exists in its neutral form during the extraction process. Therefore, the acetate buffer solution with a  $\text{pH}$  of 4.5 was chosen. This buffer ( $\text{pH} = 4.5$ ) enables the protonation of the acidic groups in trifluralin, thus preserving the neutral form of the compound and increasing its affinity towards the extraction solvent, i.e., sample matrix to the aqueous phase, then to the ACN phase. To this hypothesis, the amount of trifluralin extracted to the ACN layer needs to be enhanced to

improve the method performance, which means aqueous phase volume would be investigated to achieve both sensitivity and precision (discussed in later parts).

Water can have both positive and negative effects on the QuEChERS. Water can easily penetrate the sample structure to dissolve analytes and create favorable conditions for their extraction into organic solvents. The analytes will be efficiently extracted with an appropriate amount of water.<sup>18,21</sup> Conversely, using too much water in this process can affect the volume ratio of water and solvent or form emulsions, which reduces the extraction efficiency. Different sample matrices or analytes have different dissolution ratios and require different amounts of water. In this study, we investigated the volume of water used in the process of extracting analytes from the sample matrix, and the results are presented in the form of a graph, as shown in *Fig. 2(a)*.

The findings presented in *Fig. 2(a)* demonstrate that the recovery of trifluralin exhibits a gradual increase as the amount of water is increased from 2 to 10 mL. For lower water volumes (2 mL and 5 mL), the water was not sufficient to penetrate the sample matrix and/or not able to quantitatively leach the analyte to the extracts. However, higher water volume might result in the analyte partitioning in a larger extract. Then, single extraction for fixed ACN volume at 10 mL performed lower efficiency, i.e., a



*Fig. 2.* Effect of (a) water phase volume on the QuEChERS extraction procedure and (b) the impact of different sorbents on the clean-up efficiency.

decrease in recovery at 15 mL, compared to 10 mL, the highest recovery, of the water phase (82.5 % vs. 96.1 %). The study of Anastassiades et al. also recommended a range of water volume from 2 to 5 mL per gram of sample for shrimp samples using the QuEChERS.<sup>18</sup> As a theoretical assumption, an amount of 5 g of shrimp sample in the present study would require a water volume ranging from 8 to 25 mL, which included the investigation range and the optimized water phase in the present study. Therefore, a solvent ratio of 1:1 v/v (10 mL:10 mL) water to ACN was adequate for the quantitative extraction and applied for further experiments.

Overall, these results highlight the importance of optimizing the volume of water used in the QuEChERS for extracting trifluralin from the samples, demonstrating that an appropriate volume ratio of water and ACN can enhance extraction efficiency.

### 3.2. Dispersive solid-phase extraction (dSPE) for sample clean-up

Dispersive solid-phase extraction (dSPE) is commonly used for removing potential matrix interferences through the adsorption mechanism of various sorbent materials. The sorbent materials typically used for dSPE include primary-secondary amine (PSA), octadecyl (C<sub>18</sub>), graphitized carbon black (GCB), and Florisil.<sup>22,23</sup> In the present study, C18, PSA, and a combination of C18 and PSA were attempted for dSPE, in which PSA was used to eliminate organic acids and polar interferences, while C18 was used to remove other non-polar interferences. *Fig. 2(b)* shows that the combination of PSA and C18 results in higher extraction efficiency in terms of recovery. This result was consistent with previous reports in the literature.<sup>24-26</sup> As mentioned, PSA is a primary-secondary amine had the ability to retain and concentrate polar and acidic compounds, such as organic acids, sulfonic acids, and phenols.<sup>27</sup> C18 is octadecyl silica, and it can adsorb non-polar compounds such as fatty, protein, volatile oil, and liposoluble substances. Therefore, the combination of PSA and C18 sorbents in the sample preparation of fish and shrimp can effectively remove fatty acids and the

light yellow color that often appears after extraction. Although GCB has been widely applied in many studies due to its effective color removal ability, it can also strongly adsorb compounds containing benzene groups, which is not suitable for trifluralin analysis.<sup>28</sup>

### 3.3. Analytical method performance

#### 3.3.1. Limit of detection and quantification (LOD and LOQ)

The limits of detection (LOD) and quantification (LOQ) were estimated by simultaneously analyzing 11 separate shrimp samples spiked with trifluralin of 1.0 µg kg<sup>-1</sup>. The standard deviation (SD) for these samples was calculated to apply the following relationships:  $LOD = 3.3 \times SD/a$  and  $LOQ = 10 \times SD/a$ , whereas *a* is the slope of the established calibration curve.<sup>29</sup> The LOD and LOQ values are present in *Table 1*, which demonstrates remarkably low concentrations as well as the favorable sensitivity of the analytical method and is suitable for trifluralin

*Table 1.* Analytical method performance

Parameter				
LOD		0.3 (µg kg <sup>-1</sup> )		
LOQ		1.0 (µg kg <sup>-1</sup> )		
Regression equation		$y = 0.4499x - 0.0004$		
R <sup>2</sup>		0.9993		
Spiked concentration		LOQ	2LOQ	3LOQ
RSD <sub>r</sub> (%)	Fish	1.16	0.86	1.19
	Shrimp	1.96	1.23	1.31
	Breaded shrimp	1.56	1.09	1.33
RSD <sub>R</sub> (%)	Fish	1.29	1.50	1.48
	Shrimp	2.01	1.23	1.32
	Breaded shrimp	1.91	1.09	1.35
Recovery (%)	Fish	94.2	95.5	93.6
	Shrimp	92.7	94.0	94.8
	Breaded shrimp	92.4	94.5	94.3
ME (%)	Fish	-5.40 ± 0.88		
	Shrimp	-7.47 ± 0.49		
	Breaded shrimp	-8.74 ± 0.27		

The sample was spiked at the LOQ (Limit of Quantification) level with a concentration of 1.0 µg kg<sup>-1</sup>, at the 2LOQ level with a concentration of 2.0 µg kg<sup>-1</sup>, and at the 3LOQ level with a concentration of 3.0 µg kg<sup>-1</sup>.

ME (%) is the Matrix Effects expressed as a percentage.

analyses in different food matrices. To the best of our knowledge, there were no regulations regarding the allowable content of trifluralin in fish and shrimp by EU. However, according to EU Commission Regulation No. 600/2010, the maximum residue limit (MRL) for trifluralin in terrestrial animal products<sup>30</sup> is set at  $10 \mu\text{g kg}^{-1}$ , in which the LOQ estimated in the present study was lower ( $1.0 \mu\text{g kg}^{-1}$  vs.  $10 \mu\text{g kg}^{-1}$ ).

### 3.3.2. Calibration curve

In the present study, the quantification was conducted based on the calibration curve, which was established according to the linear relationship between the trifluralin standard concentrations, and their respective chromatographic peak areas normalized by the internal standard peaks. The working range is from  $0.50 \mu\text{g L}^{-1}$  to  $3.0 \mu\text{g L}^{-1}$  (Table 1) and demonstrates the goodness of linearity ( $R^2 = 0.9993$ ).

### 3.3.3. Intra- and inter-day precision

Three representative sample matrices, i.e., fish, shrimp, and breaded shrimp, spiked with trifluralin at three different concentration levels of LOQ, 2LOQ, and 3LOQ were used to evaluate the intra-day precision or repeatability of the method. Six replicates ( $n = 6$ ) were performed for each sample, and the  $RSD_r$  (%) value was used for the assessment. Meanwhile, one-way ANOVA analysis was used to calculate the  $RSD_R$

(%) value for evaluating the inter-day precision or reproducibility of the method (in three different days and six replicates for each day). The results for method precision evaluation are performed in Table 1, which indicates agreements with Appendix F of AOAC (2016)<sup>17</sup> for standard method performance requirements (in ppb concentration ranges).

### 3.3.4. Recovery tests

The recovery tests were conducted for three representative aquatic product samples, including fish, shrimp, and breaded shrimp. The spiked trifluralin concentrations were also in three different levels of LOQ, 2LOQ, and 3LOQ ( $1.0 \mu\text{g kg}^{-1}$ ,  $2.0 \mu\text{g kg}^{-1}$ , and  $3.0 \mu\text{g kg}^{-1}$ ). The recoveries shown in Table 1 are all lower than 100%, which might be due to the potential analyte loss during the sample preparation, e.g., QuEChERS, dSPE. However, those values were in accordance with Appendix F of AOAC (2016) for ppb ranges, indicating the analytical procedure could be applied for further sample analysis.

### 3.3.5. Quality control chart

In order to control the stability of the analytical procedure, the Shewhart quality control chart or QC chart was constructed based on the analysis of the real sample, in which the fish matrix was chosen as a representative. The QC chart was obtained from 21

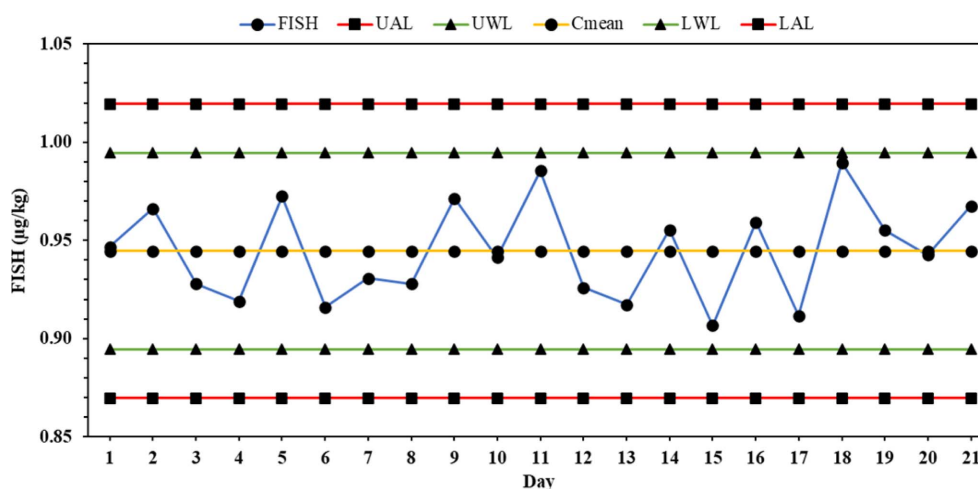


Fig. 3. Shewhart quality control chart.

analysis days (Fig. 3), including the mean concentration ( $C_{\text{mean}}$ ) and warning values, i.e., upper warning limit (UWL), lower warning limit (LWL), upper action limit (UAL), and lower action limit (LAL). These values were calculated according to the principles of  $\pm 2SD$  and  $\pm 3SD$ , in which SD is the standard deviation of three replicates, for warning and action limits, respectively. The spiked fish sample to build the QC chart is called the QC sample, then the QC sample should be analyzed simultaneously with other samples to check the stability of the analytical system. To ensure the accuracy of the analytical results, the QC sample should be between the LWL and UWL.<sup>31</sup>

The analysis should be temporarily stopped, and the whole procedure, e.g., QuEChERS and GC-MS/MS measurement, has to be checked if there is one of the following situations for QC sample results, including (i) out of the LAL-UAL, (ii) more than two in three continuous values beyond the LWL-UWL, (iii) more than nine continuous values located at the same side of the mean line (above or under), and (iv) six continuous points going up or down.<sup>31</sup>

For the present study, it could be seen from Fig. 3 that the analytical procedure meets its favorable stability for different day analyses, i.e., all action and warning limits are close to the mean value. Besides,

the obtained results from the individual analysis of the QC sample are in the range of LWL-UWL. There are also none of the nine continuous points located on the same side of the mean line.

### 3.3.6. The matrix effects

The matrix effect (%ME) is calculated as the ratio of the slope of the matrix-matching calibration curve to the slope of the calibration curve prepared in the pure solvent, using the following formula:

$$\%ME = \left( \frac{\text{Slope of calibration curve in the matrix}}{\text{Slope of calibration curve in pure solvent}} - 1 \right) \times 100\%$$

The matrix effects were evaluated by applying specific criteria: ME = 0 to indicate no matrix effects, ME < 0 to indicate ion suppression matrix effects, and ME > 0 to indicate ion enhancement matrix effects. These criteria allowed us to assess the impact of the sample matrix on the measurement of analytes. Matrix effects can introduce variability in the analyte response, potentially affecting the accuracy and reliability of the analysis. By categorizing the matrix effects into these three categories, we were able to identify and characterize the nature of the observed matrix effects. Furthermore, it is noteworthy that when the ME

Table 2. Concentrations of trifluralin in aquatic products

No.	Sample	Determined concentration ( $\mu\text{g}/\text{kg}$ )		Recovery $\pm$ SD (%)	RSD (%)
		Original sample	Spiked samples		
1	Fish-1	Not detected	0.929 $\pm$ 0.015	92.9 $\pm$ 1.5	1.57
2	Fish-2	Not detected	0.926 $\pm$ 0.019	92.6 $\pm$ 1.9	2.02
3	Fish-3	Not detected	0.932 $\pm$ 0.015	93.2 $\pm$ 1.5	1.65
4	Fish-4	Not detected	0.943 $\pm$ 0.024	94.3 $\pm$ 2.4	2.53
5	Fish-5	Not detected	0.942 $\pm$ 0.009	94.2 $\pm$ 0.9	0.91
6	Shrimp-1	Not detected	0.949 $\pm$ 0.015	94.9 $\pm$ 1.5	1.53
7	Shrimp-2	Not detected	0.934 $\pm$ 0.011	93.4 $\pm$ 1.1	1.14
8	Shrimp-3	Not detected	0.930 $\pm$ 0.011	93.0 $\pm$ 1.1	1.22
9	Shrimp-4	Not detected	0.916 $\pm$ 0.011	91.6 $\pm$ 1.1	1.18
10	Shrimp-5	Not detected	0.918 $\pm$ 0.011	91.8 $\pm$ 1.1	1.17
11	Breaded shrimp-1	Not detected	0.927 $\pm$ 0.010	92.7 $\pm$ 1.0	1.05
12	Breaded shrimp-2	Not detected	0.917 $\pm$ 0.004	91.7 $\pm$ 0.4	0.46
13	Breaded shrimp-3	Not detected	0.941 $\pm$ 0.002	94.1 $\pm$ 0.2	0.21
14	Breaded shrimp-4	Not detected	0.941 $\pm$ 0.029	94.1 $\pm$ 2.9	3.12
15	Breaded shrimp-5	Not detected	0.929 $\pm$ 0.010	92.9 $\pm$ 1.0	1.07



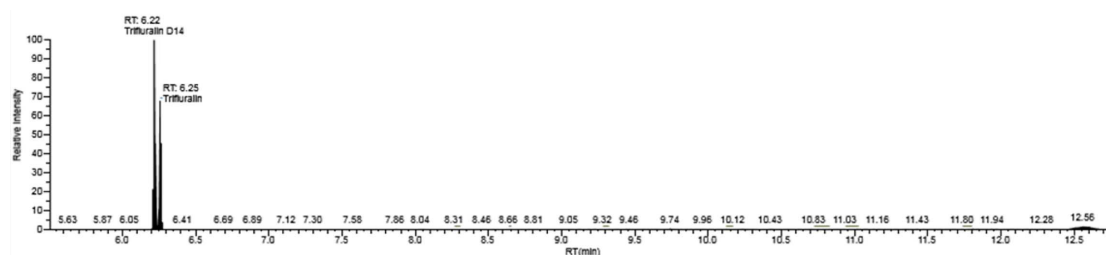


Fig. 4. Chromatogram (SRM mode) of trifluralin in a shrimp sample spiked with trifluralin standard at the 2LOQ level ( $2.0 \mu\text{g kg}^{-1}$ ).

value falls within the range of 0 to  $\pm 20\%$ , it can be assumed that there are no significant matrix effects present. These criteria were utilized to evaluate the influence of the sample matrix on the analyte measurements and determine the extent of ion suppression or enhancement.<sup>32</sup>

The results (Table S1) showed that the %ME values obtained were  $(-5.40 \pm 0.88)\%$  for the fish matrix,  $(-7.47 \pm 0.49)\%$  for the shrimp matrix, and  $(-8.74 \pm 0.27)\%$  for the breaded shrimp matrix. All the values obtained for the three matrices were less than 0, indicating ion suppression matrix effects. Additionally, the |ME%| values for all three matrices were below 10 %, suggesting that the matrix had no significant impact on the analysis method.

### 3.4. Application of the validated method for aquatic products

The validated method was applied to determine the trifluralin concentration in the collected samples. Besides, spiked samples were conducted simultaneously to ensure accuracy. %RSDs were also calculated for three replicates ( $n = 3$ ). The results in Table 2 show that no trifluralin was detected for all available samples since they were commercial products. However, spiked samples (a representative chromatogram of a shrimp sample spiked with trifluralin, SRM mode, is performed in Fig. 4) demonstrate their favorable recoveries (over 90 %) and proper repeatability for ppb ranges.

## 4. Conclusions

The present study described the QuEChERS and SPE as a sample preparation procedure for the analysis

of trifluralin in aquatic products at trace concentrations. The water phase volume during the QuEChERS and different sorbents for dSPE were investigated to achieve the optimized conditions in terms of recoveries. The sample amount of 5 g was used for the aqueous phase and acetonitrile phase ratio of 10:10 (mL), and no severe analyte loss was observed during the whole sample preparation, especially the dSPE employing a mixture of C18 and PSA. GC-MS/MS was used as a measurement method, demonstrating high sensitivity for the analysis in ppb ranges. The analytical method was validated based on the guideline in Appendix F of AOAC (2016), showing good agreement. Due to many steps in the analytical method, a QC chart was constructed to ensure stability among different runs. No trifluralin was detected by the method for commercial samples; however, favorable recoveries and repeatability were obtained. Therefore, the proposed method can be used as a tool for trifluralin analysis (trace concentrations) in different aquatic products in terms of both routine analysis and research activities.

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