RESEARCH ARTICLE

Rapid determination of abamectin B1a residues in fishery products by HPLC-QTOF-MS

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Abamectin B1a is a commonly employed antiparasitic agent in aquaculture. Excessive residues of this agent in aquatic products may threaten human health. Traditional detection methods have several disadvantages including serious matrix interference, complex sample pretreatment, and insufficient sensitivity. This research developed a sensitive, rapid, and reliable analytical method for determining abamectin B1a residues in fish liver tissues. A high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (HPLC-QTOF-MS) method was proposed. Chromatographic separation was performed on an EclipsePlus C18 column with gradient elution using methanol as the organic phase and 5 mM ammonium acetate containing 0.1% formic acid as the aqueous phase. Mass spectrometric detection was conducted in positive electrospray ionization (ESI+) mode with full-scan acquisition. The results demonstrated 88.26% mean recovery with good linearity (Y = 1837.24X − 206.5, r = 0.9997). The limit of quantification (LOQ) and limit of detection (LOD) were 1.0 μg/kg and 0.5 μg/kg, respectively. The proposed method was applied to 20 commercial fish samples of four species, and abamectin B1a was detected in three samples at concentrations of 5.8, 11.2, and 17.2 μg/kg. The proposed method was efficient, simple, and reliable, provided strong technical support for the quantitative and qualitative detection of abamectin B1a residues in aquatic products, and was suitable for routine safety and quality monitoring.

Keywords: HPLC-QTOF-MS; fishery products; abamectin B1a; simultaneous determination.

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Introduction

Abamectin is a macrolide broad-spectrum antiparasitic agent, comprising avermectin B1a and B1b, derived from the natural compounds known as avermectins. While avermectin is the parent compound produced by soil bacteria such as *Streptomyces avermitilis* [1], abamectin is the commercialized product extensively used for agricultural livestock deworming and crop pest control [2]. However, abamectin is highly toxic to aquatic organisms, especially crustaceans and fish with low median lethal concentrations (LC_{50}). Its residues can accumulate in organisms through the food chain, posing potential risks to ecological environment and human health [3]. Based on China's National Food Safety Standard (GB 2763-2021) [4], the maximum residue limit (MRL) of abamectin in aquatic products is 0.1 mg/kg, placing high requirements on abamectin residue detection accuracy in aquatic products.

Currently, extensive research has been conducted on methods for detecting abamectin residues. Among these, chromatographic techniques and coupled chromatography-mass spectrometry occupy a dominant position in this field of study. High-performance liquid chromatography (HPLC) is commonly applied for the regular monitoring of abamectin in by-product and agricultural samples due to their cost-effectiveness and straightforward operations. Li *et al.* introduced an HPLC-UV method to measure abamectin residues in tea with a limit of detection (LOD) of 1.2 μg/kg. In complex samples, however, this method showed poor resistance to matrix interference, making it susceptible to providing false-positive results [5]. HPLC-tandem mass spectrometry (HPLC-MS/MS) has emerged as the primary approach for the analysis of abamectin residues due to its exceptional specificity and sensitivity. Zhang *et al*. proposed an HPLC-MS/MS analytical technique to measure abamectin residues in milk with average recovery rates ranging from 85.3% to 92.1%, meeting trace residue detection requirements [6]. In addition, Wang *et al*. simultaneously quantified several antiparasitic agents including ivermectin and abamectin in vegetable samples by integrating quick, easy, cheap, effective, rugged, and safe (QuEChERS) sample pretreatment with HPLC-MS/MS [7]. Researchers have also investigated alternative detection platforms such as enzyme-linked immunosorbent assay (ELISA) and Raman spectroscopy [8, 9]. ELISA can rapidly screen large sample batches, but suffers from limited quantitative accuracy, while Raman spectroscopy is rarely applied in complex matrix sample detection due to its instrumental precision limitations. The research on the detection of abamectin residues has mainly focused on terrestrial agricultural and sideline products like vegetables, tea, and livestock products with relatively scarce research on aquatic products. In addition, existing aquatic product detection methods mostly adopt HPLC or HPLC-MS/MS technologies, which have certain limitations that HPLC has insufficient sensitivity and difficulty in meeting the precise detection requirements of trace abamectin B1a in aquatic products. Although HPLC-MS/MS has high sensitivity, it has limited capabilities in qualitatively analyzing structural elucidation and unknown impurities, making it unable to meet the precise qualitative requirements of complex matrix samples in food crime cases [10]. HPLC-quadrupole time-of-flight mass spectrometry (HPLC-QTOF-MS) combines the robust separation performance of HPLC with the superior resolution and high mass accuracy of QTOF-MS, which can simultaneously conduct qualitative and quantitative analyses with a single injection and provide accurate structural characterization and strong anti-interference capability [11, 12], demonstrating significant advantages in unknown compound screening and trace pollutant detection. However, no published studies have yet reported the application of this technique to the detection of abamectin B1a residues in aquatic products, highlighting a significant research gap in this field.

This research proposed an HPLC-QTOF-MS detection method for abamectin B1a residues detection in fish liver tissues, optimized the detection conditions, verified the methodological performance, and applied the established method for actual samples screening. The results of this research provided a scientific basis for the safety and quality supervision of aquatic products.

Materials and methods

Standard solution preparation

Abamectin B1a standard (purity ≥ 98%) (Yuansi Standard Technology Co., Ltd., Shanghai, China) was accurately diluted with acetonitrile to prepare a 2 mg/L stock standard solution and stored at 4 – 8°C. The working solution in different concentrations was further diluted from stock solution with acetonitrile.

Sample pretreatment

Two live specimens of each Chinese perch (*Siniperca chuatsi*) and sea bass (*Lateolabrax japonicus*) species were purchased from a local

seafood supermarket located in Guangzhou, Guangdong, China. The liver tissues were collected and minced within 30 minutes and then thoroughly mixed. The homogenized tissues were transferred to polyethylene bottles and stored at −24 to −30°C. Sample pretreatment was adapted from China's National Standard GB 23200.121-2021 (National Food Safety Standard - Determination of 331 Pesticides and Their Metabolite Residues in Plant-derived Foods by Liquid Chromatography-Mass Spectrometry) with modifications to incorporate acetonitrile extraction coupled with lipid removal [13]. Specifically, 2 g homogenized liver samples were placed into 50 mL centrifuge tubes and mixed with 4 g anhydrous magnesium sulfate ($MgSO_4$), 1 g sodium chloride (NaCl), and 10 mL acetonitrile. The mixtures were vigorously vortexed for 4 minutes and centrifuged at 4,000 rpm for 5 minutes. The supernatants were decanted into 15 mL centrifuge tubes loaded with 0.3 g PSA, 0.3 g C_{18}, and 0.6 g $MgSO_4$. After vortexing for 2 minutes, mixtures were centrifuged again at 4,000 rpm for 5 minutes. 4 mL aliquots of the purified supernatants were evaporated to dryness under gentle nitrogen stream at 40°C using Lichen LC-DCY-24G Dry Nitrogen Evaporator (Lichen Scientific Instruments Co., Ltd., Shaoxing, Zhejiang, China). The residues were re-dissolved in 0.4 mL aliquots of acetonitrile-water mixture at the ratio of 20:80 (v/v) and passed through a 0.22 µm organic-phase filter membrane. The resulting filtrate was then collected for subsequent experiments.

HPLC quadrupole time of flight mass spectrometry analysis

Agilent 1290/6540 LC-QTOF-MS (Agilent Technologies, Santa Clara, CA, USA) was employed for experimental analysis of this study by using an EclipsePlus C18 column (1.8 µm, 3.0 × 150 mm) with the column temperature at 35°C. Chromatographic separation was performed under two mobile phase conditions with five replicate injections performed per phase [14]. Mobile phase A consisted of aqueous solution containing 5 mM ammonium acetate and 0.1% (v/v) formic acid, while phase B contained methanol. Flow rate was set at 0.4 mL/min and injection volume was 1 µL. Gradient elution program was set as 0.00 – 2.00 min for 95% phase A and 5% phase B, 5.00 – 10.00 min for 5% phase A and 95% phase B, and 10.01 – 12.00 min for 95% phase A and 5% phase B. Mass spectrometric detection was performed using a dual Agilent jet stream (AJS) electrospray ionization source (Dual AJS ESI). 2 mg/L abamectin B1a standard solution was subjected to full-scan analysis in both negative electrospray ionization (ESI^-) and positive electrospray ionization (ESI^+) modes. The optimized parameters for MS were 35 psi nebulizer gas pressure, 8 L/min drying gas flow rate, 350°C drying gas temperature, 8 L/min sheath gas flow rate, 375°C sheath gas temperature, 3,500 V capillary voltage, and 500 V nozzle voltage. Full-scan ranges were set at m/z 100 – 1,000 for MS1 and 50 – 1,000 for MS2 with high-resolution mode (4 GHz) (High Res Mode) being enabled throughout the analysis. Tandem mass spectrometry (MS2) analysis was conducted using 50 and 60 V collision energies (CE). Fragment ions of the target analyte in MS2 was acquired using product ion scan mode. Based on experimental requirements, two fragment ions with high and stable signal intensities were selected as qualitative qualifier ions [15]. MS parameters for abamectin B1a were set as that the ion at m/z 751.3969 was used as the qualitative ion, and sodium adduct ion at m/z 895.4814 was used as the quantitative ion.

Determination of abamectin B1a recovery in fish liver

Five sets of spiked solutions with concentrations of 2, 5, 10, 20, and 50 µg/L were prepared by spiking 2 mg/L abamectin B1a standard solution into the blank extracts to evaluate abamectin B1a recovery in fish liver. After pretreatment, all spiked solutions were analyzed. Each concentration was injected six times per day for three consecutive days. Meanwhile, five standard solution sets with the same concentrations were prepared using acetonitrile as solvent and subjected to the same instrumental analysis procedure. Intra-day

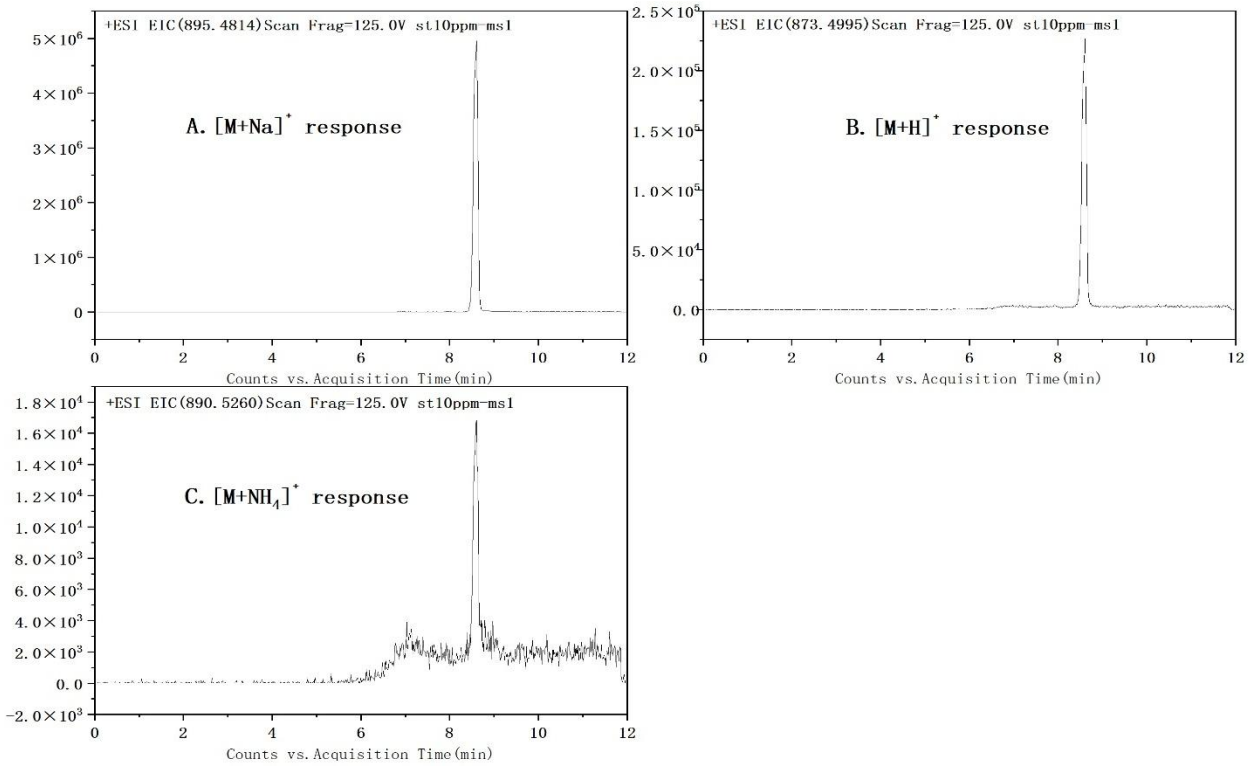

Figure 1. MS of abamectin B1a solution by HPLC-QTOF-MS.

relative standard deviation (RSD), inter-day RSD and recovery were calculated. Six blank fish liver homogenate aliquots were fortified with 2 mg/L abamectin B1a standard solution to prepare matrix-matched calibration standards with final concentrations of 5, 10, 20, 50, 100, and 200 µg/L. All fortified samples were processed through pretreatment procedure and analyzed through HPLC-QTOF-MS with three replicate injections per sample. Linear regression was performed by plotting the peak area of abamectin B1a against its fortified concentration in the matrix.

Market sample detection

The method developed in this research was applied to analyze 20 commercial fish samples of four species obtained from markets in densely populated areas with one of each species from each market.

Results and discussion

Optimization of chromatographic conditions

Data analysis revealed that mobile phase A provided enhanced chromatographic resolution with a larger peak area and a shorter retention time of 8.605 min for abamectin B1a. Therefore, this mobile phase was selected for all subsequent target compound separations.

Analysis of mass spectrometric response

The results revealed that the target analyte presented higher signal intensity in ESI^+ mode, where full-scan MS1 analysis was conducted for the identification of quasi-molecular ions including $[M + H]^+$, $[M + Na]^+$, and $[M + NH_4]^+$. Among them, $[M + Na]^+$ ion presented the highest signal intensity (Figure 1A) followed by $[M + H]^+$ ion (Figure 1B), while $[M + NH_4]^+$ ion exhibited the weakest response (Figure 1C). Therefore, $[M + Na]^+$ adduct ion (m/z 895.4814) was adopted as the precursor ion for subsequent MS analyses of abamectin B1a. Comparative evaluation of MS2 spectra at collision energies of 50 and 60 V revealed that 50 V collision energy generated a

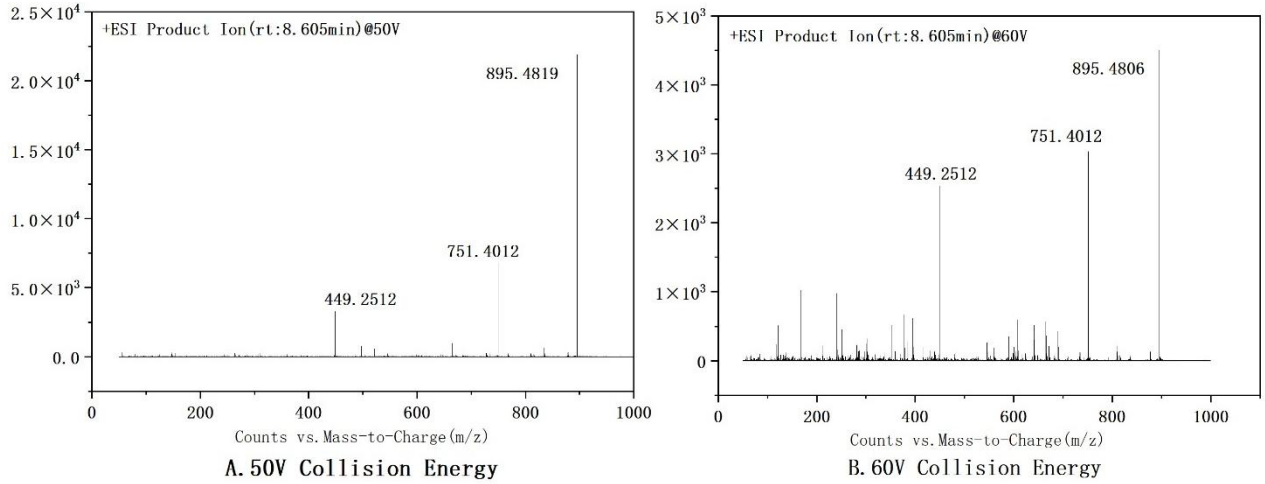

Figure 2. Secondary ions at 50 V and 60 V collision energy of abamectin B1a.

balanced ratio of precursor-to-fragment ion signal intensities alongside high fragment ion abundances (Figure 2). Therefore, 50 V was selected as the optimal collision energy for subsequent analyses. At 50 V collision energy, the fragment ion with maximum abundance was witnessed at m/z 751.4012. In comparison to the precursor ion at m/z 895.4814, this fragment ion presented a mass loss of 144.0802, revealing that the C-O bond was cleaved during the collision-induced dissociation process. The major fragment ions were detected at m/z 751.4012 (theoretical value of 751.3969) and m/z 449.2512 (theoretical value of 449.2510). Abamectin B1a molecular structure was then deduced based on organic compound MS fragmentation rules and the proposed fragmentation pathway. Abamectin B1a sodium adduct ($[M + Na]^+$, m/z 895.4814) fragmentation pathway was elucidated that the precursor ion first lost its terminal disaccharide moiety *via* glycosidic bond cleavage, creating the characteristic product ion at m/z 751.3969 (qualitative ion). Subsequent macrocyclic lactone ring fragmentation generated the secondary fragment ion at m/z 449.2510 (Figure 3). This stepwise fragmentation confirmed the structural identity of abamectin B1a and supported the reliability of the qualitative and quantitative analyses.

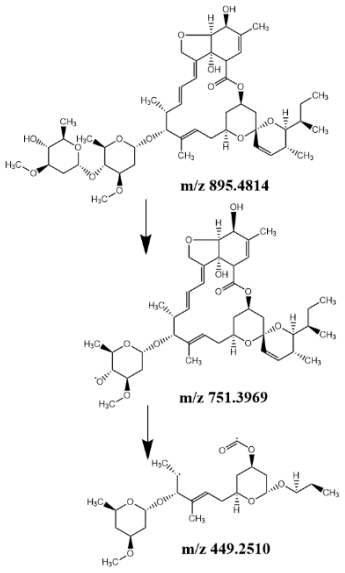

Figure 3. Fragmentation pathways of debris ions.

Method recovery and precision tests

The recoveries obtained were 84.60, 90.55, 93.24, 89.81, and 83.08%, respectively, with an average recovery of 88.26%. Method precision was investigated through intra-day and inter-day tests. The results demonstrated that intra-day RSD values were 4.8, 4.7, 2.1, 3.5, and 4.1% for the five spiked levels, respectively. Corresponding inter-day RSD values were 2.9, 3.0, 1.9, 1.2, and 1.5%, respectively. All intra-day RSD values were less than 8.5%, while all inter-day RSD values were below 3.0%, demonstrating excellent repeatability and precision of the developed method. The average abamectin B1a

Table 1. Precision test of abamectin B1a in liver.

Agent spiking (μg/L)	Parallel determination results (mg/L)						Intra-day RSD (%)	Inter-day RSD (%)
	1	2	3	4	5	6		
2	1.7656	1.7326	1.7842	1.5691	1.6842	1.7638	4.8	2.9
	1.6472	1.8690	1.4858	1.7501	1.5872	1.6832		
	1.8531	1.7033	1.9427	1.7732	1.5282	1.8231		
5	4.3281	4.6583	4.7380	4.1984	4.5873	4.7236	4.7	3.0
	4.8326	4.7368	4.5731	4.3871	4.8562	4.4675		
	4.3425	4.6408	4.6832	4.7239	4.7044	4.5863		
10	8.8952	9.3722	9.3925	9.3701	9.3728	9.4825	2.1	1.9
	9.5233	9.5806	9.6372	9.6737	8.9213	9.3225		
	9.6266	9.3282	9.0723	9.2518	9.1783	8.8622		
20	17.2733	17.2823	18.3013	18.7690	18.5723	18.0155	3.5	1.2
	18.7508	17.6233	18.1986	17.6304	17.8633	17.9920		
	17.3536	18.3722	18.0602	18.8344	18.3852	18.7151		
50	38.3088	42.4265	42.5102	39.8022	40.7382	40.8428	4.1	1.5
	43.8230	38.6022	40.2982	44.8663	41.5822	43.0337		
	45.0343	37.9221	42.7338	44.0117	35.7492	41.5536		

recovery rate across the five concentration levels was 88.26%. The intra-day RSD values ranging from 2.1% to 8.5% and inter-day RSD values ranging from 1.2% to 3.0% confirmed that the proposed method met the analytical criteria for the detection of pesticide residues including acceptable RSD values and satisfactory recovery rates (Table 1).

Matrix effect

Fish liver tissues are rich in phospholipids, lipids, and other components, resulting in the possibility of matrix interference with the target analyte [16]. Matrix effect (ME) was defined as the percentage ratio of matrix-matched calibration curve slope to that of the solvent calibration curve, a metric applied for the evaluation of matrix-induced enhancement or suppression effects [17]. ME evaluation criteria were that ME < 90% indicated a matrix-induced suppression effect on the analyte response. ME > 110% suggested a matrix-induced enhancement effect. 90% < ME < 110% implied no significant ME. The results showed that the calculated ME value was 91.7%, which indicated that, after the optimized pretreatment, fish liver matrix components exerted negligible enhancement or suppression effects on abamectin B1a response.

Linear regression equation, limit of quantification (LOQ), and limit of detection (LOD)

The equation of the obtained matrix-matched calibration curve was expressed as Y = 1,837.24X − 206.5 (r = 0.9997), revealing a strong linear correlation in the concentration range of 0.005 – 0.20 mg/L, equivalent to 2.5 – 100 μg/kg in liver tissue. For comparison, a solvent-based calibration curve was also obtained using the same procedure, giving the equation of Y1 = 1,842.8X1 − 217.3 (r = 0.9996). Weighted least squares method was applied to perform weighted linear regression, using a 1/x weighting factor. In this model, x denoted the fortified concentration (μg/L) and y corresponded to the mean peak area of the target analyte at varying fortified concentrations. LOD was defined as the concentration yielding a signal-to-noise ratio (S/N) of 3, while LOQ was defined as the concentration at an S/N of 10 [18, 19]. The results revealed the LOD and LOQ values of 0.5 and 1.0 μg/kg for abamectin B1a, respectively.

Analysis of market sample detection

The results showed that abamectin B1a was detected in three of the 20 samples at concentrations of 5.8, 11.2, and 17.2 μg/kg with

Table 2. Random inspection of abamectin B1a in fishery products.

Market Source	Sample	Species	Detection result (μg/kg)
Market 1	Sample1	*Chinese perch*	ND
	Sample2	*Sea bass*	ND
	Sample3	*Crucian carp*	11.2
	Sample4	*Grass carp*	ND
Market 2	Sample5	*Chinese perch*	ND
	Sample6	*Sea bass*	ND
	Sample7	*Crucian carp*	ND
	Sample8	*Grass carp*	ND
Market 3	Sample9	*Chinese perch*	ND
	Sample10	*Sea bass*	ND
	Sample11	*Crucian carp*	ND
	Sample12	*Grass carp*	5.8
Market 4	Sample13	*Chinese perch*	ND
	Sample14	*Sea bass*	ND
	Sample15	*Crucian carp*	17.2
	Sample16	*Grass carp*	ND
Market 5	Sample17	*Chinese perch*	ND
	Sample18	*Sea bass*	ND
	Sample19	*Crucian carp*	ND
	Sample20	*Grass carp*	ND

the detection rate of 15% (Table 2). These results revealed that the overall safety and quality status of commercially available fish products was moderate, denoting that market supervision authorities still needed to strengthen supervision over pesticide application in aquaculture and related industries.

Conclusion

This research proposed a trace detection method based on HPLC-QTOF-MS for the detection of the highly toxic substance abamectin B1a in fish and aquatic products. The results showed that optimization of mobile phase composition significantly enhanced both chromatographic peak symmetry and separation efficiency. Combined with MS conditions using electrospray ionization in positive ion full-scan mode, accurate quantitative and qualitative analyses of abamectin B1a were achieved. Pretreatment method efficiently eliminated matrix interference. Under the optimized conditions, the developed method presented excellent linearity in 0.005 – 0.20 mg/L range, equivalent to 2.5 – 100 μg/kg in fish liver tissue. The average recovery rate, LOD, and LOQ fully satisfied the analytical requirements for the detection of the trace levels of abamectin B1a in aquatic products. The proposed approach fully utilized the advantages of strong anti-interference capability and high mass accuracy of HPLC-QTOF-MS, which filled the gap in using this technology for the detection of abamectin B1a in aquatic products and provided reliable technical support for the investigation of food crime cases by public security laboratories, quality control by regulatory authorities, and relevant research carried out by scientific research institutions. This research still had certain limitations, which included that pretreatment process was time-consuming and had not yet been automated batch processing, restricting its applicability to large-batch sample analyses. In addition, the study only focused on fish liver tissues, neglecting fish muscle, gills, and other aquatic product species, therefore, the applicability of the

proposed method needed to be further expanded. Further, the interference from abamectin B1a metabolites in aquatic products on the detection results had not been thoroughly evaluated, and there was room for improvement in the specific identification of target analyte in complex matrices. Future research could optimize the sample pretreatment workflow to enhance batch analysis capabilities and sample throughput by incorporating rapid pretreatment techniques such as QuEChERS and dispersive liquid-liquid microextraction in conjunction with automated instrumentation. The scope of investigation should be expanded to apply this method to a wider variety of aquatic products and different tissue types, which would validate the method's applicability and facilitate the establishment of unified detection standards for abamectin B1a across various aquatic product categories. By leveraging the structural elucidation capabilities of high-resolution mass spectrometry, in-depth studies should be conducted on the degradation products and metabolic pathways of abamectin B1a. Concurrently, qualitative analysis parameters should be optimized to further enhance the accuracy and specificity of the method when applied to complex matrices, thereby providing more comprehensive technical support for ecological risk assessment and food safety regulation.

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