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RESEARCH PAPER

Development, validation, and implementation of an ultratrace analysis method for the determination of moenomycin A, in aquatic animal products

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Abstract

Moenomycin A, an antimicrobial growth promoter widely used as an additive in aquaculture feedstuffs, has been restricted for use in the European Union and China due to its potential risk of promoting resistant strains of pathogenic bacteria and causing residues in aquatic animal products. Although methods for analyzing moenomycin A in feedstuffs have been developed, no established method exists for aquatic matrices. In this study, we present, for the first time, a sensitive and validated high-performance liquid chromatography-tandem mass spectrometry (HPLC–MS/MS) method for the determination of moenomycin A in aquatic animal products. Samples were extracted using methanol and purified with the QuEChERS method employing C18 sorbent. The aliquot was dried under a nitrogen stream, reconstituted with methanol-water solvent, and analyzed by HPLC–MS/MS. The developed method exhibited good linearity ($r^2 > 0.995$) over a wide concentration range (1–100 μg/L) and a low limit of detection (1 μg/kg). Average recoveries ranged between 70 and 110% at spiked concentrations of 1, 50, and 100 μg/kg, with associated intra- and inter-day relative standard deviations of 1.25 to 7.32% ($n = 6$) and 2.91 to 10.08% ($n = 3$), for different representative aquatic animal production, respectively. To the best of our knowledge, this is the first reported HPLC–MS/MS method for the quantification of moenomycin A in aquatic animal products. The new approach was effectively employed in the analysis of moenomycin A across various aquatic samples.

Keywords Moenomycin A · Aquatic animal products · HPLC–MS/MS · Drug residues · Dispersive solid-phase extraction · Validation

Introduction

Moenomycin, also known as flavomycin, bambermycin, or flavophospholipol, is a phosphoglycolipid antibiotic produced by various *Streptomyces* spp. strains. The compound inhibits bacterial growth by disrupting peptidoglycan synthesis and subsequently interfering with the formation of bacterial cell walls [1]. Although moenomycin is generally effective against most gram-positive and some gram-negative bacteria, certain protective bacteria, such as *lactobacilli* and *bifidobacteria*, demonstrate resistance [2, 3]. In 2002, the Ministry of Agriculture of China approved

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moenomycin as a new veterinary drug. It has been used as a feed additive in aquaculture for growth promotion and to improve digestion and assimilation of animal nutrition [4–6]. However, the use of antibiotics can lead to residues in animal products and induce cross-resistance of human pathogens to related antibiotics [7]. Furthermore, unmetabolized drugs can enter the environment through animal waste, contaminating soils, and water sources [8–10]. As a result, the European Commission restricted the use of moenomycin as a feed additive in 2006, and the Ministry of Agriculture and Rural Affairs of China banned it in 2019, with the effectivity from 2020. Over-exploited domestic fisheries with intensive and high-density culture practices potentially increase the risk of moenomycin misuse in aquaculture [11]. This raises concerns about the potential health risks associated with the accumulation of moenomycin in humans, emphasizing the need for accurate monitoring of moenomycin residues in aquatic animal products intended for human consumption.

Five major components of the moenomycin complex have been structurally characterized: moenomycin A, moenomycin A12, moenomycin C4, moenomycin C3, and moenomycin C1 [12–16]. The moenomycin premix available in markets consists of these five chemicals, with moenomycin A comprising more than half of the mixture. Consequently, a confirmatory method for determining moenomycin A can effectively indicate the use of moenomycin drug in farming.

Previous publications have reported methods for analyzing moenomycin and moenomycin A [17, 18]. Official methods, such as microbiological tests, have been utilized by state-owned laboratories to assess moenomycin presence in medicated feed and soil environments [19, 20]. However, these tests are more suitable for sample screening and lack the necessary selectivity for complex matrices and explicit drug identification. High-performance liquid chromatography (HPLC) coupled with ultraviolet (UV) detection has also been employed for detecting moenomycin residues in feedstuffs [21]. While more effective than microbiological approaches in determining the fate and distribution of the five moenomycin components, HPLC methods still lack the required selectivity and sensitivity for quantitative analysis. Fast-atom-bombardment mass spectrometry (FAB–MS) and high-performance liquid chromatography-tandem mass spectrometry (HPLC–MS/MS) with electron spray ionization (ESI) have been used to analyze moenomycin residues in feeds and animal excrements [18, 22, 23]. However, FAB–MS requires high moenomycin concentrations, rendering it unsuitable for practical trace analysis. As a result, HPLC–MS/MS has been widely adopted for moenomycin analysis. Nevertheless, most HPLC–MS/MS methods primarily focus on determining moenomycin in feeds, rendering them unsuitable for analyzing aquatic matrices due to their complexity and diversity.

To address this gap, we developed and validated an ultratrace method for the analysis of moenomycin A in aquatic animal products using HPLC–MS/MS. This novel method represents the first of its kind and features easy sample extraction, rapid purification with QuEChERS, and minimal matrix interference. Furthermore, our approach exhibits a wide linear range, high specificity and sensitivity, and precise quantification for measuring moenomycin A residue in aquatic animal products. Lastly, we successfully applied the newly developed method to analyze moenomycin A in real samples across various aquatic animal products, demonstrating its potential for use in monitoring programs and risk assessments for official control focusing on animal drug residue analysis.

Materials and methods

Chemicals and reagents

All chemicals and reagents used in this study were of analytical or chromatographic grade. Moenomycin A standard (> 95% purity) was procured from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). A stock solution of moenomycin A was prepared at 1 µg/mL in methanol, with further dilutions for recovery experiments and calibration curve preparation. Methanol (MeOH) and acetonitrile (ACN) of chromatographic grade were purchased from Merck (Darmstadt, Germany). Ammonium hydroxide (28% in H_2O) and ammonium acetate were sourced from Sinopharm Group Chemicals Limited (Shanghai, China). Ultrapure water (18.2 MΩ) was produced using a Milli-Q water purification system (Millipore Co., Bedford, MA, USA). The octadecyl silicon dioxide (C18, 50 µm, 60 Å) and ethylenediamine-N-propyl silane silica gel (PSA, 40–63 mm, 6 nm) were obtained from Agela Technologies (Tianjin, China). Enhanced matrix removal-lipid (EMR-Lipid) was acquired from Agilent Technology (CA, USA). Hydrophobic polytetrafluoroethylene (PTFE) filters (0.22 µm) were purchased from Tianjin Branch Billion Lung Experimental Equipment Co., Ltd. (Tianjin, China).

Sample preparation

Aquatic animal products were procured from local supermarkets in China. The sampling procedures for all species were prepared according to the National Standard of the People's Republic of China, Practice of sampling plans for aquatic products (GB/T 30891-2014) [24]. For grass carp and large yellow croaker, the edible parts were sliced and combined as one sample after the head, fishbone, and guts were removed. The edible parts of Chinese mitten crab and soft-shell turtle were extracted and collected. The whole soft tissues and body fluids of mussel should be assembled. The muscles of shrimp were picked up without the shrimp head, shell, and intestinal gland. Then, the collected tissues were mixed, homogenized for 5 min by a high-pressure homogenizer (Genizer, Los Angeles, USA; 1.5 kW) and stored at − 20 °C in the dark prior to the analysis.

A homogenized sample (2 g) was weighed into a 50-mL centrifugal tube and extracted with 5 mL of 10:90 (v:v) ammonium hydroxide–methanol solution via vortex mixing for 5 min. The sample was then sonicated (160 W) in a water bath at 50 °C for 10 min and centrifuged at 5000 g for 10 min at 4 °C. The supernatant was decanted into another tube, and the residue was re-extracted following the same procedure. The combined supernatant was subjected to dispersive solid-phase extraction (d-SPE) using 200 mg of C18 sorbent, vortexed for 30 s, and centrifuged at 3000 × *g* for 5 min. The supernatant (5 mL) was transferred to a glass centrifuge tube, cleaned with 5 mL of *n*-hexane, and dried under a gentle nitrogen stream at 40 °C. The residue was reconstituted in 1 mL of MeOH/water (1:1) and filtered through a 0.22-μm hydrophobic PTFE filter membrane for HPLC–MS/MS analysis. The blank samples of all species were initially tested for the absence of moenomycin A. Moreover, the blank sample of grass carp was used in the method optimization.

Matrix-matched calibration solutions were prepared alongside the samples. Appropriate standard solutions were added to the extraction solution of the blank samples before drying with nitrogen. The solutions were then concentrated and dissolved in MeOH/water (1:1, 1 mL) to generate final concentrations of 1, 2, 10, 20, 50, and 100 μg/L for the matrix-matched calibration standards.

LC–MS/MS analysis

The HPLC–MS/MS system, consisting of an HPLC (LC-20A, Shimadzu Corporation, Japan) and an AB Sciex Qtrap 5500 tandem quadrupole mass spectrometer (Danaher Corporation, Washington, DC, USA) with an electrospray ionization (ESI) source, was used for determination of moenomycin A in multiple reaction monitoring (MRM) mode. The highest intensity of product ion was used as the quantitative ion, and the second one was applied to confirm the analyte. The MS/MS parameters for moenomycin A are summarized in Table 1. Chromatographic separation was performed on an ACQUITY HSS T3 column (2.1 mm × 100 mm, 1.8 μm, Waters, Milford, MA, USA) with an injection volume of 10 μL. Chromatographic analysis was conducted at a flow rate of 0.4 mL/min and a column temperature of 40 °C, using mobile phase A (water containing 10 mmol/L ammonium acetate) and mobile phase B (acetonitrile). The gradient elution was as follows: maintain 10% B for 0.5 min, increase to 95% B over 2.5 min, hold at 95% B for 1.0 min, decrease to 10% B in 0.5 min, and allow 1.5 min for re-equilibration.

Table 1 MS/MS parameters of moenomycin A

Compounds	Precursor ion (*m/z*)	Product ions (*m/z*)	Collision energy (eV)	Retention time (min)
Moenomycin A	789.9	575.6*	− 36	2.73
		554.1	− 45	

*Quantitative ion

Negative ionization mode mass spectrometry parameters included a spray voltage of − 4500 V, a CUR pressure of 40 psi, GS1 and GS2 pressures of 55 psi, an ion source temperature of 550 °C, and a CAD of medium.

Method validation

The method's linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and ruggedness were evaluated and validated in-house according to SANTE/11312/2021 guidelines [25]. Matrix calibration curves for six aquatic animal product varieties (grass carp (*Ctenopharyngodon idella*), large yellow croaker (*Larimichthys crocea*), shrimp (*Penaeus vannamei*), Chinese mitten crab (*Eriocheir sinensis*), mussel (*Mytilus edulis*), and soft-shell turtle (*Pelodiscus Sinensis*)) were constructed by plotting the peak area against the concentration of the corresponding calibration standards at six concentration levels in a range of 1–100 μg/L. Linearity was assessed using the coefficient of determination (r^2) and regression analysis, employing a linear regression model with $1/x^2$ weighting. Accuracy was determined by calculating mean recoveries at three different spiked concentrations (1, 50, and 100 μg/kg) with six parallel measurements in each aquatic matrix. Precision was assessed by calculating relative standard deviations (RSDs) for intra-day and inter-day variations. The intra-assay precision is presented over three concentrations ($n = 6$), and the inter-assay precision is calculated at each spiking concentration in triplicate. LOD and LOQ were determined based on peak-to-peak signal-to-noise (*S*/*N*) ratios of 3 and 10, respectively, by measuring blank samples spiked with varying concentrations of moenomycin A from low to high, ensuring detectable quantitative and qualitative ions. Method ruggedness study was tested by altering eluent concentration (± 2 mM), column oven temperature (± 3 °C), vortex time in extraction (± 1 min), and the amount of *n*-hexane (± 1 mL). The ruggedness was assessed by monitoring deviations on chromatographic peaks, retention time, and recovery.

Matrix effect (ME) was evaluated by comparing moenomycin A signals at 10 µg/kg in matrix and solvent using a standard solution prepared in the sample solution and pure solvent, according to Eq. 1 [26]. ME% values of 0 were considered to have no matrix effect, while positive and negative values indicated matrix-induced enhancement and suppression, respectively. $\text{Response}_{\text{matrix}}$ and $\text{Response}_{\text{solvent}}$ represent the signal of standards in matrix and pure solvent, respectively.

$$\text{ME\%} = \frac{\text{Response}_{\text{matrix}} - \text{Response}_{\text{solvent}}}{\text{Response}_{\text{solvent}}} \times 100\% \tag{1}$$

Results and discussion

HPLC–MS/MS conditions

To obtain sufficient signals for mass characterization, a 500 µg/L standard solution was utilized for parameter optimization. In previous reports, the negative ion mode displayed more intense signals than the positive ion mode [18]. In our study, mass scans were performed in negative ion mode with a flow rate of 10 µL/min. Considering that the singly charged precursor ion $[M–H]^-$ at *m/z* 1580.7 exceeds the mass range of MS instrument (5 ~ 1000 amu), the double-charged precursor ion $[M–2H]^{2-}$ at *m/z* 789.9 was adopted. The characteristic product ions were detected at *m/z* 575.8, 554.1, 566.9, and 545.4 (Fig. 1c). The product ions at *m/z* 575.8 and 554.1 were selected as quantitative and qualitative ions based on their signal intensity, and the corresponding chromatograms are shown in Fig. 1a and b, respectively. Moreover, ionization conditions and collision energy were optimized, with detailed information presented in Table 1.

To achieve high sensitivity and good peak shape, the performance of ACQUITY HSS T3 (100 mm × 2.1 mm, 1.8 µm) and Kinetex F5 (100 mm × 2.1 mm, 2.6 µm) columns was evaluated. Due to the enhanced retention capability of the reverse-phase interface, these two columns are more suitable for highly polar compounds. Both columns exhibited higher sensitivity for moenomycin A and excellent peak shape, while low stability was observed using the F5 column. This effect resulted in a calibration curve with a poor coefficient of determination ($r^2 = 0.97868$) in solvent (Fig. 2a). However, a satisfactory calibration curve with $r^2 > 0.999$ was obtained using the T3 column (Fig. 2b). Therefore, the T3 column was selected for the chromatographic separation of moenomycin A.

Sample extraction conditions

The choice of extraction solvent is critical in the pretreatment of samples, as it influences the extraction efficiency of the analyte. Considering the properties of moenomycin A, we examined methanol, 80% methanol-water, acetonitrile, and various ratios of ammonium hydroxide/methanol solutions, as they can dissolve polar substances and achieve high extraction efficiency in complex substrates like aquatic animal products.

A 50-µL aliquot of 1 µg/mL moenomycin A was spiked into blank samples, followed by vortex mixing for 1 min. The samples were then extracted with 10 mL of reagents, and the solution was filtered for HPLC–MS/MS analysis. These results were compared to moenomycin A spiked in the blank matrix solution. The chromatographic peak area was analyzed to determine the optimum extraction reagent (expressed as recovery). As depicted in Table 2, the extraction recoveries of methanol and acetonitrile were 52.5% and 44.6%, respectively. Although moenomycin A can dissolve in water, the recovery rate of methanol-water was decreased to 46.9%. This observation may be ascribed to that the interaction force between the hydroxyl group of water and moenomycin A is lower than that of methanol due to the weakly ionized characteristic of water. Remarkably, the presence of ammonium hydroxide significantly improved the recovery of moenomycin A, reaching 87.4 ~ 90.9%. This improvement can be attributed to ammonium hydroxide promoting ionization of moenomycin A in the solution and enhancing its solubility in methanol. Consequently, a 10% ammonium hydroxide/methanol solution (10 mL of 28% NH_4OH solution to 90 mL of methanol) was selected as the final extraction solvent for moenomycin A in aquatic animal products.

Additionally, the volume of the extraction reagent and the number of extraction repetitions were evaluated to achieve optimal extraction efficiency. First, 5, 10, 15, and 20 mL of 10% ammonium hydroxide/methanol were employed to extract moenomycin A spiked into blank samples once. These volumes were also tested by dividing them into two equal parts to perform the extraction twice. As depicted in Fig. 3, the recovery using a 5 mL extraction solution was considerably low due to its limited volume. For single extractions using 10, 15, and 20 mL of ammonium hydroxide/methanol solution, the recoveries progressively increased from 90.9 to 93.9%. Although not a substantial improvement, it can be inferred that a larger volume of the solution can dissolve more analyte from the samples. Using 10 mL of 10% ammonium hydroxide/methanol for double extraction yielded the highest extraction efficiency of 96.4%, indicating that adding more solvent may help extract residual analyte from the sample [27, 28]. Wang reported that the extraction of virginiamycin M1 in various tissues

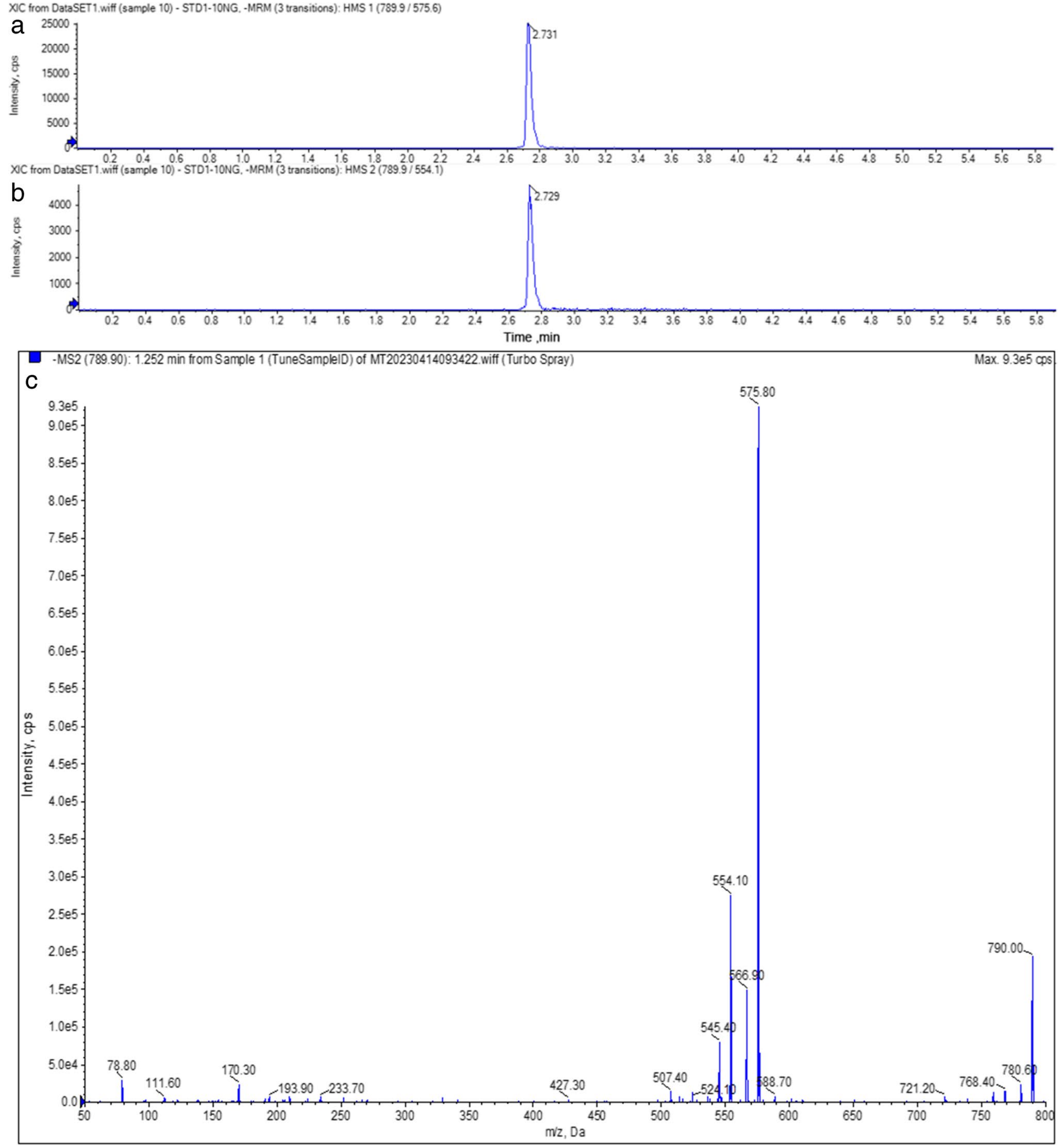

Fig. 1 Extracted ion chromatograms of moenomycin A (10 µg/L) at *m/z* 575.6 (**a**), *m/z* 554.1 (**b**) with ACQUITY HSS T3 column, and MS2 spectrum in the negative ion mode (**c**)

of chicken and swine exceeded 70% for a single extraction and more than 90% for double extraction [28], consistent with our observations in this study. Consequently, 10 mL of 10% ammonium hydroxide/methanol used in two extraction steps was chosen to achieve high-efficiency extraction of moenomycin A from the sample.

Sample cleanup conditions

Methanol, the extraction solvent, tends to introduce numerous interfering substances, such as pigments and proteins, in aquatic animal products due to its high polarity. Therefore, it is essential to clean up the extract to minimize interference

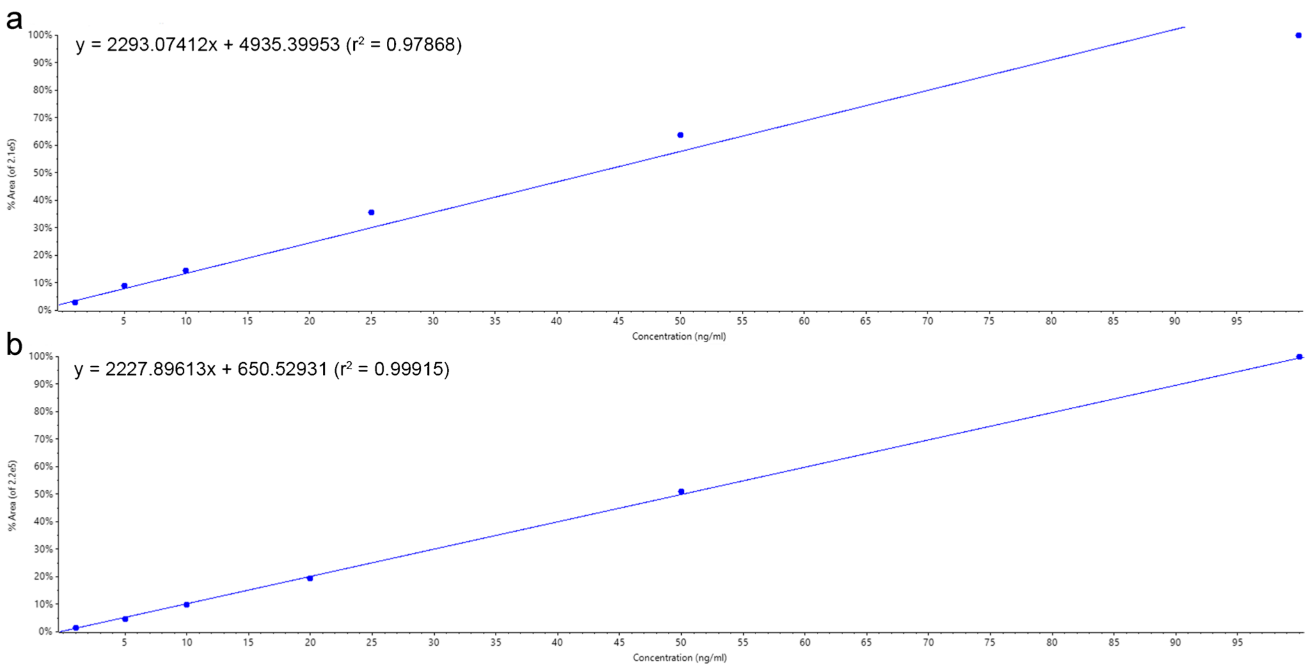

Fig. 2 Calibration curve of moenomycin A with F5 (**a**) and T3 (**b**) columns

Table 2 Extraction recovery of moenomycin A using different solvents

Reagents	Recovery (%)						Mean recovery (%)
	1	2	3	4	5	6	
Methanol	52.3	50.2	53.4	55.1	50.8	53.2	52.5 ± 1.80
80% methanol-water	47.8	45.7	44.0	48.6	48.3	46.8	46.9 ± 1.76
5% ammonium hydroxide/methanol	92.1	84.6	89.7	90.4	83.2	84.5	87.4 ± 3.75
10% ammonium hydroxide/methanol	88.7	85.3	98.1	96.4	87.5	89.2	90.9 ± 5.15
20% ammonium hydroxide/methanol	85.9	88.4	88.3	90.5	91.7	91.6	89.4 ± 2.27
acetonitrile	44.3	46.8	36.8	50.7	48.8	40.2	44.6 ± 5.29

during chromatographic separation and reduce matrix effect on mass spectrometry analysis. For this purpose, Xu investigated three types of SPE cartridges for sample extract cleanup using Oasis HLB (hydrophilic-lipophilic-balanced reversed-phase sorbent), Bond Elut C18 (hydrophobic, bonded silica sorbent), and Phenomenex Strata-X (reversed-phase functionalized polymeric sorbent) [17]. These columns have successfully purified various antibiotics [29, 30]. However, our study found that these SPE cartridges can also adsorb moenomycin A, resulting in low recoveries ranging from 30 to 60%. Furthermore, the traditional SPE approach requires preconditioning, loading, washing, and elution, which consume both time and reagents. Xu reported that adding acetonitrile to the extracted solution could precipitate proteins, reducing protein interference [17]. However, the optimized addition of 20 mL of acetonitrile resulted in a time-consuming process for the subsequent concentration procedures.

This study further assessed dispersive solid-phase extraction (d-SPE) based on the QuEChERS approach for the purification process to achieve higher recoveries, lower interferences, and faster operations. Common d-SPE sorbents used in purification include C18, PSA, and EMR, which have been widely applied to various matrices such as poultry, crops, and aquaculture samples [31–34]. The original QuEChERS method utilized PSA absorbents to eliminate polar compounds, such as sugars and fatty acids from a nonpolar matrix due to the primary secondary amine groups on the surface of PSA absorbents [35]. Additionally, C18 was introduced to enhance the cleanup efficiency in samples containing fat [36]. The EMR sorbent is capable of removing the major lipid classes through

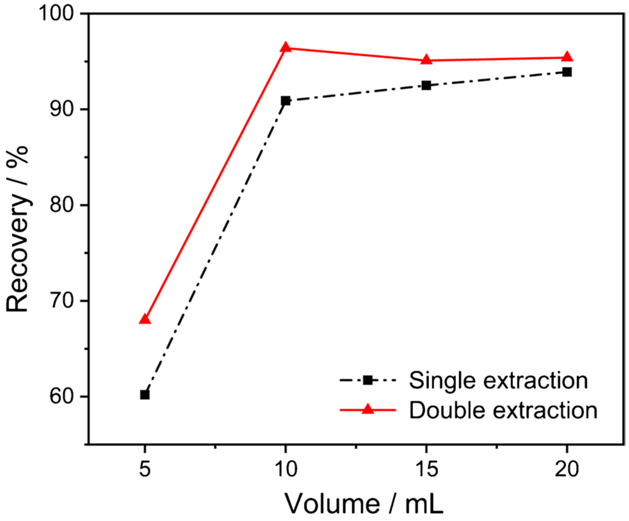

Fig. 3 Recovery of moenomycin A in different volumes of 10% ammonium hydroxide/methanol with single and double extraction (the volume for double extraction was tested by dividing the volume into two equal parts to perform the extraction twice)

a combination of size-exclusion and hydrophobic interactions without compromising extraction efficiency [37]. Therefore, we prepared and examined d-SPE cartridges with different combinations of C18, PSA, and EMR. Cleanup results were evaluated based on their recoveries. Additionally, we studied different sorbent amounts (0, 100, 200, 300, and 500 mg) to determine the optimal conditions. Blank sample extract solutions (10 mL) were spiked with 50 ng of moenomycin A, followed by the QuEChERS method using various sorbents. Then, the solution was centrifuged and analyzed for HPLC–MS/MS. Figure 4 illustrates the results obtained by optimizing the sorbent and dosage. The relationship between moenomycin A recoveries and the quantities of C18, PSA, and EMR sorbents revealed that 200 mg of C18 provided the most optimal purification condition. In contrast, the EMR sorbent exhibited substantial moenomycin A adsorption. The PSA sorbent demonstrated higher moenomycin A adsorption than the C18 sorbent. Consequently, 200 mg of C18 was selected as the absorbent in the d-SPE purification of the final QuEChERS method.

Matrix effect

The matrix effect can cause difficulties in qualitative and quantitative analysis in LC–MS/MS with electrospray ionization (ESI-MS), leading to signal suppression or enhancement of the analyte. This effect mostly depends on the analyte properties, matrices, sample preparation, and instrumental parameters [38, 39]. The presence of matrix effect can result in poor sensitivity, accuracy, and linearity. Generally, internal standards, extract dilution, and matrix-matched calibration curve can be applied to compensate for matrix effect with satisfactory recovery and precision [40–42].

For moenomycin A, the matrix effects of six types of aquatic animal products exhibited signal suppression. As shown in Fig. 5, grass carp, shrimp, large yellow croaker, soft-shell turtle, and mussel displayed low/negligible matrix effects (– 20 ~ 0%), while Chinese mitten crab showed medium matrix effects (– 20 ~ – 50%) [31]. Additionally, the sample treated with d-SPE using C18 sorbent significantly eliminated matrix interferences (Fig. 4). Considering the matrix effect in Chinese mitten crab, matrix-matched

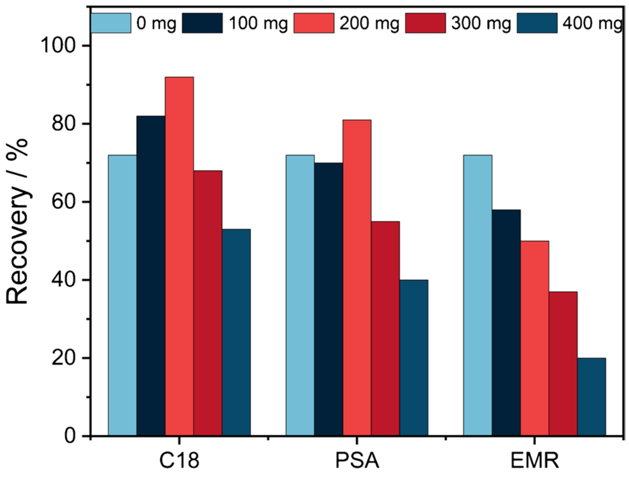

Fig. 4 Recoveries of three sorbents at different amounts by the QuEChERS method

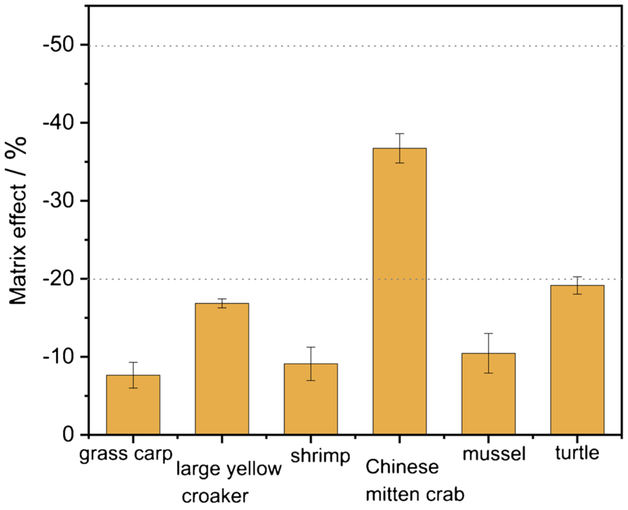

Fig. 5 Matrix effects of moenomycin A after sample preparation of six types of aquatic animal products

Table 3 Matrix calibration curves, linear range, and coefficients of determination of moenomycin A in six aquatic matrices

Matrixes	Calibration	r^2	Linear range (μg/L)
Grass carp	$Y = -1036.33752 + 5.55936E4*X$	0.9981	1–100
Large yellow croaker	$Y = 90.14534 + 5.26163E4*X$	0.9985	
Shrimp	$Y = -273.68087 + 9.28513E4*X$	0.9973	
Chinese mitten crab	$Y = 2672.71683 + 1.10487E5*X$	0.9962	
mussel	$Y = -997.04519 + 6.43810E4*X$	0.9981	
Soft-shell turtle	$Y = -1013.02090 + 5.60921E4*X$	0.9983	

calibration standards were employed to achieve accurate quantitative analysis by HPLC–MS/MS.

Linearity

The linearity was evaluated using a 6-point calibration curve, covering the range from 1 to 100 μg/L. The calibration curves for all matrices, linear range, and r^2 values are displayed in Table 3. For the six aquatic matrices, the r^2 of the matrix calibration curves were all greater than 0.995. This result indicates that our approach provides excellent linearity for moenomycin A.

Transition ions for quantification in different matrices

As the transition ions used for quantification would be present in real blank samples, which can deviate the quantitation accuracy, transition ions for MRM detection were evaluated in six blank matrices as well

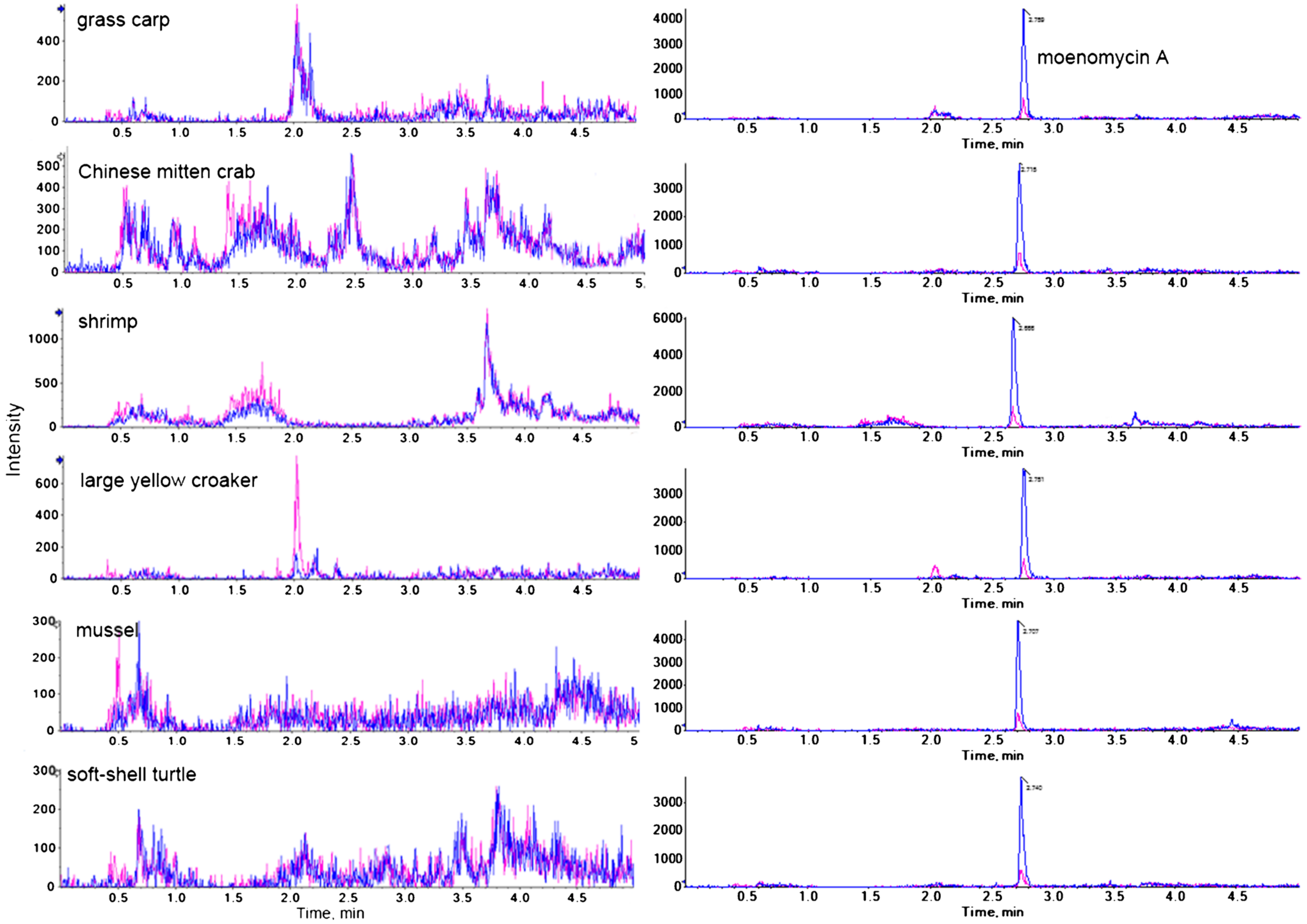

Fig. 6 HPLC–MS/MS chromatograms of six blank matrix samples (left) and moenomycin A spiked at 1.0 μg/kg in various matrices after preparation (right)

as in their corresponding spiked samples. Figure 6 illustrates the chromatograms of six blank matrices and moenomycin A at a concentration of 1.0 μg/kg in different matrices following the preparation process. There were no notable peaks seen at the retention time corresponding to moenomycin A in the chromatograms of the six blank samples. Therefore, the results of this study indicate that the MRM ions employed for the identification of moenomycin A exhibited obvious selectivity, and which would not affect the accuracy during quantitation.

Limits of detection and quantification

The LOD and LOQ were determined by parallel analyzing six matrix-matched blank samples spiked with moenomycin A at low concentration levels ($n=6$). The LOD represents the minimum concentration of the analyte that can be reliably detected and confirmed as a positive sample by the method employed. Therefore, LOD was determined by the qualitative transition 789.9 > 554.1 from the signal-to-noise (S/N) ratio of 3 and $S/N=10$ was considered the LOQ by quantitative ion. The LOD and LOQ were validated at 1 μg/kg and 2 μg/kg, respectively. These results indicate that the developed method exhibits high sensitivity for the determination of moenomycin A. In this investigation, the LOD and LOQ by HPLC–MS/MS are significantly lower than those commonly used in feedstuffs [18], chicken litter [8], and poultry tissues [17] (Table 4). To the best of our knowledge, this is the most sensitive method for the determination of moenomycin A compared with previously published methods.

Accuracy and precision

The accuracy and precision of the method were evaluated using blank samples of grass carp, large yellow croaker, shrimp, Chinese mitten crab, mussel, and soft-shell turtle, spiked at three levels of moenomycin A. The accuracy was expressed as the recovery of detected values relative to the spiked concentration of moenomycin A. The precision was expressed as the RSD of the measured concentrations, including intra- and inter-day precision. Results for recoveries and intra-precision are presented as averages over concentrations of 1, 50, and 100 μg/kg ($n=6$) on the same day. The inter-precision is calculated in triplicate for each concentration. As shown in Table 5, recoveries of moenomycin A at three concentration levels and different matrices ranged from 78.75 to 109.5%. The Chinese mitten crab exhibited the lowest recovery, potentially due to the high lipid content in its roe which may have hindered efficient extraction [43].

The precision, in terms of RSD, was obtained from the same experiments of recovery. The inter-day precision suggests that the method's performance can achieve similar results across different days or batches of analysis, indicating reproducibility. For the intra-day precision, it expresses consistent results within the same day or batch, denoting repeatability. The intra- and inter-precision, based on three levels and six matrices, were all less than 15%. These results revealed that the established method for the measurement of moenomycin A in various aquatic animal products is accurate and precise.

Stability

Additionally, the stability of moenomycin A in a standard solution was assessed. Moenomycin A stock solution of 100 μg/kg proved stable for at least 6 months at − 40 °C, while the diluted standard solution at 1 μg/kg stored at 4 °C exhibited no apparent loss for 3 months. However, the solution concentration decreased to 81.2% ($n=3$) of 1 μg/kg without darkness storage at room temperature for 30 days (see Electronic Supplementary Material Fig. S1), indicating the poor stability of moenomycin A at room temperature under light exposure. Consequently, the accuracy of determination might be affected if the degradation of the standard chemicals for calibration goes unnoticed.

Ruggedness

The method was found resistant to altered concentration and temperature for reference standard except at 8 mM of ammonium acetate. It was observed that decrease in eluent

Table 4 Comparison of analytical methods for the determination of moenomycin A

No	Matrix	Purification method	LOQ (μg/kg)	Calibration range (μg/L)	Recoveries (%)	RSD (%)	Ref
1	Feed stuffs	HLB-Oasis cartridges	100	10–1000	83.9–94.2	< 23	18
2	Chicken litter	tC_{18}-SPE cartridge	–	50–5000	–	–	8
3	Poultry tissues	Acetonitrile precipitation	10	20–400	66.5–89.4	< 10.2	17
4	Aquatic edible part	C18 d-SPE	2	1–100	78.8–109.5	< 10.6	This work

Table 5 Recovery and precision results for the determination of moenomycin A in different aquatic animal products

Matrix	Spiked level (μg/kg)	Mean recovery (*n* = 6, %)	Intra-RSD (*n* = 6, %)	Inter-RSD (*n* = 3, %)
Grass carp	1	100.7	3.18	5.11
		97.60	6.82	
		99.30	5.23	
	50	98.66	3.26	2.91
		95.46	1.25	
		96.32	3.04	
	100	101.4	4.68	4.26
		96.82	2.55	
		97.11	4.03	
Large yellow croaker	1	93.90	2.98	5.17
		95.80	3.89	
		98.05	7.32	
	50	96.26	4.71	4.07
		99.41	3.34	
		96.34	3.88	
	100	94.22	5.64	4.17
		92.68	3.91	
		95.45	2.69	
Shrimp	1	95.90	4.42	5.07
		95.57	5.21	
		96.48	6.31	
	50	95.77	4.66	4.64
		97.31	3.81	
		92.04	4.23	
	100	93.62	5.27	4.14
		92.68	3.29	
		95.83	3.54	
Chinese mitten crab	1	83.10	3.61	3.40
		85.57	2.35	
		83.80	3.93	
	50	80.67	6.73	5.20
		84.93	3.55	
		84.27	4.19	
	100	78.75	5.46	4.56
		81.33	4.88	
		80.86	2.92	
Mussel	1	90.33	5.41	10.08
		93.30	6.84	
		109.5	2.87	
	50	96.74	4.15	4.79
		100.9	2.77	
		97.56	6.47	
	100	99.41	5.42	4.36
		96.83	3.83	
		101.6	2.66	
Soft-shell turtle	1	94.28	2.69	4.36
		98.25	4.17	
		96.25	5.42	
	50	95.84	2.65	3.92
		96.47	4.57	
		92.73	3.66	
	100	90.68	2.83	4.66
		95.42	2.94	
		96.19	5.66	

concentration to 8 mM affected the RSD to 8.33, whereas other peaks were found resistant to altered concentration and temperature (Table 6). Additionally, the vortex time in extraction (± 1 min) and the amount of *n*-hexane (± 1 mL) for cleanup were also examined for the variation of recoveries. The RSDs for these variations did not exceed 10%.

Application to real samples

To validate the proposed methodology, a total of 47 samples comprising grass carp, common carp, snakehead, crucian, bighead carp, bream, turbot, and oyster collected from diverse provinces across China were analyzed by the developed method (see Electronic Supplementary Material Table S2). The intensity ratio between qualitative and quantitative ions is a crucial factor in assessing suspect positives, as it serves as an important indicator of their reliability and accuracy [44]. The identification of positive samples necessitates the simultaneous confirmation of the following two conditions: (i) the intensity ratios of the samples were validated by similar concentrations of standards, and their relative deviations in ion ratio tolerance limits must comply with the regulatory requirements outlined in the SANTE/11312/2021 guidance (see Electronic Supplementary Material Table S1) [25]; (ii) the relative deviations of retention time between positives and standards are less than ± 2.5%. Based on our LC–MS/MS equipment, the ratio criteria of 15% are used to evaluate positives (see Electronic Supplementary Material Fig. S2). Accordingly, the results identified one positive sample containing residues of moenomycin A in total of 47 samples (see Electronic Supplementary Material Table S2 and Fig. S3).

Table 6 Validation parameters for ruggedness

Standard	Buffer concentration (mmol) (RSD)				Column temperature (°C) (RSD)				Precision for area (RSD)	Precision for RT (RSD)
	8	9	11	12	37	39	41	43		
Moenomycin A	8.33	2.75	0.52	2,14	0.49	0.26	0.19	0.41	0.34	0.11

**n* = 3. *RT*, retention time

Conclusions

In summary, this study developed and validated an ultratrace analysis method for moenomycin A in edible tissues of aquatic animal products using HPLC–MS/MS. The optimized extraction, combined with the application of the QuEChERS method, resulted in an effective, rapid, and easy-to-operate approach, which was successfully applied for the qualitative and quantitative determination of moenomycin A in aquatic animal products. With an LOD of 1 μg/kg, significantly lower than those for feedstuffs, the method is well-suited for routine analysis of moenomycin A in aquatic animal products. The developed method demonstrated specificity, good linearity, high sensitivity, satisfactory precision, and accuracy. It has also been successfully employed to analyze moenomycin A in 47 aquatic samples collected in China. Furthermore, this method will be further validated and optimized for the determination of moenomycin A in poultry samples in future work.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Zehl M, Pittenauer E, Rizzi A, Allmaier G. Characterization of moenomycin antibiotic complex by multistage MALDI-IT/RTOF-MS and ESI-IT-MS. J Am Soc Mass Spectrom. 2006;17:1081–90. https://doi.org/10.1016/j.jasms.2006.04.019.
2. Pfaller MA. Flavophospholipol use in animals: positive implications for antimicrobial resistance based on its microbiologic properties. Diag Microbiol Infect Dis. 2006;56:115–121. https://doi.org/10.1016/j.diagmicrobio.2006.03.014.Get rights and content.
3. Van Heijenoort J. Formation of the glycan chains in the synthesis of bacterial peptidoglycan. Glycobiology. 2001;11:25R-36R. https://doi.org/10.1093/glycob/11.3.25R.
4. Welzel P. Syntheses around the transglycosylation step in peptidoglycan biosynthesis. Chem Rev. 2005;105:4610–60. https://doi.org/10.1021/cr040634e.
5. Stembera K, Vogel S, Buchynskyy A, Ayala JA, Welzel P. A surface plasmon resonance analysis of the interaction between the antibiotic moenomycin A and active ingredient -binding Protein 1b. ChemBioChem. 2002;3:559–65. https://doi.org/10.1002/1439-7633(20020603)3:6%3c559::AID-CBIC559%3e3.0.CO;2-%23.
6. Eichhorn P, Aga DS. Characterization of moenomycin antibiotics from medicated chicken feed by ion-trap mass spectrometry with electrospray ionization. Rapid Commun Mass Spectrom. 2005;19:2179–86. https://doi.org/10.1002/rcm.2044.
7. Paik J, Kern I, Lurz R, Hakenbeck R. Mutational analysis of the Streptococcus pneumoniae bimodular class a active ingredient-binding proteins. J Bacteriol. 1999;181(12):3852–6. https://doi.org/10.1128/JB.181.12.3852-3856.1999.
8. Pérez S, McJury BE, Eichhorn P, Aga DS. Determination of the antimicrobial growth promoter moenomycin-A in chicken litter. J Chrom A. 2007;1175:234–41. https://doi.org/10.1016/j.chroma.2007.10.053.
9. Butaye P, Devrise LA, Haesebrouck F. Antimicrobial growth promoters used in animal feed: effects of less well known antibiotics on gram-positive bacteria. Clin Microbiol Rev. 2003;16(2):175. https://doi.org/10.1128/CMR.16.2.175-188.2003.
10. Ostash B, Walker S. Moenomycin family antibiotics: chemical synthesis, biosynthesis, and biological activity. Nat Prod Rep. 2010;27:1594–617. https://doi.org/10.1039/C001461N.
11. Cao L, Naylor R, Henriksson P, Leadbitter D, Metian M, Troell M, Zhang WB. China's aquaculture and the world's wild fisheries. Science. 2015;347:133–5. https://doi.org/10.1126/science.1260149.
12. Heßler-Klintz M, Hobert K, Biallaß A, Siegels T, Hiegemann M, Maulshagen A, Müller D, Welzel P, Huber G, Böttger D, Markus A, Seibert G, Stärk A, Fehlhaber HW, Heijenoort YV, Heijenoort JV. The first moenomycin antibiotic without the methyl-branched uronic acid constituent.- Unexpected structure activity relations. Tetrahedron. 1993;49(35):7667–7678. https://doi.org/10.1016/S0040-4020(01)87242-2.

13. Tseng YY, Liou JM, Cheng WC, Hsu JT, Hsu TL, Wu MS, Wong CH. Combating multidrug-resistant Helicobacter pylori with moenomycin A in combination with active ingredient or active ingredient. Front Chem. 2022;10:897578. https://doi.org/10.3389/fchem.2022.897578.
14. Donnerstag A, Hennig L, Findeisen M, Welzel P, Haessner R. ^{1}H NMR as a tool for the structure elucidation of moenomycin antibiotics. Magn Reson Chem. 1996;34(12):1031–5. https://doi.org/10.1002/(SICI)1097-458X(199612)34:12%3c1031::AID-OMR10%3e3.0.CO;2-7.
15. El-Abadla N, Lampilas M, Hennig L, Findeisen M, Welzel P, Müller D, Markus A, Heijenoort JV. Moenomycin A: the role of the methyl group in the moenuronamide unit and a general discussion of structure-activity relationships. Tetrahedron. 1999;55(3):699–722. https://doi.org/10.1016/S0040-4020(98)01063-1.
16. Zhang L, Chen CC, Ko TP, Huang JW, Zheng Y, Liu W, Wang I, Malwal SR, Feng X, Wang K, Huang CH, Hsu STD, Wang AHJ, Oldfield E, Guo RT. Moenomycin biosynthesis: structure and mechanism of action of the prenylftransferase MoeN5. Angew Chem Int Edit. 2016;55(15):4716–20. https://doi.org/10.1002/anie.201511388.
17. Xu H, Zhang H, Wang F, Zhang X, Cai X. Determination of moenomycin A residues in poultry tissues by liquid chromatography-tandem mass spectrometry. J Food Safety Quality. 2014;5(12):3784–3789. http://chinafoodj.ijournals.cn/ch/reader/view_abstract.aspx?file_no=20141112004&flag=1.
18. Gallo P, Fabbrocino S, Serpe L, Fiori M, Civitareale C, Stacchini P. Determination of the banned growth promoter moenomycin A in feed stuffs by liquid chromatography coupled to electrospray ion trap mass spectrometry. Rapid Commun Mass Sp. 2010;24(7):1017–24. https://doi.org/10.1002/rcm.4478.
19. Commission E. Eight Commission Directive of 15 June 1978 establishing Community methods of analysis for the official control of feeding stuffs (78/633/EEC). Off J Eur Commun. 1978;L206:43.
20. Gavalchin J, Katz SE. The persistence of fecal-borne antibiotics in soil. J AOAC Int. 1994;77:481. https://doi.org/10.1039/ja994090011n.
21. Chen B, Li R, Guo Y, Yang K, Chen G, Ma X. Purification and preparation of moenomycin A from fermentation broth by multidimensional chromatography. Chromatographia. 2016;79:667–74. https://doi.org/10.1007/s10337-016-3086-0.
22. Fehlhaber HW, Girg M, Seibert G, Hobert k, Welzel P, Heijenoort YV, Heijenoort JV. Tetrahedron. 1990;46(5):1557–1568. https://doi.org/10.1016/S0040-4020(01)81965-7.
23. Li J, Sha M, Li X, Yin D. Determination of flavomycin A in feed by liquid chromatography tandem mass spectrometry. Chin J Vet Med. 2012;46(9):22−25. http://zgsyzz.ivdc.org.cn/zhonggsyzz/ch/reader/view_abstract.aspx?file_no=20120326001&flag=1#.
24. GB/T 30891, 2014. Practice of sampling plans for aquatic products. General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China; Standardization Administration of the People's Republic of China.
25. European Commission. Analytical quality control and method validation procedure for pesticide residues analysis in food and feed. SANTE/11312/2021; 2021.
26. Yuan JP, Sun YM, Zhao J, Yao YX. Rapid determination of hexabromocyclododecane enantiomers in animal meat by matrix solid phase dispersion coupled with LC–MS/MS. Food Chem. 2022;394:133405. https://doi.org/10.1016/j.foodchem.2022.133405.
27. Lee SW, Choi JH, Cho SK, Yu HA, El-Aty AMA, Shim JH. Development of a new QuEChERS method based on dry ice for the determination of 168 pesticides in paprika using tandem mass spectrometry. J Chrom A. 2011;1218:4366–77. https://doi.org/10.1016/j.chroma.2011.05.021.
28. Wang X, Wang M, Zhang K, Hou T, Zhang L, Fei C, Xue F, Hang T. Determination of virginiamycin M1 residue in tissues of swine and chicken by ultra-performance liquid chromatography tandem mass spectrometry. Food Chem. 2018;250:127–33. https://doi.org/10.1016/j.foodchem.2018.01.024.
29. Chaudhary DV, Patel DP, Shah PA, Shah JV, Sanyal M, Shrivastav PS. Determination of lercanidipine in human plasma by an improved UPLC–MS/MS method for a bioequivalence study. J Pharm Anal. 2016;6(2):87–94. https://doi.org/10.1016/j.jpha.2015.09.001.
30. Chen M, Zhang Y, Wang F, Zheng N, Wang J. Simultaneous determination of C18 fatty acids in milk by GC-MS. Separations. 2021;8(8):118. https://doi.org/10.3390/separations8080118.
31. Alcantara-Duran J, Moreno-Gonzalez D, Garcia-Reyes JF, Molina-Diaz A. Use of a modified QuEChERS method for the determination of mycotoxin residues in edible nuts by nano flow liquid chromatography high resolution mass spectrometry. Food Chem. 2019;279:144–9. https://doi.org/10.1016/j.foodchem.2018.11.149.
32. Zhao L, Lucas D, Long D, Richter B, Stevens J. Multi-class multi-residue analysis of veterinary drugs in meat using enhanced matrix removal lipid cleanup and liquid chromatography-tandem mass spectrometry. J Chrom A. 2018;1549:14–24. https://doi.org/10.1016/j.chroma.2018.03.033.
33. Zhao JH, Hu LX, He LX, Wang YQ, Liu J, Zhao JL, Liu YS, Ying GG. Rapid target and non-target screening method for determination of emerging organic chemicals in fish. J Chrom A. 2022;1676:463185. https://doi.org/10.1016/j.chroma.2022.463185.
34. Schenck FJ, Lehotay SJ, Vega V. Comparison of solid-phase extraction sorbents for cleanup in pesticide residue analysis of fresh fruits and vegetables. J Sep Sci. 2002;25(14):883–90. https://doi.org/10.1002/1615-9314(20021001)25:14%3c883::AID-JSSC883%3e3.0.CO;2-7.
35. Li J, Zhang J, Liu H, Wu L. A comparative study of primary secondary amino (PSA) and multi-walled carbon nanotubes (MWCNTs) as QuEChERS absorbents for the rapid determination of active ingredient and its major metabolites in fish samples by high-performance liquid chromatography–electrospray ionisation–tandem mass spectrometry. J Sci Food Agric. 2016;96:555–60. https://doi.org/10.1002/jsfa.7123.
36. Lehotay SJ, Mastovska K, Yun SJ. Evaluation of two fast and easy methods for pesticide residue analysis in fatty food matrices. J AOAC Int. 2005;88:630–8.
37. Alcántara-Durán J, Moreno-González D, García-Reyes JF, Molina-Díaz A. Use of a modified QuEChERS method for the determination of mycotoxin residues in edible nuts by nano flow liquid chromatography high resolution mass spectrometry. Food Chem. 2019;279:144–9. https://doi.org/10.1016/j.foodchem.2018.11.149.
38. Yang CW, Zhang X, Yuan L, Wang YK, Sheng GP. Deciphering the microheterogeneous repartition effect of environmental matrix on surface-enhanced Raman spectroscopy (SERS) analysis for pollutants in natural waters. Water Res. 2023;232:119668. https://doi.org/10.1016/j.watres.2023.119668.
39. Niessen WMA, Manini P, Andreoli R. Matrix effects in quantitative pesticide analysis using liquid chromatography-mass spectrometry. Mass Spectrum Rev. 2006;25(6):881–99. https://doi.org/10.1002/mas.20097.
40. Gajda A, Posyniak A, Zmudzki J, Tomczyk G. Determination of active ingredient in chicken fat by liquid chromatography with UV detection and liquid chromatography-tandem mass spectrometry. J Chrom B. 2013;928:113–20. https://doi.org/10.1016/j.jchromb.2013.03.011.

41. Chen J, Wei Z, Cao XY. QuEChERS pretreatment combined with ultra-performance liquid chromatography-tandem mass spectrometry for the determination of four veterinary drug residues in marine products. Food Anal Method. 2019;12:1055–66. https://doi.org/10.1007/s12161-018-01431-1.
42. Nasiri A, Jahani R, Mokhtari S, Yazdanpanah H, Daraei B, Faizi M, Kobarfard F. Overview, consequences, and strategies for overcoming matrix effects in LC-MS analysis: a critical review. Analyst. 2021;146:6049–6063. https://doi.org/10.1039/D1AN01047F.
43. Wang F, Lin W, Lv S, Jiang S, Lin L, Lu J. Comparison of lipids extracted by different methods from Chinese mitten crab (*Eriocheir sinensis*) hepatopancreas. J Food Sci. 2019;84(12):3594–600. https://doi.org/10.1111/1750-3841.14946.
44. Maldonado-Reina AJ, López-Ruiz R, Romero-González R, Martínez Vidal JL, Garrido-Frenich A. Assessment of co-formulants in marketed plant protection products by LC-Q-Orbitrap-MS: application of a hybrid data treatment strategy combining suspect screening and unknown analysis. J Agric Food Chem. 2022;70:7302–13. https://doi.org/10.1021/acs.jafc.2c01152.