

Review

Determination of Antibiotic Residues in Aquaculture Products by Liquid Chromatography Tandem Mass Spectrometry: Recent Trends and Developments from 2010 to 2020

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Abstract: The issue of antibiotic residues in aquaculture products has aroused much concern over the last decade. The residues can remain in food and enter the human body through the food chain, posing great risks to public health. For the safety of foods and products, many countries have issued maximum residue limits and banned lists for antibiotics in aquaculture products. Liquid chromatography tandem mass spectrometry (LC/MS/MS) has been widely used for the determination of trace antibiotic residues due to its high sensitivity, selectivity and throughput. However, considering its matrix effects during quantitative measurements, it has high requirements for sample pre-treatment, instrument parameters and quantitative method. This review summarized the application of LC/MS/MS in the detection of antibiotic residues in aquaculture products in the past decade (from 2010 to 2020), including sample pre-treatment techniques such as hydrolysis, derivatization, extraction and purification, mass spectrometry techniques such as triple quadrupole mass spectrometry and high-resolution mass spectrometry as well as status of matrix certified reference materials (CRMs) and matrix effect.

Keywords: aquaculture products; antibiotic residues; liquid chromatography tandem mass spectrometry (LC/MS/MS); sample pre-treatment; matrix effects



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1. Introduction

With the rapid development of China's aquaculture industry, China's aquaculture production now accounts for over 60% of the world's total [1]. To achieve the high yield, fish production adopts intensive and semi-intensive practices, which lead to a higher concentration of animals in small spaces and substantially increase the risk of disease [2]. Thus, antibiotics are often used as veterinary drugs and feed additives to treat and prevent aquaculture infections. The misuse or long-term use of antibiotics can lead to resistance in aquaculture products and humans, and even toxic side effects such as teratogenicity, carcinogenicity and mutagenicity in human body [3]. Consequently, many countries have gradually introduced maximum residue limits (MRLs) and prohibition lists for veterinary drugs residues in food of animal origin.

At present, antibiotics commonly used in aquaculture mainly include quinolones (QNs), sulfonamides (SAs), amphenicols (APs), nitrofurans (NFs), tetracyclines (TCs), macrolides (MALs), aminoglycosides (AGs), lincosamides, beta-lactams, etc. In 2002, the use of antibiotics such as chloramphenicol, nitrofurantoin antibiotics and nitroimidazole in food-producing animals was banned in China. In 2016, the Ministry of Agriculture and Rural Affairs of China announced a ban on the use of four QNs, lomefloxacin, pefloxacin,

ofloxacin and norfloxacin in food-producing animals. However, some veterinary drugs that have been banned, such as chloramphenicol, nitrofurans and malachite green, can still be detected in shrimp and fish samples [4]. To further strengthen the control of veterinary drugs, China issued a prohibited list of drugs and other compounds and a standard for maximum residue limits (Supplementary Materials) of veterinary drugs in animal origin food in 2019 (GB 31650-2019). The Codex Alimentarius Commission (CAC) developed the standards of MRLs for veterinary drugs in food (CAC/MRL 2-2015). Additionally, the European Commission (EU) has published the EU No 37/2010 about pharmacologically active substances and their classification regarding MRLs in foodstuffs of animal origin. As permitted veterinary drugs, many antibiotics have available MRL data in the Annex III of EU No 37/2010. For some prohibited antibiotics, the EU had set a minimum required performance level (MRPL) such as nitrofurans metabolites, chloramphenicol and sum of malachite green and leuco-malachite green at 1, 0.3 and 2 $\mu\text{g}\cdot\text{kg}^{-1}$ in aquaculture products, respectively (Commission Decision 2004/25/EC). Table S1 summarizes the MRL or MRPL of antibiotics in aquaculture products in different countries. The current prohibited antibiotics of aquaculture products in the Chinese standards are basically consistent with those in the EU standards, and both have similar MRLs for most antibiotics. Compared with those in the CAC standard, China's existing veterinary drug residue limits for aquaculture products are more comprehensive. With the improvement of limit standards, the national standard detection method of antibiotics in aquaculture products have also increasingly advanced. There are seventeen relevant standards for antibiotics in aquaculture products in China (Table S2), twelve of which are liquid chromatography tandem mass spectrometry detection methods (LC/MS/MS). According to the above, it can be concluded that LC/MS/MS will be more and more widely used in the detection of antibiotics in aquaculture products.

In 2016, Justino et al. [5] reviewed detection techniques for contaminants in aquaculture products, indicating that LC/MS/MS is becoming the dominant technique. In the same year, Santos et al. [2] summarized the current analytical methods for eight antibiotics in aquaculture fishes, detailing the legal provisions governing antibiotics in different countries and pointing out that multiclass and multiresidue detection is the future trend. In summary, based on the current trends of detection methods, this paper reviews the characteristics and research status of LC/MS/MS for the detection of antibiotics in aquaculture products during last decade (2010–2020), summarizes the sample pre-treatment methods of different antibiotics in aquaculture products, giving emphasis on hydrolysis, derivatization and extraction/purification methods and discusses representative matrix effect of antibiotics. The situation of matrix reference material in different countries is discussed as well.

2. Sample Pre-Treatments

The main steps of the analytical procedures used for determination of multi-antibiotics in Aquaculture products are shown in Figure 1. Aquaculture products are complex foods with high fat and protein, which increases the difficulty of extraction and separation. Thus, prior to analysis, extraction/clean-up and enrichment/concentration techniques are often needed to eliminate or reduce matrix effects to obtain more accurate results. The good chromatography separation and sensitive mass spectrometry response can also effectively improve the accuracy and sensitivity of the analysis. As we can see from Figure 2, most antibiotics are bound to proteins in aquaculture products and require acid hydrolysis prior to extraction, such as NFs, TCs and SAs, among which NFs requires hydrolysis along with derivatization for mass spectrometric detection to improve detection sensitivity. In addition, when using ultraviolet or fluorescence detectors to detect some antibiotics without chromogenic and fluorescent groups, it is also necessary to use derivatization reagents to give the analytes ultraviolet or fluorescent properties, for example, AGs and NFs [6,7]. However, TCs and QNs have chromogenic and fluorescent groups that do not require derivatization. In addition, most antibiotics have high polarity or boiling points and require derivatization before detection by gas chromatography (GC). For example, Santos et al. [8]

used gas chromatography tandem mass spectrometry (GC/MS) to screen chloramphenicol in trout by derivatization with silylated reagents after extraction and purification.

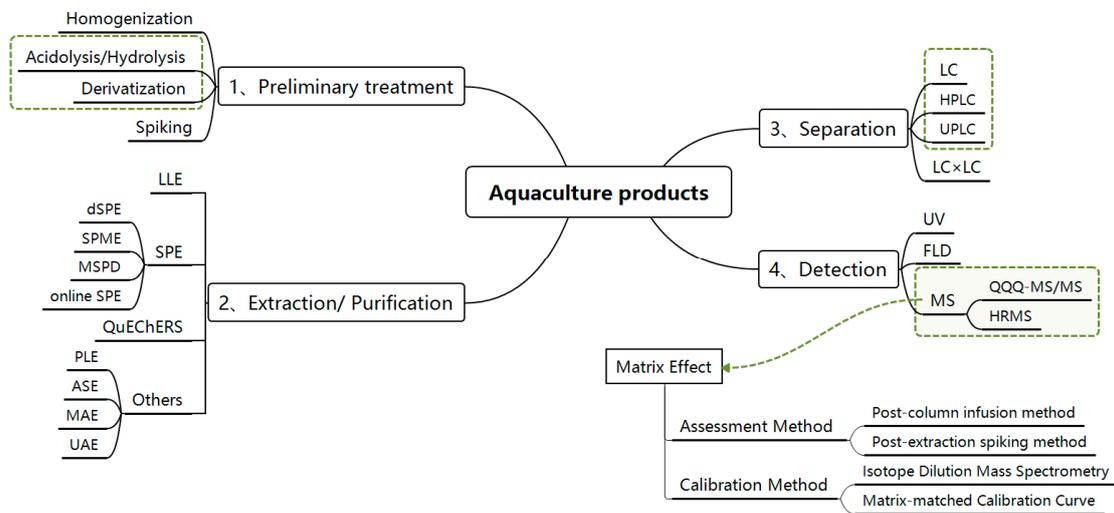


Figure 1. The main steps of the analytical procedure applied in determination of antibiotics of aquaculture products.

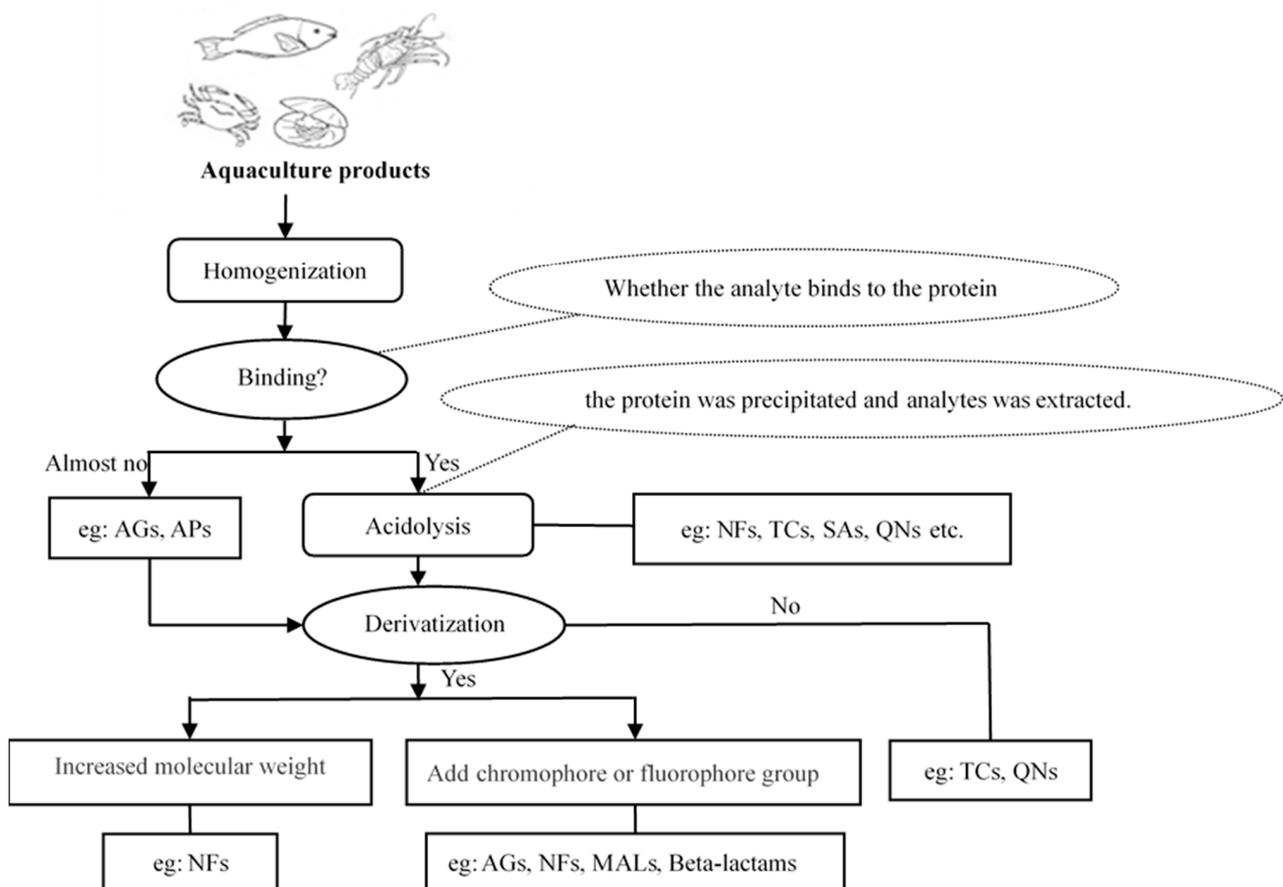


Figure 2. The current workflow of preliminary treatment for major antibiotics.

2.1. Hydrolysis and Derivatization

The hydrolysis step is highly required to convert the combined state to the free state before sample extraction and purification for those antibiotics in aquaculture products in

the form of protein binding. In order to provide a theoretical basis for establishing a more efficient pre-treatment method, many researchers further investigated the rules of binding and desorption of proteins and antibiotic drugs [9]. In 2002, M. A. Khan et al. [10] demonstrated the high affinity between bovine serum albumin (BSA) and TCs by fluorescence quenching. In 2018, Pan Lin [9] studied the effect of three different matrix components (protein, fat and water) on the extraction efficiency of TCs, and the results showed that TCs have a strong binding effect with egg albumin (CEA), which can lead to low extraction efficiency. Li et al. [11] reported that there was a strong hydrogen bonding interaction between fluoroquinolone antibiotics (FQs) and fish serum albumin (FSA) which can be broken by 50–90% acetonitrile acid solution, and when protein was precipitated with 90% acetonitrile solution, the recoveries of four FQs were >80%. Zhang Yanxi [12] chose BSA as the model carrier protein to simulate the physiological conditions of fish in vitro, and it was confirmed that sulfamethoxazole and sulfamedoxine interacted with BSA, which lead to the low recovery of SAs. The ammonium acetate buffer including 0.3% acetic acid could effectively eliminate the binding of SAs with BSA, and the recovery reached more than 90%.

In addition, the parent NFs are metabolized rapidly in animals, and the half-lives in vivo are not more than a number of hours [13]. Most of the methods published in the literature rely on the detection of metabolites. Moreover, their metabolites tend to form metabolite-protein adducts that are stable for a long time, so the acidic hydrolysis step is commonly used to liberate the covalently bound metabolites [14]. However, nitrofurans metabolites, such as semicarbazide (SEM), 3-amino-2-oxazolidone (AOZ), 1-amino-hydantoin (AHD) and 5-methylmorpholino-3-amino-2-oxazolidinone (AMOZ), are characterized by small relative molecular mass (75–201 Da) and large polarity, which makes it difficult to detect directly by mass spectrometry. In most articles, free nitrofurans metabolites were derivatized with 2-nitrobenzaldehyde (2-NBA) as the derivatizing reagent under a 37 °C shaking bath for 16 h [15–20] to increase the relative molecular mass and detection sensitivity before extraction. Although hydrolysis and derivatization require a long time, they are the key to an efficient extraction for binding antibiotics. To shorten derivatization time to 2 h, some researchers [21] increased the derivatization temperature to 60 °C in a shaking bath, but the sufficient hydrolysis time of the incurred sample was not discussed in detail. Differently, Tao et al. [22] and Wang et al. [23] adopted an ultrasound-assisted derivatization method to replace the shaking bath method (37 °C, 16 h). With 2-nitrobenzaldehyde (2-NBA) as the derivatization reagent, the NF metabolites were hydrolyzed and derivatized with a reaction temperature of 40 °C for 1 h [22]. Palaniyappan et al. [24] developed a new method of microwave-assisted derivatization, and the results were achieved in a short time of 6 min with good recovery. Moreover, different derivatization reagents have also been proposed. Luo et al. [25] used 7-(diethylamino)-2-oxochromene-3-carbaldehyde (DAOC) as the derivatization reagent to react with four NF metabolites to form hydrazone derivatives under the assistance of a microwave within 20 min, which were very stable and exhibited excellent fluorescence sensitivity with maximum excitation and emission wavelengths of 450 and 510 nm, respectively. Du et al. [6] chose 2-hydroxy-1-naphthaldehyde (HN) as a novel derivatization agent, and the synthetic derivative was easily formed and stable, which was suitable for detection by HPLC-FLD and HPLC-MS/MS.

Other than the use of Nitrofurazone (NFZ), the presence of SEM in the sample may also occur by reaction with biurea and azodicarbonamide that are commonly used for food preservation. In addition, SEM is naturally present in the shells of crayfish, shrimp, prawn and soft-shell crab. Therefore, the use of SEM as the exclusive marker for NFZ might be unreliable. 5-nitro-2-furaldehyde (NF) was used as another residual marker for nitrofurazone, and 2,4-dinitrophenylhydrazine (DNPH) was used as a derivatization reagent [26,27]. Derivatization was easily performed in an ultrasonic water bath at 30 °C for 5 min [26], greatly shortening the derivatization time.

2.2. Extraction and Purification Methods

Extraction and purification methods for antibiotics in aquaculture products mainly include liquid–liquid extraction (LLE), solid-phase extraction (SPE), QuEChERS, pressurized liquid-phase extraction (PLE), microwave-assisted extraction (MAE), etc.

2.2.1. Liquid–Liquid Extraction (LLE)

LLE was traditionally used for the extraction of antibiotics due to its simplicity and practicality. For LLE, solvent selection plays a critical role to enhance the recovery of the analyte, which improves the limit of detection (LOD), and minimize the matrix effect. The extraction solvent was chosen according to the physicochemical properties of compounds. Du et al. [6] and Zhang et al. [28] chose ethyl acetate as the extract solvent to extract four nitrofurans in shrimp, with a recovery rate of 85–107%. As for AGs with greater polarity, a simple extraction is generally performed with an aqueous buffer [29,30]. Kaufmann et al. [29] used a trichloroacetic acid aqueous solution for extraction, followed by solid-phase extraction, with recoveries of 60–85%. Additionally, different acids, bases, salts or complexing agents are usually added to improve the extraction efficiency and ionization efficiency of the analytes. Manuel et al. [31] adopted an acetonitrile solvent with 5% formic acid to extract eight quinolones in fish samples; the formic acid provided an acidic medium to facilitate the extraction of quinolone antibiotics with a recovery rate of 72–108% and intra-day reproducibility of less than 10.5%. The mixed solution of ethyl acetate and ammonia water (98:2) were used as the extraction solvent to extract three APs in tilapia; the ammonia water played a role in facilitating the extraction, and the recovery rate was 79.8–92.0% [32]. As for TCs, QNs or gentamicin, which are easily complexed with polyvalent metal cations, chelating agents are often added to the extraction solvent, then, sodium sulfate is often used in the phase separation step instead of magnesium sulfate [33–36]. Grande-Martinez et al. [37] developed a modified QuEChERS procedure to extract TCs in fish. A fish sample was extracted twice by an EDTA-McIlvaine buffer and acetonitrile, then 50 mg of C18 was added for further purification, with recoveries ranging from 80 to 105%. Shin et al. [38] performed a two-step solvent extraction method. The aqueous phase extraction solution was added with Ethylene Diamine Tetraacetic Acid (EDTA) and ammonium acetate, and acetonitrile was added with ammonium formate. The results showed that the addition of EDTA can increase the extraction recovery rate of tetracycline from less than 60% to nearly 100%. Because of the high fat in aquaculture products, the additional step of degreasing is necessary. N-hexane is the most common degreasing solvent [39–41]. Meanwhile, it is recommended to increase the centrifugation speed or add sodium chloride to overcome emulsification [38].

For the extraction of antibiotics with similar polarity, most extraction methods use single organic solvents as extractants, however, when applied to multiresidue antibiotics with different physical and chemical properties, water or a water buffer can be combined with organic solvents to expand the extraction range of analytes [39,40]. Jia et al. [42] developed a multiresidue method for the analyses of 137 veterinary drug residues. Extraction of compounds was achieved by 5 mL of an acetonitrile/water solution (84/16, *v/v*) with one hundred microliters of 0.1 M of EDTA and 1% acetic acid, and then Primary-secondary amine (PSA) and Z-Sep+ as the adsorbent of solid-phase microextraction (SPME) to purify, with a good recovery rate ranging from 82 to 112%. Figure 3 summarizes the commonly used extraction solvents for different antibiotics, including acetonitrile, methanol, ethyl acetate, buffer solutions and so on. For nitrofurans antibiotics, most of articles used ethyl acetate as the extraction solvent, and sometimes it is mixed with a small proportion of acetonitrile for extraction. For the more polar aminoglycosides, different buffer solutions were the main extraction solvents. Acidified acetonitrile is commonly used for the simultaneous extraction of quinolones, sulfonamides and tetracyclines.

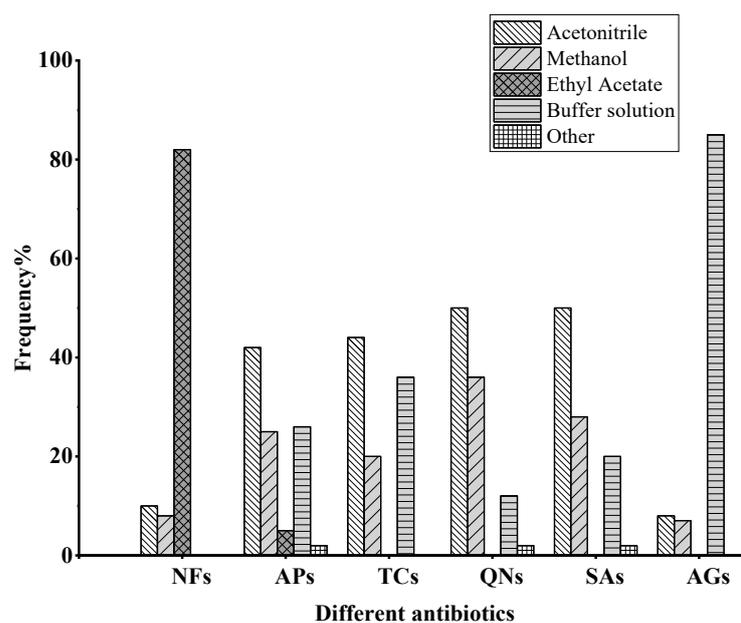


Figure 3. The proportion of different extraction solvents used for five kinds of antibiotics in articles from the recent decade.

2.2.2. Solid-Phase Extraction (SPE)

C18 [43,44], C8, Phenyl [45] and HLB [28,46] are the main reverse-phase sorbent materials used for solid-phase extraction and purification of antibiotics from aquaculture products. Furthermore, Oasis HLB SPE column is more common in the extraction of antibiotics from aquaculture products [14,22,47–49]. Evaggelopoulos et al. [46] proposed an approach to extract six penicillin antibiotics and three APs from gilthead seabream tissues. The extraction was carried out by a mixture of H₂O/acetone (50/50% *v/v*), which was repeated twice in order to increase the rates of recovery. Subsequently, the recoveries of Lichrolut RP-18 and OASIS HLB SPE column were compared. The results showed that the recovery rate of the OASIS HLB SPE column was higher, which could reach more than 95%. Liu et al. [47] used Ultrasonic-Assisted Extraction (UAE) combined the SPE method to determine the multiresidue antibiotics in fish and plasma. Fish samples were extracted with methanol and enriched using Oasis HLB solid-phase extraction columns in one step, the average recovery was 61–111%, and relative standard deviation (RSD) was less than 25%. For highly polar aminoglycoside antibiotics, ion exchange extraction columns are more suitable [50]. Gbylik et al. [51] developed a two-step extraction mean to separate seven classes of antibiotics, including aminoglycosides from fish. Firstly, the isolation of residues from the sample was applied by *m*-phosphoric acid and heptafluorobutyric acid as an ion-pair agent and acetonitrile, then, a clean-up technique was performed by polymeric weak cationic extraction column (Strata X-CW), the result of recovery was from 96 to 111%.

In recent years, increasingly new solid-phase extraction sorbents have been applied, such as graphene, multiwalled carbon nanotubes (MWCNTs), molecularly imprinted polymers (MIPs) [52–54]. Wu et al. [35] proposed two-dimensional (2D) planar graphene powder as an SPE sorbent for enrichment and cleanup of MLs from a carp sample. Finally, 15 mg of graphene was selected when the carp sample was 1 g, and the extraction recoveries ranged from 81.7 to 110.5%. With the development of analytical techniques, some new techniques based on traditional SPE have been applied in the detection of antibiotic residues in aquaculture products, such as solid-phase microextraction (SPME), matrix solid-phase dispersion extraction (MSPD), and dispersive solid-phase extraction (d-SPE). Mondal et al. [55] synthesized a novel MIL-101(Cr)-NH₂ fiber for extraction of six antibiotics (flumequine, Nalidixic acid, tilmicosin, sulfadimethoxine, sulfaphenazole and methomyl pyrimidine) from fish meat by SPME, with better reproducibility than conventional fibers, and the precision is between 1.5 and 8.3%. Pan et al. [52] extracted

AGs from fish by MSPD and compared the extraction efficiency of two sorbents, C18 and graphitized carbon black (GCB), indicating that the recoveries with the use of C18 were higher than those with GCB. Shen et al. [53] proposed a micropipette-matrix solid-phase dispersion (PT-MSPD) technique treated with a pipette tip and dispersant HLB for the detection of 14 QNs in fish tissues, and the absolute recoveries were 25% higher than those of conventional MSPD. Unlike traditional SPE, MSPD does not require tissue homogenization, precipitation, centrifugation, pH adjustment and sample transfer, avoiding the loss of samples, shortening the operation time and saving organic reagents. D-SPE is a technique in which the solid-phase extraction sorbent is dispersed in the extraction solution of the sample [56,57] and is also commonly used in the QuEChERS method [34,42], especially for the extraction of antibiotics from complex matrices. For example, Manuel et al. [31] extracted eight quinolone antibiotics from a variety of complex fish matrices by simple acidified acetonitrile liquid–liquid extraction, and then d-SPE was performed by C18 and MgSO₄. The recoveries were from 72 to 108% with good reproducibility and RSD less than 6.4%.

2.2.3. Other Techniques

To enhance environmental protection, some green extraction techniques have been gradually applied, such as pressurized liquid extraction (PLE) or accelerated solvent extraction (ASE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), etc. Compared with traditional methods, these extraction techniques take advantage of saving extraction times and reducing solvent consumption. Liu et al. [58] adopted the PLE method to extract TCs in fish and shrimp. A mixed solvent of trichloroacetic acid (TCA)/methanol (1:3) was the solvent. Equal amounts of Na₂EDTA should be added before PLE. The recoveries ranged from 75.6 to 103.5%. PLE reduced the use of solvents and extraction time compared to traditional liquid–liquid extractions. Hoff et al. [59] simultaneously detected 16 SAs in liver, comparing two extraction methods of PLE and UAE, with recoveries close to 100%, but the latter with a slight advantage of UAE in terms of solvent usage and time required. Kazakova et al. [60] extracted multiple antibiotics from lobsters by LLE and MAE methods. The best condition of MAE was 50 µL Proteinase-K and 5 µL formic acid (FA) at 50 W for about 5 min. The recoveries ranged from 71 to 100%.

In summary, LLE is a traditional and easy-to-operate but time-consuming method that is more suitable for the extraction of antibiotics with similar polarity. Compared to LLE, SPE is more automated and enables simultaneous extraction and enrichment. With the development of science and technology, some advanced materials, such as MIP materials, magnetic materials and so on, provide SPE with excellent performance. At present, SPE and LLE are still important methods for extracting and purifying antibiotics in aquaculture products. However, in our prospect, microextraction techniques and green extraction techniques are bound to become the mainstream in the near future.

3. Liquid Chromatography-Mass Spectrometry Detection Technique

The common ion sources for the liquid chromatography tandem mass spectrometry method are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). ESI sources are mainly used for polar and macromolecular compounds and have a wider range of application, so most antibiotic detection is often performed using ESI source mass spectrometry. Table 1 outlines applications of LC/MS/MS for the analysis of antibiotics in aquaculture products during the recent decade. Aldeek et al. [16] used LC-ESI-MS/MS to simultaneously detect four nitrofurans metabolites and chloramphenicol in tilapia and shrimp, and quantified using an isotopic internal standard, which could calibrate the loss of analytes during sample preparation well. The recovery rate reached 90–100%, which was 40% higher than that of the external standard method, and the RSD was less than 10%. Kung et al. [56] adopted the QuEChERS method for the detection of four sulfonamides in fish meat by HPLC-ESI-MS/MS. The recoveries were 80.2–93.5% with RSD less than 9%, the decision limit (CC_α) ranged from 1.49 to 10.09 µg·kg⁻¹, and detection

capability ($CC\beta$) ranged from 1.71 to 11.4 $\mu\text{g}\cdot\text{kg}^{-1}$. Jansomboon et al. [61] detected four sulfonamides in fish by LC-ESI-MS/MS after acidic methanol and acetonitrile liquid–liquid extraction, the detection limit was from 0.75 to 3.13 $\mu\text{g}\cdot\text{kg}^{-1}$. However, previous studies have demonstrated that the APCI source had better sensitivity for SEM. An et al. [15] found that that the detection signal generated by the APCI source was from three to fivefold higher than that of the ESI source. The APCI source also had lower background noise, which significantly enhanced the SEM signal. The LOD was 0.052–0.108 $\mu\text{g}\cdot\text{kg}^{-1}$, the LOQ was 0.25 $\mu\text{g}\cdot\text{kg}^{-1}$, and the recovery was 100.2–104.0% with good reproducibility. Similarly, Chumanee et al. [62] chose the APCI source rather than the ESI source for the detection of SEM in order to improve the sensitivity, with LODs of 0.1–0.3 $\mu\text{g}\cdot\text{kg}^{-1}$ and LOQs of 0.1–0.5 $\mu\text{g}\cdot\text{kg}^{-1}$.

With the increasing development of mass spectrometry, there is an increasing number of multiclass and multiresidue analysis (MCMR) methods for the simultaneous screening or quantification of dozens or even hundreds of different classes of residues in samples [63–67]. A subject search of the ScienceDirect database using keywords related to LC-MS, multiple residues, antibiotics and aquaculture product indicated an overall growth trend for the last 10 years (Figure 4a). Miossec et al. [68] established a UPLC-QQQ-MS/MS method for the simultaneous detection of 42 veterinary drugs in four kinds of seafood with a simple LLE using acidified methanol extraction, followed by enrichment and filtration, and the LODs ranged from 0.1 to 5.0 $\mu\text{g}\cdot\text{kg}^{-1}$ for all antibiotics except amoxicillin. Dasenaki et al. [63] used a UPLC-QQQ-MS/MS method to simultaneously detect up to 20 categories of 115 veterinary drugs, and the recoveries of 80% of the analytes ranged from 50 to 120% with RSD less than 18%. The LOQs for all analytes were less than 5 $\mu\text{g}\cdot\text{kg}^{-1}$ except for dalfoxacin, of which it was 5.6 $\mu\text{g}\cdot\text{kg}^{-1}$. Jia et al. [42] developed a UHPLC-Q/Obitrap-HRMS method for simultaneous analysis of one hundred and thirty-seven veterinary drug residues and metabolites from sixteen different classes in tilapia. Three ways of data acquisition were compared: Fall Scan/dd-MS/MS, Fall Scan/all-ion fragmentation (AIF) and Fall Scan/variable data independent acquisition (vDIA). The result showed that using vDIA instead of dd-MS/MS or AIF for nontargeted generation of fragment ions improved the selectivity and sensitivity of the analysis. The recoveries of 137 analytes ranged from 81 to 111%, $CC\alpha$ ranged from 0.01 to 2.73 $\mu\text{g}\cdot\text{kg}^{-1}$, and $CC\beta$ ranged from 0.01 to 4.73 $\mu\text{g}\cdot\text{kg}^{-1}$. Munaretto et al. [57] used LC-Q/TOF-HRMS to detect 182 pesticides, veterinary drugs and other contaminant residues and evaluated the effect of two different scanning methods (FS and dd-MS/MS). It turns out that the FS mode could detect 84% of the compounds, while dd-MS/MS scan could only detect 72%, but dd-MS/MS scan could provide fragmentation information of the target, therefore using dd-MS/MS scan for characterization and the FS mode for quantification.

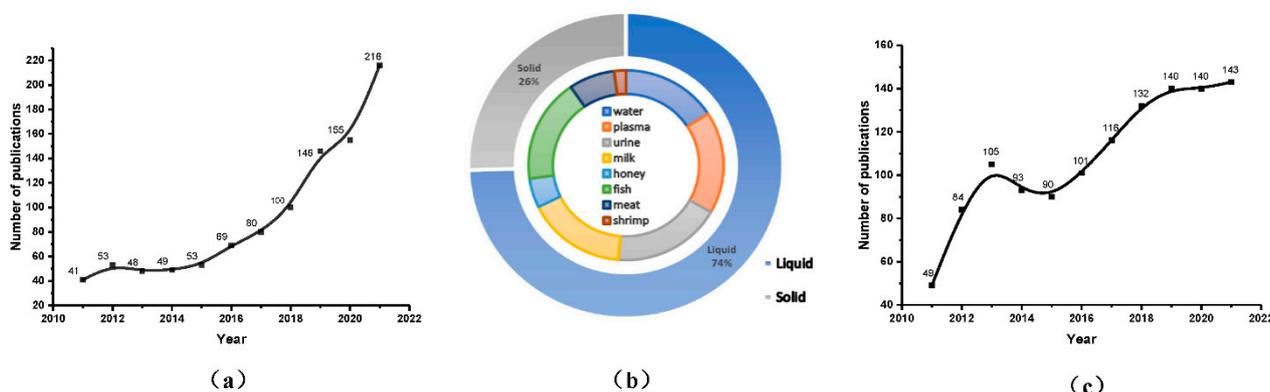


Figure 4. (a) The trend of multiresidue detection of antibiotics in last decade. (b) The application and trend of on-line solid-phase extraction in different matrixes. (c) The trend of using the on-line SPE-LC-MS method in detection of antibiotics in last decade.

Table 1. The applications of LC/MS for the analysis of antibiotics in aquaculture products during last decade.

Analyte (Number)	Preliminary Treatment	Extraction and Purification	Recovery	Detection Method	LODs/CC α ($\mu\text{g}\cdot\text{kg}^{-1}$)	LOQs/CC β ($\mu\text{g}\cdot\text{kg}^{-1}$)	Ref.
NFs (4)	Acidolysis (HCl), derivatization (2-NBA, shock, 37 °C, 16 h)	SPE: HLB	88~112%	UPLC-ESI-MS/MS	0.5	1.5	[14]
NFs (4)		EtOAc; Hex	101.6~105.9%	LC-APCI-MS/MS	0.05–0.2	0.25	[15]
NFs (4), APs (2)		H ₂ O; EtOAc	85~110% (APs)	UPLC-ESI-MS/MS	0.1–1	0.25–1	[16]
NFs (8), CAP		EtOAc; Hex, SPE by HLB	97~108% (expect PSH, DNSH, NPIR)	UHPLC-ESI-HRMS	0.01–0.1 ^a	0.01–0.18 ^b	[18]
NFs (4)		EtOAc; Hex	84~115%	LC-ESI-MS/MS	0.1–0.8 ^a	0.3–0.9 ^b	[19]
NFs (8)		EtOAc; Hex	91.6~107.3 %	UHPLC-ESI-MS/MS	0.01–0.2; NSTY (2.0)	0.04–0.5; NSTY (5.0)	[41]
NFs (4)	Acidolysis (TCA), derivatization (2-NBA, ultrasound, 40 °C, 1 h)	ASE: MEOH/5% TCA (1/1, <i>v/v</i>); SPE: HLB	77.2~97.4%	LC-ESI-MS/MS	0.07–0.13 ^a	0.31–0.49 ^b	[22]
SEM	Acidolysis (HCl), derivatization (DNPH, ultrasound, 30 °C, 5 min)	EtOAc; SPE: neutral alumina and HLB	80.8~104.4%	UPLC-ESI-MS/MS	0.05	0.1	[26]
APs (3)	/	ACN/H ₂ O (1:1, <i>v/v</i>), EtOAc; MSPD: C18	82.4~99.8%	UPLC-ESI-MS/MS	0.02–0.06 ^a	0.11–0.16 ^b	[52]
APs (3)	/	EtOAc:NH ₃ ·H ₂ O (98:2, <i>v:v</i>)	80~92%	HPLC-ESI-MS/MS	0.019–54.9 ^a	0.068–64.88 ^b	[32]
FF, FFA	/	EtOAc:NH ₃ ·H ₂ O (98:2, <i>v:v</i>); Hex, SPE: Phenyl	96.3%(FF);83.0%(FFA)	UPLC-ESI-MS/MS	6 (FF) 1 (FFA)	25 (FF) 3 (FFA)	[45]
SAs (18)	Acidolysis	UAE: 0.1% FA in ACN; On-line SPE: MCX and HLB	71.5~102%	Online-SPE- UHPLC—MS/MS	1.46–15.5	4.90–51.6	[48]
SAs (16)	Acidolysis	ASE: 0.2% FA in ACN; Hex	about 100%	HPLC-QqLIT- MS/MS	10	25	[59]

Table 1. Cont.

Analyte (Number)	Preliminary Treatment	Extraction and Purification	Recovery	Detection Method	LODs/CC α ($\mu\text{g}\cdot\text{kg}^{-1}$)	LOQs/CC β ($\mu\text{g}\cdot\text{kg}^{-1}$)	Ref.
SAs (14)	/	ACN; d-SPE: C18	80.2~93.5%	HPLC-ESI-MS/MS	1.49–10.9 ^a	1.71–11.4 ^b	[56]
SAs (14)	/	PT-MSPD: HLB	83~90%	UPLC-ESI-MS/MS	2.3–16.4	6.9–54.7	[53]
TCs (5)	/	EDTA-McIlvaine, ACN; SPE: C18	80~105%	LC-ESI-MS/MS	0.5–1.3	1.7–4.4	[37]
AGs (13)	/	0.05 g/mL TCA in water; SPE: MCX	45~85%	UHPLC-ESI-MS/MS	/	2–25	[29]
AGs (9)	/	USA: PBS; SPE: Poly-Sery and MCX	65~110%	HPLC-ESI-MS/MS	2–10 ^a	6–25 ^b	[30]
MAAs (5)	/	MeOH; SPE: Graphene; Hex	81.7~110.5%	UPLC-Qtrap- MS/MS	0.09–0.72	0.3–0.77	[35]
MAAs (6)	/	MeOH; Hex	77~109%	HPLC-QTOF-HRMS	5.8–27	17–82	[36]
QNs, SAs, TCs	Acidolysis	1% FA in water, ACN	83~100%	UHPLC-ESI-MS/MS	107–114 ^a	112–129 ^b	[40]
NFs, NIIMs, CAP, MG	Acidolysis (HCl), derivation (2-NBA, 60 °C, 2 h)	EtOAc, ACN, Hex, MgSO ₄	77.2~125.6%	HPLC-ESI-MS/MS	0.07–1.65 ^a	/	[21]
TCs, APs, SAs, TMP, FQNs, MALs	/	MEOH; SPE: HLB	61~111%	UHPLC-ESI-MS/MS	/	0.03–6.67	[49]

/: not mentioned in the article; EtOAc: ethyl acetate; MEOH: Methanol; ACN: acetonitrile; Hex: n-Hexane; FA: formic acid; TCA: trichloroacetic acid; PBS: phosphate buffered saline; LLE: liquid–liquid extraction; SPE: solid–liquid extraction; d-SPE: dispersive solid-phase extraction; MSPD: Matrix solid-phase dispersion; ASE: accelerated solvent extraction; UAE: ultrasonic assisted extraction; NFs: nitrofurans; APs: amphenicols; SAs: sulfonamides; MALs: macrolides; TCs: tetracyclines; AGs: aminoglycosides; NIIMs: nitroimidazoles; CAP: chloramphenicol; FF: florfenicol; FFA: florfenicol amine; SEM: semicarbazide; TMP: trimethoprim; MG: malachite green; HRMS: high resolution mass spectrometer; APCI: atmospheric pressure chemical ionization; ESI: electrospray ionization; HLB: hydrophilic lipophilic balance; LOD: limit of detection; LOQ: limit of quantitation. ^a using CC α to replace LODs; ^b using CC β to replace LOQ.

In order to improve the analysis efficiency and automation level, many instruments have realized the coupling of on-line solid-phase extraction and liquid mass spectrometry, like the solid-phase extraction column and the chromatographic column combined through a valve [69]. The target is directly eluted from the solid-phase extraction column to the chromatographic column, which not only simplifies the experimental steps and avoids the loss of analytes, but also greatly improves the sensitivity of the analytical method. A subject search of the ScienceDirect database using keywords related to on-line SPE-LC-MS and antibiotics indicated an overall growth trend for the last 10 years (Figure 4c). However, this method is still mostly used for liquid samples such as environmental water, plasma and urine, while the application of solid samples is less frequent, accounting for only about 26% (Figure 4b). Ma et al. [70] performed rapid determination of 15 sulfonamide antibiotic residues in pork and fish by Online SPE-LC-MSMS with simple extraction using acetonitrile solution containing 2% formic acid and cleanup using the Oasis HLB on-line column (10 mm × 1 mm, Waters, Milford, MA, USA). The recoveries ranged from 78.3 to 99%, RSD was less than 10%, and the LOQs were found to be 0.25–5 µg·kg⁻¹. Hurtado et al. [71] achieved on-line analysis of 13 analytes including sulfonamides and tetracyclines in catfish. Three kinds of on-line solid-phase extraction columns (C8, C18, GP) are compared. Among them, the recovery of GP was the best, reaching 80% to 99%. The LODs and LOQs were found to be less than 0.1 µg·kg⁻¹ and 2.4 µg·kg⁻¹, respectively. On-line SPE can achieve extraction and purification better, reduce matrix effects by diluting samples, avoid loss of analytes and improve sensitivity, thus it has become a hotspot of analytical work. However, the practical application of on-line SPE still has certain limitations due to the few types and high prices of instruments and columns.

4. Matrix Effect

The matrix effect (ME) is a prevalent phenomenon in mass spectrometry analysis, manifested as signal suppression or enhancement, which can affect method sensitivity, precision and accuracy. In recent years, an increasing amount of research about LC-MS/MS method has evaluated the impact of the matrix effect on detection and proposed solutions to reduce or eliminate it. The method of qualitative evaluation of the matrix effect is the post-column infusion method [72], which is assessed by observing the variation in the ESI response of the injected analyte. A constant amount of standard solution of analyte is delivered by an infusion pump. A blank sample extract is injected on the LC column. Then, both of them are mixed through a straight tee into the ion source of the mass spectrometer. Finally, it is easy to identify chromatographic regions most likely to experience matrix effects [73]. The method of quantitative evaluation of the matrix effect is the post-extraction spiking method, which is used to compare the response of the pure solution calibration solution (A) with the matrix-matching standard solution (B) of the same concentration. ME can be measured using the following equation: $ME\% = B/A \times 100$.

Grande-Martínez et al. [37] evaluated the ME of five TCs in salmon using the post-extraction spiking method and reduced ME by optimizing sample preparation methods. Due to the high fat content of the salmon matrix, the ME of TCs ranged from 61 to 140%. Then, the author adopted d-SPE with Z-Sep+ to remove matrix interference, and the ME reduced to 95–105% and was almost negligible. Grabicova et al. [65] also used the post-extraction spiking method to assess the matrix effects of 74 drugs in five different fish tissues (liver, kidney, brain, muscle and plasma), and the results showed that the ME was various in different tissues. Tissues with higher lipid content (liver, kidney and brain) were more affected by the matrix, suppressing 50–60% of the response signal. Signal enhancement occurred mostly in muscle and plasma, and matrix signal suppression effects were mostly seen in other tissues. Matrix-matched calibration solution calibration factors were used to calculate analyte concentrations in Grabicova's study. Miossec et al. [68] evaluated the ME of 42 veterinary drugs in four matrices (cod, red mullet, flounder and shrimp). The majority of compounds have matrix suppression effects, while erythromycin A has greater matrix enhancement. Different compounds also have certain differences in different

matrices, red mullet has a stronger matrix suppression effect than the other three. Since not every compound had access to its suitable isotopic internal standard, the matrix-matched calibration method was used to compensate for ME. Kim et al. [74] optimized the chromatographic separation gradient and used the isotope internal standard calibration method to compensate the influence of ME of four nitrofurans metabolites. In summary, ME seriously affects the analytical results of LC/MS/MS methods, thus, it is critical to overcome or reduce the effect of ME as much as possible. There are some useful methods to reduce or eliminate ME, including optimizing the sample preparation method [37,75], chromatographic mass spectrometry conditions and parameters [76] and the quantitative calibration method [65,68], especially the isotope dilution mass spectrometry method (IDMS) calibration method [74,77]. With the increasing use of mass spectrometry in the field of analytical chemistry, IDMS is playing an increasingly important role due to its greater accuracy than other calibration methods and its ability to compensate for matrix effects. However, the IDMS method also has some disadvantages, including the cost and availability of suitable isotopic materials, and differences in the physical and chemical properties between the analyte and the isotopic analogue, which can affect the ions generated in the mass spectrometer. Optimizing the sample preparation method is also the most effective way to reduce or eliminate ME, because this method could essentially reduce the matrix in the sample.

5. Antibiotic Food Matrix CRMs

Animal-derived foods such as aquaculture products have high protein and fat content, which have complex matrix interference, thus, matrix-certified reference materials (CRMs) are important for quality control in daily laboratory testing. Up to 2021, China has released a total of 22 matrix CRMs for antibiotic residue analysis (Table S3), involving five types of matrices: fish, honey, chicken, milk powder and egg. The target substances include: nitrofurans metabolites, quinolones, amphenicol, sulfonamide, nitroimidazole. Other countries have also released some related matrix CRMs, for instance, a nitrofurans marker residue in freeze-dried shrimp (MX012A, MXB12B) issued by the National Metrology Institute of Australia (NMIA, Canberra in Australia). The Korea Research Institute of Standards and Science (KRISS) released CRMs of enrofloxacin residues in chicken meal (108-03-003 (130708)) and ciprofloxacin residues in chicken meal (108-03-004 (130715)). The National Research Council Canada (NRC, Ottawa in Canada) issued CRM of veterinary drug residue in bovine (A33-11-02-BOTS). In summary, there are relatively many studies on antibiotic matrix reference materials in China, covering typical matrixes and target substances. However, the current quantity is far from meeting the quality control requirements of antibiotics in aquaculture products.

6. Conclusions

Advanced chemical analysis technology is essential for the development of food analysis. At present, LC/MS/MS technology is widely used for antibiotic detection in aquaculture products. Meanwhile, the trend is shifting towards multiresidue and multi-class detection. Considering the different properties of antibiotics, a suitable pre-treatment method is the key to improving the detection limit of the high-throughput analysis method. The ME should be observed when MS is used. Therefore, the preparation of new materials for sample pre-treatment, the assessment and elimination of matrix effects, the development of matrix CRMs and the combined use of on-line solid-phase extraction and liquid chromatography mass spectrometry are still the main hotspots for trace detection of antibiotics in aquaculture products.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/separations9020035/s1>, Table S1: Maximum Residue Limits ($\mu\text{g}\cdot\text{kg}^{-1}$) of antibiotics in aquaculture products in various countries, Table S2: Standard methods for the detection of antibiotics in aquaculture products in China, Table S3: Matrix certified reference materials for antibiotic in different countries.

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