

Review

Recent Advances in Monitoring Microbial Toxins in Food Samples by HPLC-Based Techniques: A Review

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Abstract: This study examines the significant impact of bacterial, algal, and fungal toxins on foodborne illnesses, and stresses the importance of advanced detection techniques, such as high-performance liquid chromatography (HPLC)-based methodologies. It emphasizes the urgent need for further advancements in these techniques to ensure food safety, as they offer significant benefits, including low detection limits and the ability to be combined with other techniques to detect a wide range of toxins. In this regard, HPLC has emerged as a versatile and sensitive analytical technique for this purpose. Various HPLC methods, often enhanced with detectors such as ultraviolet (UV), fluorescence (FD), and mass spectrometry (MS), have been developed to identify and quantify microbial toxins in a wide variety of food samples. Recent advancements include HPLC-FD methods that utilize the natural fluorescence of certain aflatoxins, improving detection sensitivity. HPLC-MS/MS and UHPLC-MS/MS techniques offer high selectivity and sensitivity, making them suitable for detecting a wide range of toxins in trace quantities. The adaptability of HPLC, combined with innovative detection technologies and sample preparation methods, holds significant potential for enhancing food safety monitoring and reducing the global burden of foodborne diseases.

Keywords: microbial toxins; phycotoxins; mycotoxins; endotoxins; exotoxins; cyanotoxins; HPLC techniques; food intoxication



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1. Introduction

Globally, it is estimated that nearly 600 million people (almost 1 in 10) get sick from eating contaminated food each year, leading to 420,000 deaths. This results in the loss of 33 million healthy life years and a staggering \$110 billion annually in productivity and medical expenses, especially in low- and middle-income countries due to unsafe food consumption [1]. Foodborne diseases are divided into foodborne infections and foodborne intoxications. The first is caused by ingesting food contaminated with microorganisms that cause illnesses (pathogens), which then multiply and cause infection. On the other hand, foodborne intoxication, or food poisoning, as it is commonly known, occurs when food containing toxins released by pathogens is consumed, leading to illness [2]. In particular, the biggest concern is that microbial toxins are recognized as heat-resistant, thus

persisting even after standard cooking methods, making food safety control more complex in developing nations [3,4]. These toxins are generated during the metabolic processes of microbes, mainly fungi, bacteria, and species of algae. Environmental conditions during the collection, processing, transportation, or storage of food can promote the production of these toxins, leading to product contamination that affects human health [5].

The most common pathogens and their toxins include the following:

Fungi produce toxins known as mycotoxins, which are secondary metabolites [6]. They are characterized to be heat-resistant to typical cooking temperatures (100 to 210 °C) and times (under 60 min) [7] causing severe illnesses, such as ergotism, gastrointestinal disturbances and damage, and esophageal and liver cancer [8]. They are classified based on their characteristics, including chemical structure (e.g., indole alkaloids, lactones, a furanocoumarin, coumarins, etc.), biosynthesis route (e.g., polyketides pathway, amino acid-derived), or biological effects (e.g., nephrotoxin, teratogens, mutagens, carcinogens, and allergens) [9]. The most common mycotoxins include citrinin (*Aspergillus terreus*, *A. niveus*, *A. oryzae*, *Penicillium camemberti*), ergot alkaloids (species of *Claviceps*), fumonisins (*Fusarium proliferatum*, *F. nygamai*, *F. verticillioides*), zearalenones (*Fusarium graminearum*, *F. culmorum*, *F. equiseti*, etc.), patulin (*Penicillium griseofulvum*, *P. expansum*) [8], ochratoxin A (*Aspergillus niger*, *A. alliaceus*, *A. melleus*, etc) [9], and the well-known aflatoxins (*Aspergillus flavus*, *A. bombycis*, *A. ochraceoroseus*) [10].

Bacteria produce endotoxins or exotoxins, molecules that help the survival of the bacteria by weakening its host and enhancing colonization [11]. The toxins are transported through the bloodstream or lymphatic system causing various symptoms, such as fever, diarrhea, and cardiovascular disorders, which alter the immune and nervous systems in the host [4]. Exotoxins are released outside the bacteria and are classified as type I (or superantigens), type II (or membrane-disrupting toxins), and type III (or A-B toxins). Type I toxins consist of lipopolysaccharides and lipoteichoic acid molecules that cause a high immune reaction, leading to a shock in the host [12]. Type II corresponds to phagocytes or enzymes (phospholipases and hemolysins) that participate in the lyses of the host cells [11]. Finally, type III is based on two subunits (polypeptide chains), composed of an enzyme (subunit A) and its binding substrate (subunit B) that binds to receptors on the human cell membrane and inactivates it [11]. Exotoxins are associated with specific diseases like botulism, tetanus, and diphtheria. Some examples of bacteria that can produce toxins include *Bacillus* spp., *Clostridium* spp., *Staphylococcus aureus*, *Campylobacter* spp., *Salmonella* spp., *Escherichia coli*, and *Listeria* spp. [13]. Meanwhile, endotoxins consist of lipopolysaccharides and phospholipids located in the outer of the cell wall of Gram-negative bacteria, surrounding their peptidoglycan layer [12]. When the macrophages provoke bacterial lysis, it causes a release of these endotoxins [11]. For example, when the lipid A component is released upon bacterial cell lysis, it results in symptoms like fever, diarrhea, weakness, and blood coagulation [14].

Algae, which includes certain types of microalgae (phytoplankton and seaweeds), is responsible for the production of phycotoxins, which are commonly related to harmful algal blooms (HABs) events [15]. This phenomenon is characterized by the proliferation of toxic microalgae, frequently during temperate seasons, which can last up to months [15]. The most prevalent phycotoxins include saxitoxins, okadaic and domoic acids, azaspiracids, spirolides, pinnatoxins, gymnodimines, palytoxins, ciguatoxins, brevetoxins, and tetrodotoxins [16]. These algae toxins provoke a significant number of intoxications each year when consuming contaminated fish, resulting from cutaneous irritation or diarrhea to respiratory, gastrointestinal, or neurological illness [17]. In particular, some acute illnesses related to phycotoxins are paralytic shellfish poisoning (PSP, related to *Alexandrium*, *Gymnodinium*, and *Pyrodinium* species that produce saxitoxins), neurotoxic shellfish poisoning (NSP, associated to brevetoxins of *Karenia brevis*), diarrhetic shellfish poisoning (DSP, related to genera *Dinophysis* and *Prorocentrum* that produced okadaic acid), Ciguatera fish poisoning (CFP, caused by ciguatoxins from *Gambierdiscus* species.), and amnesic shellfish poisoning (ASP, related to domoic acid produce by *Nitzschia* species) [18].

Other microalgal species related to toxins production are *Azadinium* and *Amphidoma* spp. in azaspiracids that cause azaspiracid poisoning (AZP), *Ostreopsis* spp. that produces palytoxins, *Alexandrium ostenfeldii* and *A. peruvianum* that releases spirolides or *Protoceratium*, *Gonyaulax* and *Lingulodinium* species that produce yessotoxins [19].

Cyanobacteria are a type of photosynthetic bacteria found in water bodies worldwide. When cyanobacteria die, they release toxins into the water (cyanotoxins), which can cause harm to humans, animals, and aquatic organisms [20]. These cyanotoxins include microcystins, cylindrospermopsins, anatoxins, cyanopeptolins, nodularins, anabaenopeptins, and saxitoxins, depending on the cyanobacteria responsible for their released [21]. The most common pathogenic cyanobacteria include *Anabaena*, *Nostoc*, *Hapalosiphon*, *Fischerella*, *Anabaenopsis*, *Aphanizomenon*, *Gloeotrichia*, *Cylindrospermopsis*, *Scytonema*, *Raphidiopsis*, *Cuspidothrix*, *Nodularia*, *Stigonema*, *Calothrix*, *Cylindrospermum*, and *Desmonostoc* [22]. These toxins can accumulate in the food chain and can be introduced into humans through the consumption of contaminated fish, posing significant health risks such as liver cancer, gastrointestinal damage, and neurotoxic effects [23].

Regardless of the group of microorganisms the toxin comes from, it commonly reaches humans through various foodborne mechanisms (reviewed in [24]), including the following:

- Direct contact can occur due to contaminated hands of food handlers or infected animals and contaminated surfaces;
- Cross-contamination can occur due to pathogens transferred from raw foods or utensils used without proper cleaning, as well as to improper storage;
- Water contamination can occur by irrigating crops with contaminated water or using contaminated water during food processing, washing, or cooking;
- Airborne transmission can occur by spreading pathogens through the air in dust particles, water droplets, or poorly maintained or contaminated ventilation systems.
- Soil contamination can be caused by naturally present pathogens or by using untreated manure as fertilizers;
- Human factors, such as poor hygiene or sickness of food handlers, which can transmit pathogens to food through coughing, sneezing, or direct contact; Pests, which can include insects and rodents;
- Contaminated ingredients that could become contaminated during the supply chain.

Regulations have been established to legislate the presence of these biotoxins worldwide to address the public health issues caused by them. For example, the European Commission Directive (2003/100/EC) established 0.005–0.02 mg Kg⁻¹ of aflatoxins B1 as a maximum concentration in feed [25]. In terms of cyanotoxins, the Ministry of Health of Brazil established in the Portaria N° 2.914, 12 December 2011, the concentration of 1.0 µg L⁻¹ for microcystins and 3.0 µg L⁻¹ for saxitoxin [26]; the National Resource Management Ministerial Council of Australia reported in Australian Drinking Water Guidelines a permitted concentration of 1.3 µg L⁻¹ of microcystin LR [27]. Meanwhile, the Ministry of Health of Italy and South Africa established in the “Decreto legislativo 31/2001” [28] and “Water Services Act, 1997” [29], respectively, a concentration of 0.8 µg/L for microcystin LR. Finally, the Ministry of Health of China (Standards for Drinking Water Quality) [30], Spain (Real Decreto 140/2003) [31], and Japan (Waterworks Act) [32] reported a limit of 1.0 µg L⁻¹ of microcystin LR.

Therefore, significant research has been conducted to develop analytical methods to detect the presence of biotoxins, encompassing immunochemical and chromatographic techniques [33]. Methods based on chromatography have been mostly standardized. In particular, gas chromatography (GC), high-performance liquid chromatography (HPLC), enzyme-linked immunosorbent assay (ELISA), and bioassays [33] have been widely reported. However, there are also emerging techniques, such as biosensors, PCR [34], and Next-Generation Sequencing (NGS) [35].

Among these methods, HPLC stands out as a modern form of liquid chromatography that utilizes small-particle columns through which the mobile phase is pumped at high pressure. This versatile analytical technique extensively analyzes pharmaceuticals,

biomolecules, polymers, and numerous organic and ionic compounds [15]. Depending on the characteristics of the analyte, HPLC can be combined with other techniques or employed with different detectors to overcome challenges in detecting analytes. Some of the most prevalent detectors that can be coupled to HPLC include ultraviolet (UV) and fluorescence detectors (FL), with the latter being particularly sensitive [36]. Furthermore, the integration of HPLC with immunoaffinity processes can improve properties such as the recovery percentage to purify the samples [37]. Additionally, mass spectrometry (MS) can significantly enhance the method's sensitivity, allowing for detecting analytes at very low concentrations [38]. Thanks to these properties, the HPLC methodologies implemented for detecting and quantifying microbial toxins have proven to achieve incredibly low limits of detection (LOD) and limits of quantification (LOQ).

This research will concentrate on methodologies that utilize HPLC to identify microbial toxins in food samples from 2020 to the present. Figure 1 summarizes the newly developed HPLC-based approaches for identifying fungal and microbial toxins.

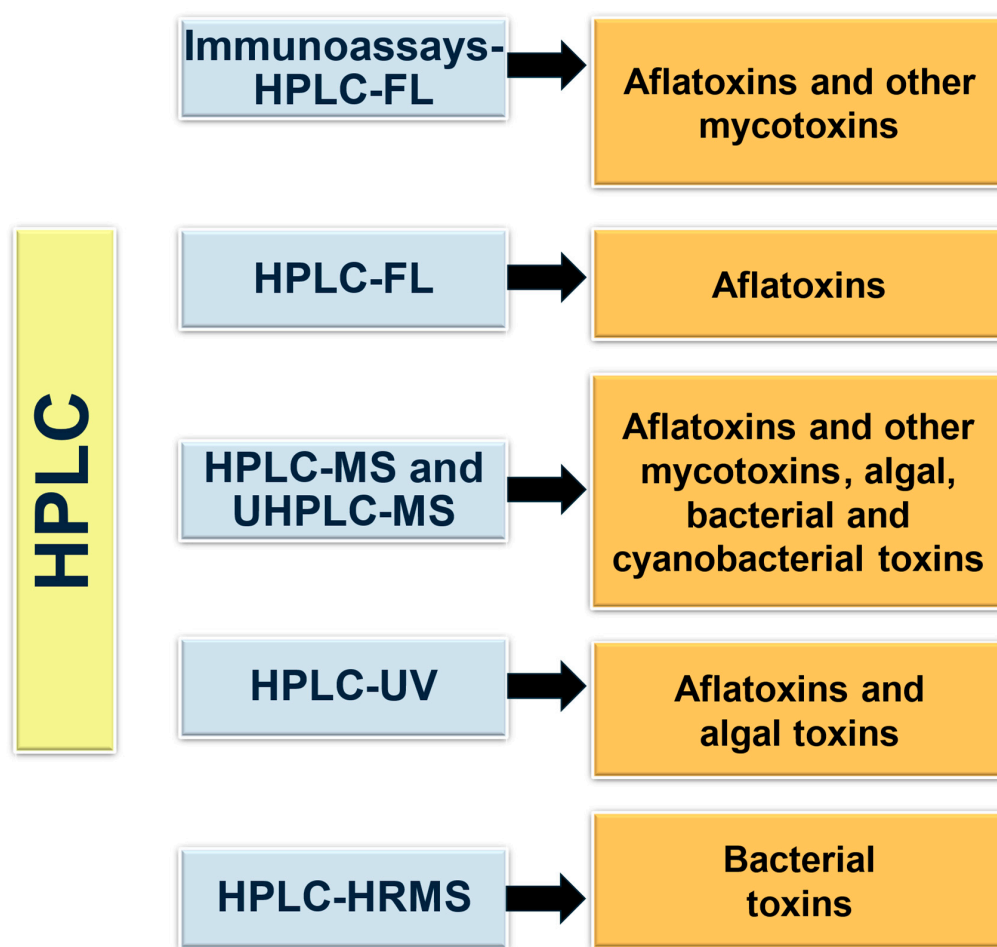


Figure 1. HPLC-based techniques for the detection of fungal and microbial toxins in food samples.

2. HPLC-Based Methodologies for Microbial Toxin Detection in Food Samples

2.1. Aflatoxins and Other Mycotoxins

Numerous species of fungi have been found to produce over 300 mycotoxins, including penicillic acid, botryodiplodin, chaetoglobosin, communesin, and penitrem [39]. One of the most well-known types of mycotoxin is aflatoxins, which are primarily produced by *Aspergillus parasiticus* and *Aspergillus flavus* but are also known to be produced by other fungal genera, such as *Penicillium* and *Fusarium* [40]. These mycotoxins can be found on crops and are commonly found in improperly stored foods. It is estimated that mycotoxins contaminate one-fourth of the world's crops during growth or storage [41]. Additionally,

aflatoxins can contaminate processed foods like meat [40]. Aflatoxins have low solubility in non-polar solvents but are freely soluble in moderately polar organic solvents like chloroform and methanol, with a solubility in water ranging from 10 to 20 mg L⁻¹ [42]. Although there are approximately 20 derivatives of aflatoxins, types B₁, B₂, G₁, and G₂ are the most prevalent natural toxins found in food and animal feed [43].

Traditionally, aflatoxins have been linked to liver cancer and bile duct hyperplasia [44]. However, exposure to these mycotoxins has also been associated with the development of cancer in the kidney, pancreas, bladder, bone, and other organs. Aflatoxins can also cause lung and skin cancers through inhalation and direct contact. Chronic exposure to aflatoxins can lead to immunosuppression, teratogenicity, mutagenicity, cytotoxicity, and estrogenic effects in mammals. Additionally, they are believed to contribute to nutritional disorders like kwashiorkor and growth faltering by interfering with the absorption of micronutrients, protein synthesis, and metabolic enzyme activities [45]. The Panel on Contaminants in the Food Chain (CONTAM Panel) has established a limit of 78 ng kg⁻¹ of body weight per day to minimize the extra cancer risk to 1% [42]. The European Union has also set maximum permissible limits for various crops, such as 4.0 µg kg⁻¹ for wheat, 5.0 µg kg⁻¹ for coffee beans, 5.0 µg kg⁻¹ for corn, and 4.0 µg kg⁻¹ for dried figs [46,47]. Regarding Ochratoxin A, another major mycotoxin, the maximum permissible concentration has been established at 5 µg kg⁻¹ [47].

As seen in Table 1, most of the current methods developed for mycotoxins are focused on aflatoxins, with the most used column for HPLC being C-18, despite aflatoxins being insoluble in non-polar solvents, except chloroform [48]. Various authors proposed different solvent combinations as mobile phases, including acetonitrile, water, ethanol, acetic acid, toluene, formic acid, and ammonium nitrate, to modify the retention time. In some cases, a gradient mixture was necessary for proper separation. Fluorescence detection (FD) was the most used for aflatoxins and other mycotoxins. The analytical parameters such as LOD, LOQ, and linear range will be discussed in the following subsections. All the recoveries in the validated methods were found to be acceptable according to the American Society of Chemistry, which considers a recovery a range from 80 to 120% and standard deviations less than or equal to ± 20% at each fortification level [49].

Table 1. HPLC-based techniques for detecting fungal toxins (mycotoxins) in food and their analytical parameters.

Fungal Toxins (Mycotoxins)	Sample Type and Preparation	HPLC-Based Technique and Chromatographic Conditions	Analytical Parameters	Permissible Limits
Aflatoxins B ₁ , B ₂ , G ₁ , and G ₂ [50]	Peanut butter 12.5 g of locally made peanut butter samples were combined with 2.5 g of NaCl, and 62.5 mL of 70% methanol was blended and then filtered using a No. 1 Whatman filter. Subsequently, 30 mL of water was added to 15 mL of the filtrate. After this, 15 mL were passed through a solid-phase extraction cartridge and rinsed with 10 mL water. Aflatoxins were eluted with 1 mL of ethanol and then 1 mL of water	HPLC-UV Aflatoxins were separated with a C-18 column with a mobile phase of 40:50:10 methanol-water-acetonitrile solution. The UV detection was carried out at 365 nm, with a flow rate of 0.7 mL min ⁻¹ . The standards for aflatoxins G ₂ , G ₁ , B ₂ , and B ₁ had 1, 4, 1, and 4 µg mL ⁻¹ concentrations, respectively.	Not validated method Total aflatoxin content ranged between 373.6–6741.6 µg kg ⁻¹ , and aflatoxin B ₁ content was 54.3–805.8 µg kg ⁻¹ [50]	Sum of B ₁ , B ₂ , G ₁ , and G ₂ in peanuts: 15 µg kg ⁻¹ [47] Sum of B ₁ , B ₂ , G ₁ , and G ₂ in processed peanuts: 4 µg kg ⁻¹ [47]

Table 1. Cont.

Fungal Toxins (Mycotoxins)	Sample Type and Preparation	HPLC-Based Technique and Chromatographic Conditions	Analytical Parameters	Permissible Limits
Aflatoxins B ₁ , B ₂ , G ₁ , and G ₂ , and M ₁ [51]	Rice and flour 5 g of powdered sample was dispersed in 10 mL 70:30 methanol-water and sonicated for 20 min. The supernatant was completed to 45 mL with water.	HPLC-FD A C18 column (150 × 4.6 mm; 5 µm) was used at 40 °C. A sample size of 25 µL was injected, and the mobile phase consisted of 65% methanol and 35% water, with a flow rate of 1.2 mL min^{−1}.	Validated method LOD: 0.009–0.015 ng mL^{−1} Linearity: 0.05–8 ng mL^{−1}	
	Milk 20 mL samples were centrifuged at 5000 rpm for 15 min, filtered with a 0.22 µm syringe filter, and diluted with 25 mL of deionized water. A MOF-based structure was selected as adsorbent in the extraction and pre-concentration of aflatoxins			
Aflatoxins B ₁ , B ₂ , G ₁ , and G ₂ [52]	Merkén spice Sample processing not specified.	HPLC-FD A HPLC-FLD with post-column derivation with a Kobra electrochemical cell (R-Biopharm Rhone, Glasgow, UK) was used.	Validated method LOD: 0.3 ng g^{−1} LOQ: 1.0 ng g^{−1} Linearity: 0.6–6.2 ng g^{−1} (B₂ and G₂), and 1.96–20 ng g^{−1}	Sum of B₁, B₂, G₁, and G₂ for cereals: 4 µg kg^{−1} [47] Sum of B₁, B₂, G₁, and G₂ for mixture of spices: 10 µg kg^{−1} [47] Aflatoxin M₁ in milk: 0.05 µg kg^{−1} [47]
Aflatoxins B ₁ , B ₂ , M ₁ , and M ₂ [53]	Milk 2.5 mL of sample were defatted by centrifugation at 4000 rpm for 10 min. The sample was then vortex-mixed for 3 min and passed through an in-syringe dispersive micro-solid phase extraction (ISDµSPE) as a sample pretreatment.	HPLC-FD A C18 column Hypersil gold (250 × 4.6 mm; 5 µm) was used at 40°C. The sample size was 25 µL, and the flow rate was 1.2 mL min^{−1}. A gradient was applied using a mobile phase consisting of a mix of 13:74:13 methanol-water-acetonitrile for 6 min, followed by 2 min of a 20:60:20 mix. A 9-min hold followed this, then the initial conditions were restored for 2 min and held until 35 min. The fluorescence detector was set at 360 and 440 nm for excitation and emission wavelengths, respectively.	Validated method LOD: 0.003–0.005 ng mL^{−1} LOQ: 0.01–0.02 ng mL^{−1} Linearity: 0.01–1.0 ng mL^{−1} for Aflatoxins M₁, M₂, and B₂, and 0.02–1.0 ng mL^{−1} for Aflatoxin B₁	

Table 1. Cont.

Fungal Toxins (Mycotoxins)	Sample Type and Preparation	HPLC-Based Technique and Chromatographic Conditions	Analytical Parameters	Permissible Limits
Aflatoxins M ₁ [54]	Raw milk Samples were filtrated with cellulose nitrate 0.45 µm membrane filters and centrifuged. The supernatant was filtered with 13 mm × 0.2 µm microfilters.	HPLC-FD A C18 column (250 × 4.6 mm; 5 µm) and immunoaffinity columns were used to isolate Aflatoxins M ₁ . A fluorescence detector at 365 and 430 nm was used with a flow rate of 1 mL min ^{−1} at room temperature. The mobile phases used were isocratic: (A) methanol, (B) acetonitrile, and (C) water.	Validated method LOD: 11.99 ng kg ^{−1} and 16.95 ng kg ^{−1} for aflatoxins M ₁ and M ₂ , respectively. Linearity: 0.6–6.2 ng g ^{−1} (B ₂ and G ₂), and 1.96–20 ng g ^{−1} (B ₁ , and G ₁)	
Aflatoxins B ₁ , B ₂ , G ₁ , and G ₂ . Ochratoxin A [55]	Wheat 50 g of sample were extracted with 100 mL of an 8:2 methanol-water mixture and then filtered. Subsequently, 10 mL of filtrate was diluted with 10 mL of phosphate buffer solution (PBS).	HPLC-FD For aflatoxins, an Inertsil ODS-3C C-18 column (250 × 4.6 mm; 5 µm) was utilized at 40 °C. The sample volume was 100 µL, with a flow rate of 1 mL min ^{−1} and a mobile phase consisting of a 6:2:3 water-acetonitrile-acetic acid mix. Post-column derivatization involved using 350 µL of 4M nitric acid and 120 mg of potassium bromide for every 1000 mL mobile phase. Detection occurred via fluorescence at wavelengths 333 and 460 nm. For Ochratoxin A, a mobile phase comprising 47:51:2 acetonitrile-water-acetic acid was employed.	Validated method LOD: 0.014–0.030 µg kg ^{−1} LOQ: 0.045–0.098 µg kg ^{−1}	Sum of B ₁ , B ₂ , G ₁ , and G ₂ for cereals: 4 µg kg ^{−1} [47] Sum of B ₁ , B ₂ , G ₁ , and G ₂ in maize: 10 µg kg ^{−1} [47] All foods ready for human consumption: not exceed 10 µg kg ^{−1} of aflatoxin, of which aflatoxin B ₁ shall not be more than 5 µg kg ^{−1} [56] Ochratoxin A in unprocessed cereals: 5 µg kg ^{−1} [47]
Aflatoxins B ₁ , B ₂ , G ₁ , and G ₂ . Ochratoxin A [40]	Processed meat 10 g of minced samples were mixed with 40 mL of a 60:40 acetonitrile-water mix and 0.2 g of NaCl. Then, 4 mL of the mixture were diluted in 44 mL of 2% tween-20-PBS. After that, 0.5 mL of the treated sample was mixed with 0.5 mL of acetonitrile for the cleanup process.	HPLC-FD 100 µL of extract was injected into a C-18 Thermo LC-Si column (250 × 4.6 mm) at 40 °C. The column had a fluorescence detector set at 365 and 435 nm. The mobile phase, consisting of toluene, ethyl acetate, formic acid, and methanol in a 90:5:2.5:2.5, was delivered at a flow rate of 2 mL min ^{−1} .	Validated method LOD for aflatoxin B ₁ : 16.5–26.6 µg kg ^{−1} LOD for ochratoxins: 3.8–17 µg kg ^{−1} Linearity: 0.1–20 µg kg ^{−1}	

Table 1. Cont.

Fungal Toxins (Mycotoxins)	Sample Type and Preparation	HPLC-Based Technique and Chromatographic Conditions	Analytical Parameters	Permissible Limits
Aflatoxins B ₁ , B ₂ , G ₁ , and G ₂ [46]	<u>Wheat, corn, dried fig, or dried coffee beans</u> 5 g of ground sample were mixed with 80% ethanol, filtered, and diluted in phosphate buffer solution (PBS).	HPLC-FD A sample of 50 mL was injected into Aflaclean™ immunoaffinity columns at a flow rate of 0.5 mL min ⁻¹ . The mobile phase consisted of a 60:30:10 water-methanol-acetonitrile solution. Detection was carried out on a UV-Vis at 365 nm.	Validated method LOD: 0.02–0.10 µg kg ⁻¹ LOQ: 0.04–0.45 µg kg ⁻¹	
		HPLC-FD A 40 µL sample was injected into a silica column (LC-Si, 5 µm, 25 cm × 4.6 cm) heated to 30°C at a flow rate of 1.5 mL min ⁻¹ , using a mobile phase consisting of a 1:1 mixture of solution A (toluene-ethyl acetate-methanol 45:3:2 mL) and solution B (toluene-ethyl acetate-formic acid 45:3:2 mL).	Validated method LOQ: 2.0, 0.5, 1.0, and 0.5 ng g ⁻¹ for aflatoxins B ₁ , B ₂ , G ₁ , and G ₂ , respectively	Sum of B ₁ , B ₂ , G ₁ , and G ₂ in corn and dried figs: 10 µg kg ⁻¹ [47] Sum of B ₁ , B ₂ , G ₁ , and G ₂ in peanuts: 15 µg kg ⁻¹ [47] Sum of B ₁ , B ₂ , G ₁ , and G ₂ for cereals: 4 µg kg ⁻¹ [47]; Sum of B ₁ , B ₂ , G ₁ , and G ₂ for processed ground peanuts: 4 µg kg ⁻¹ [47]
		HPLC-FD IMMUNOPREP cleanup cartridges and a C18 column (150 × 4.6 mm; 5.0 µm) were utilized, along with a fluorescence detector set at 362 and 455 nm and a flow rate of 1.8 mL min ⁻¹ . A gradient of solution A (24:52:21 acetonitrile-methanol-water) and solution B (80:20 methanol-water) was employed. The sample size was 1000 µL, and the process was conducted at 30 °C.	Validated method LOQ for peanut, sorghum, rice, and flattened rice: 0.125 ng g ⁻¹ . LOQ for peanut butter, almond, and wheat-based cookies: 0.5 ng g ⁻¹ . Linearity: 0.0125–10 ng g ⁻¹	
Aflatoxins B ₁ , B ₂ , G ₁ , and G ₂ [57]	<u>Raw peanuts</u> 25 g of peanuts were blended with 125 mL solution containing 70% methanol, 30% water, and 5.0 g of NaCl. The mixture was then filtered and passed through an Aflatest® IAC column.			
Aflatoxins B ₁ , B ₂ , G ₁ , and G ₂ [58]	<u>Rice, flattened rice, sorghum, raw and processed peanut, almond, peanut butter, or wheat-based cookies</u> 12.5 g of peanut samples + 12.5 g of water were mixed. 25 g of other samples were extracted with 80% methanol.			

Table 1. Cont.

Fungal Toxins (Mycotoxins)	Sample Type and Preparation	HPLC-Based Technique and Chromatographic Conditions	Analytical Parameters	Permissible Limits
Aflatoxins B ₁ , B ₂ , G ₁ , and G ₂ [59]	Peanut kernel 50 g of ground sample were mixed with 5 g of NaCl, 200 mL of 80:20 ethanol-water, and 100 mL of n-hexane for 30 min. The mixture was then filtered, and 20 mL of the sample was diluted with 130 mL of deionized water.	HPLC-FD 100 µL of the sample was injected into a reverse-phase C18 column (25 cm × 4.6 mm; 5 µm) at 40°C. The mobile phase consisted of water-methanol-acetonitrile (6:3:2) with 120 µL of potassium bromide and 350 µL of 4 N nitric acid. The flow rate was set at 1 mL min ^{−1} with FARLIB® used as the post-column derivatization cell. A fluorescence detector was employed at 352 nm and 435 nm.	Validated method LOD: 0.06–0.15 µg kg ^{−1} LOQ: 0.20–0.50 µg kg ^{−1} Linearity: 0.4–7.2, 0.08–1.44, 0.4–7.2, and 0.08–1.44 µg L ^{−1} for aflatoxins B ₁ , B ₂ , G ₁ , and G ₂ , respectively	Sum of B ₁ , B ₂ , G ₁ , and G ₂ in corn and dried figs: 10 µg kg ^{−1} [47] Sum of B ₁ , B ₂ , G ₁ , and G ₂ in peanuts: 15 µg kg ^{−1} [47] Sum of B ₁ , B ₂ , G ₁ , and G ₂ for cereals: 4 µg kg ^{−1} [47]; Sum of B ₁ , B ₂ , G ₁ , and G ₂ for processed ground peanuts: 4 µg kg ^{−1} [47]
		HPLC-MS and HPLC-MS/MS HPLC method not available. A G1315B diode array detector with 500 nL and 10 mm pathlength coupled to a single quadrupole mass spectrometer with an electrospray ionization source was used. An ESI mode was chosen for instrument performance. The capillary voltage was set at 3000 V, and the analyte ionization voltage was 150 eV. N ₂ was employed as nebulizing gas at 35 psig with a drying flow of 12 L min ^{−1} at 350 °C.	Validated method LOD: 0.0188–0.1250 µg kg ^{−1} LOQ: 0.037567–0.3750 µg kg ^{−1} Linearity: 0.375–20 µg kg ^{−1}	Sum of B ₁ , B ₂ , G ₁ , and G ₂ in rice: 10 µg kg ^{−1} [47] Sum of B ₁ , B ₂ , G ₁ , and G ₂ for cereals: 4 µg kg ^{−1} [47] All foods ready for human consumption should not exceed 10 µg of aflatoxin, of which aflatoxin B ₁ shall not be more than 5 µg kg ^{−1} [56] Ochratoxin A in unprocessed cereals: 5 µg kg ^{−1} [47]
Fungal toxins (mycotoxins)	Sample type and preparation	HPLC-based Technique and chromatographic conditions	Analytical parameters	Permissible limits
Aflatoxins B ₁ , B ₂ , G ₁ , and G ₂ [60]	Rice Samples were treated with a magnetic covalent organic framework.	HPLC-MS and HPLC-MS/MS HPLC method not available. A G1315B diode array detector with 500 nL and 10 mm pathlength coupled to a single quadrupole mass spectrometer with an electrospray ionization source was used. An ESI mode was chosen for instrument performance. The capillary voltage was set at 3000 V, and the analyte ionization voltage was 150 eV. N ₂ was employed as nebulizing gas at 35 psig with a drying flow of 12 L min ^{−1} at 350 °C.	Validated method LOD: 0.0188–0.1250 µg kg ^{−1} LOQ: 0.037567–0.3750 µg kg ^{−1} Linearity: 0.375–20 µg kg ^{−1}	Sum of B ₁ , B ₂ , G ₁ , and G ₂ in rice: 10 µg kg ^{−1} [47] Sum of B ₁ , B ₂ , G ₁ , and G ₂ for cereals: 4 µg kg ^{−1} [47] All foods ready for human consumption should not exceed 10 µg of aflatoxin, of which aflatoxin B ₁ shall not be more than 5 µg kg ^{−1} [56] Ochratoxin A in unprocessed cereals: 5 µg kg ^{−1} [47]

Table 1. Cont.

Fungal Toxins (Mycotoxins)	Sample Type and Preparation	HPLC-Based Technique and Chromatographic Conditions	Analytical Parameters	Permissible Limits
Aflatoxins B ₁ , B ₂ , G ₁ , and G ₂ [37]	Commercial rice 20 g of ground sample were spiked at 1.43 µg kg⁻¹, and then 100 mL of an 80:20 methanol-water solution was added. The mixture was stirred at 950 rpm for 1 h. After sedimentation, the supernatant was centrifuged at 5000 rpm for 45 min. Next, 7 mL of the treated sample was diluted with 43 mL PBS and filtered through a syringe filter.	HPLC-MS and HPLC-MS/MS A C18 column was used (15 × 0.3 mm; 4 µm). The sample size was 10 µL, with a flow rate of 10 µL min⁻¹. The column was coupled to a diode array and a single quadrupole mass spectrometer. The mobile phase comprised a mixture of formic acid 0.05% and acetonitrile. Diode array and mass spectrometer detectors (cHPLC-DAD/MS) Model 1100 Series, (Agilent Technologies, Madrid, Spain) were used. Also, a G1315B diode array detector (Agilent Technologies, Madrid, Spain) with 500 nL and 10 mm pathlength coupled to a single quadrupole mass spectrometer with an electrospray ionization source (Model 6120 Series, Agilent Technologies) were used. Positive ESI mode was used, the capillary voltage was set at 3000 V, and analyte ionization voltage at 150 eV. N₂ was employed as nebulizing gas at 35 psig with a drying flow of 12 Lmin⁻¹ at 350 °C.		
			Validated method LOD: 0.0967–0.3267 µg kg⁻¹ LOQ: 0.3167–1.0667 µg kg⁻¹ Linearity: 1–25 µg L⁻¹ for all analytes	Sum of B₁, B₂, G₁, and G₂ in rice: 10 µg kg⁻¹ [47] Sum of B₁, B₂, G₁, and G₂ for cereals: 4 µg kg⁻¹ [47] All foods ready for human consumption should not exceed 10 µg of aflatoxin, of which aflatoxin B₁ shall not be more than 5 µg kg⁻¹ [56] Ochratoxin A in unprocessed cereals: 5 µg kg⁻¹ [47]

Table 1. Cont.

Fungal Toxins (Mycotoxins)	Sample Type and Preparation	HPLC-Based Technique and Chromatographic Conditions	Analytical Parameters	Permissible Limits
Aflatoxins B ₁ , B ₂ , G ₁ , and G ₂ [61]	Cultured fish Fish muscle and liver samples were extracted by an ultrasound-assisted extraction procedure using a 60:40 acetonitrile–0.1 M KH ₂ PO ₄ aqueous buffer (pH 6.0) mixture.	HPLC-MS and HPLC-MS/MS Sample size: 20 µL, flow rate of 60 µL min ^{−1} , mobile phase: 0.1:99.9 formic acid–methanol. A 3200 Q TRAP LC/MS/MS (ABSciex, Concord, Canada) with an electrospray ionization source was used, ions spray voltage (IS), 5500 kV; ion source temperature of 300 °C; N ₂ as nebulizer gas and curtain gas, 40 psi; N ₂ as collision gas.	Validated method LOD: 0.2967–0.61 µg kg ^{−1} [61]	
Aflatoxins B ₁ , B ₂ , G ₁ , and G ₂ . Ochratoxin A and B. Enniatins A, B, A ₁ , and B ₁ [62]	Maize Samples were treated with a magnetic covalent organic framework.	HPLC-MS and HPLC-MS/MS A C18 column was used (100 × 3 mm; 1.8 µm) at 40 °C. The sample size was 2 µL and detected through a triple-quadrupole mass spectrometer. Mobile phase: gradient mixture of Solvent A (100% acetonitrile) and B (0.1:99.9 formic acid–5 mmol L ^{−1} ammonium formate), flow rate of 0.4 mL min ^{−1} . An Agilent 6460 triple-quadrupole mass spectrometer equipped with an electrospray ionization source was used. Mode, positive multiple reaction monitoring (MRM+); drying gas temperature of 300 °C; sheath gas temperature of 250 °C; drying gas flow rate of 5 L min ^{−1} ; sheath gas flow rate, 10 L min ^{−1} ; nebulizer pressure of 45 psi; capillary voltage of 3500 V; and nozzle voltage of 0 V. High-purity N ₂ was used as the drying gas.	Validated method LOD: 0.0267–1.67 µg kg ^{−1} LOQ: 0.07–5.57 µg kg ^{−1} Linearity: 0.05–20 µg kg ^{−1}	Sum of B ₁ , B ₂ , G ₁ , and G ₂ in rice: 10 µg kg ^{−1} [47] Sum of B ₁ , B ₂ , G ₁ , and G ₂ for cereals: 4 µg kg ^{−1} [47] All foods ready for human consumption should not exceed 10 µg of aflatoxin, of which aflatoxin B ₁ shall not be more than 5 µg kg ^{−1} [56] Ochratoxin A in unprocessed cereals: 5 µg kg ^{−1} [47]

Acronyms used in Table 1: **HPLC-UV**: high-performance liquid chromatography with ultraviolet detection; **HPLC-FD**: high-performance liquid chromatography with fluorescence detection; **HPLC-MS**: high-performance liquid chromatography with mass spectrometry detection; **HPLC-MS/MS**: high-performance liquid chromatography with tandem mass spectrometry detection; **HPLC-HMRS**: high-performance liquid chromatography with high-resolution mass spectrometry detection; **LOD**: limit of detection; **LOQ**: limit of quantification.

2.1.1. HPLC-UV

Mycotoxin UV detection has been historically discussed in the literature [63]. However, this methodology is not widespread in routine analyses as it is not sufficiently sensitive to reach trace levels [64]. For example, Uzeh and Adebawale [50] analyzed to identify aflatoxins B₁, B₂, G₁, and G₂ in locally processed peanut butter. They discovered that aflatoxin B₁ was present in all samples, while aflatoxins B₂, G₂, and G₁ were found in 71.43%, 85.71%, and 57.14% of the samples. The total aflatoxin content in the peanut butter samples ranged from 373.6 to 6741.6 µg kg⁻¹, exceeding the maximum permissible limit of 20 µg kg⁻¹ recommended by the (USFDA; [65]), as well as the limit of ≤ 4 µg kg⁻¹ set by the European Union (EU). Additionally, the content of aflatoxin B₁ ranged from 54.3 to 805.8 µg kg⁻¹, surpassing the EU limit of 2 µg kg⁻¹ [66]. In another study, the concentration of mycotoxins (deoxynivalenol, HT-2 toxin, T-2 toxin, and ochratoxin A) was determined by high-performance liquid chromatography (HPLC) with a UV detector in samples from bee pollen, propolis, and honey. The concentrations detected were between 1.601 and 0.704 µg/kg⁻¹ [67].

2.1.2. HPLC-FD

The natural fluorescence traits of B-group and G-group aflatoxins have been used to develop detection and differentiation methods. B-group aflatoxins exhibit blue under UV light, while the G-group displays yellow-green fluorescence. As a result, fluorescence detectors in HPLC (HPLC-FD) methodologies have been extensively explored for aflatoxin detection. For example, in a study by Algammal et al., [40] aflatoxin B₁ was found in concentrations ranging from 16.5 to 26.6 µg kg⁻¹, while ochratoxins were present in concentrations ranging from 3.8 to 17 µg kg⁻¹ [40]. Turksoy and Kabak [55] also employed HPLC with a fluorescence detector to detect aflatoxins B₁, B₂, G₁, G₂, and ochratoxin A in wheat samples. Aflatoxins B₁ and B₂ were detected in samples as low as 0.35 and 0.094 µg kg⁻¹, respectively. None of the samples in this study contained aflatoxin G₁ and aflatoxin G₂. For ochratoxins, levels were detected from 3.8 to 17 µg kg⁻¹. The LOD ranged from 0.014 µg to 0.030 µg kg⁻¹, and the LOQ ranged from 0.045 to 0.098 µg kg⁻¹ [55]. Maggira et al. analyzed raw milk samples to detect aflatoxins M₁ and M₂ using an HPLC-FD method in a separate study. The method had LODs of 11.99 and 16.95 ng g⁻¹, Intraday recovery of 90 to 100% and 91 to 119%, and RSD < 2% and < 6.1% for aflatoxins M₁ and M₂, respectively [54].

Sample pretreatment is often advantageous for purifying and concentrating an analyte while reducing interferences from complex matrices [68]. In this sense, solid phase extraction has been commonly used before qualitative and quantitative aflatoxin measurements. For example, Shuib and Shab [53] determined aflatoxins M₁, M₂, B₁, and B₂ in milk using a new sample pretreatment technique, in-syringe dispersive micro-solid phase extraction, coupled with HPLC-FD. They achieved a LOD from 0.003 to 0.005 ng mL⁻¹, LOQ from 0.01 to 0.02 ng mL⁻¹, recoveries from 73.0 to 109.6%, with relative standard deviations (RSDs) less than 17.3% [53]. Similarly, Aliluo et al. [51] developed a fluorometric method for rapidly screening aflatoxins B₁, B₂, G₁, and M₁ after their magnetic MOF-808/graphene oxide composite extraction. This method monitored the total aflatoxins content of food samples with a linear range of 0.05 to 8 ng mL⁻¹, with a LOD ranging from 0.009 to 0.015 ng mL⁻¹ with RSD ranging from 2.9 to 4.2% [51]. Similarly, it has been reported that specificity and sensitivity are greatly enhanced when combining immunoaffinity extractions with HPLC-FD [69]. For instance, Omar et al. [46] developed an Enzyme-Linked Immunosorbent Assay (ELISA) for quantifying total aflatoxins in food samples [46]. They analyzed wheat, corn, dried fig, and dried coffee beans and found average values ranging from 1.14 to 4.12 µg kg⁻¹, with LODs from 0.02 to 0.10 µg kg⁻¹ and LOQs from 0.04 to 0.45 µg kg⁻¹. Another study conducted by Shen and Singh [57] determined aflatoxins in raw peanuts using an immunoaffinity column as a sample clean-up method followed by normal-phase HPLC-FD analysis. They achieved a LOQ of 2.0 ng g⁻¹, 0.5 ng g⁻¹, 1.0 ng g⁻¹, and 0.5 ng g⁻¹ of aflatoxin B₁, B₂, G₁, and G₂ of peanut, respectively, with lin-

earities (R^2) of the four types of aflatoxin greater than 0.999 and inter-day recovery rates at LOQ of 109%, 115%, 113%, and 77%, respectively [57]. Additionally, Dhanshetty et al. [58] developed a method with Automated Immunoaffinity Cleanup and Inline HPLC–FD to detect aflatoxins B₁, B₂, G₁, and G₂ in various samples. The LOQ was 0.125 ng g^{−1} for peanut, sorghum, rice, and flattened rice, while it was 0.5 ng g^{−1} for peanut butter, almond, and wheat-based cookies [58]. All this goes accord to the standard method for the detection and quantification of aflatoxins B₁, B₂, G₁, and G₂, which was verified by Aliakbarzadeh et al. [59], who carried out an immunoaffinity purification followed by HPLC–FD according to requirements of ISO/IEC 17025. In this study, they achieved LODs from 0.06 to 0.15 µg kg^{−1}, LOQs from 0.20 to 0.50 µg kg^{−1}, average recoveries of 81.2 to 83.4%, and a repeatability of 4.99 to 5.89% [59].

Several studies have highlighted the necessity of post-column derivatization to enhance the inherent fluorescence properties of aflatoxins, thereby improving the HPLC–FD method's sensitivity. For instance, Palma et al. analyzed the spice merken for quantifying aflatoxins B₁, B₂, G₁, and G₂ by HPLC–FD with post-column derivatization. Post-column derivatization showed significant enhancement of the sensitivity of B₂–G₂ (4-fold) and B₁–G₁ (2-fold) when compared with that of pre-column derivatization (Figure 1). They achieved excellent linearity across concentration ranges of 0.6 to 6.2 ng g^{−1} for aflatoxins B₂ and G₂, and 1.96 to 20 ng g^{−1} for aflatoxins B₁ and G₁. The LODs and LOQs for B₁, B₂, G₁, and G₂ were calculated at 0.3 and 1.0 ng g^{−1}, respectively. The recoveries varied from 71% for G₂ to 86% for B₁ [52], demonstrating that HPLC–FD is a suitable tool for detecting aflatoxins in food and beverage samples at low concentrations [54].

2.1.3. Novel Technologies: HPLC-MS/MS, LC-HRMS, and UPLC-MS/MS

Due to the innovations in chromatographic technologies, HPLC with tandem Mass Spectrometry (HPLC-MS/MS) has become the common method for determining aflatoxins in food because of its high selectivity, sensitivity, and reproducibility. In this sense, Wei et al. conducted rapid extraction of aflatoxins B₁, B₂, M₁, and M₂, ochratoxins A and B, and enniatins A, B, A₁, and B₁ from maize using magnetic covalent organic framework before an analysis with an HPLC-MS/MS detection. This approach yielded good linearity within a range of 0.05 to 50 µg kg^{−1}, high sensitivity (LOD of 0.02 to 1.67 µg kg^{−1}), and satisfactory recoveries ranging from 73.8 to 105.3%, with relative standard deviations of less than 8.5% [62]. In a similar methodology, Wang et al. [60] analyzed aflatoxins B₁, B₂, G₁, and G₂ in rice samples. Their study demonstrated good linearity in the concentration range of 0.375 to 20 µg kg^{−1}, an LOD of 0.0188 to 0.1250 µg kg^{−1}, and an LOQ of 0.0375 to 0.3750 µg kg^{−1}. The average recovery values for the four aflatoxins ranged from 85.1% to 111.0% [60]. In a related study, Romero-Sánchez et al. [37] conducted a comprehensive analysis of aflatoxins B₁, B₂, G₁, and G₂ in commercial rice using immunoaffinity column clean-up before HPLC-MS/MS. They achieved recovery values of 86 to 92%, standard deviations between 5 and 11%, LOD values between 0.09 and 0.32 µg kg^{−1}, and LOQs between 0.31 and 1.06 µg kg^{−1} [37]. In another study, Jayasinghe et al. [61] developed a miniaturized vortex-assisted dispersive molecularly imprinted polymer micro-solid phase extraction and HPLC-MS/MS for detecting aflatoxins in cultured fish. They achieved a LOD ranging from 0.29 to 0.61 µg kg^{−1}, with intraday and inter-day relative standard deviations lower than 20% and analytical recoveries within the 80% to 100% range for all aflatoxins [61]. Wang et al. [70] analyzed aflatoxins B₁, B₂, G₁, and G₂ with pretreatment with nanofiber-packed solid-phase extraction in samples of peanuts, white fruits, badam, cashew nuts, and various fodder and animal tissues and simultaneously determined with HPLC-MS/MS. The authors achieved LODs ranging from 0.07 to 0.17 ng g^{−1}, intra-day and inter-day RSDs for spiked samples of 1.3 to 8.0% and 1.9 to 5.8%, and recoveries from 60.1 to 98.4% [70]. On the other hand, a UPLC-MS/MS method was used to simultaneously analyze the aflatoxin (B₁, B₂, G₁, G₂), fumonisins (B₁, B₂), deoxynivalenol, zearalenone, ochratoxin A, T-2, and HT-2 in cereal samples (e.g., rice, corn, wheat). The employed extraction solvent was a mixture of phosphate buffer solution (PBS) and 70% methanol.

The recovery percentages ranged between 70% and 120%, and the limit of quantification (LOQ) was found to be between 0.5 and 20 $\mu\text{g kg}^{-1}$ [71]. UHPLC-HRMS analysis was utilized to simultaneously detect up to 24 mycotoxins in coix seeds, which are an important food in China. The mycotoxins include zearalenone, deoxynivalenol, nivalenol, fumonisin B1, B2, B3, aflatoxin B1, sterigmatocystin, and tenuazonic acid. The recovery percentages ranged from 74.2% to 101.1%, and the limits of quantification (LOQ) ranged from 0.5 to 100 $\mu\text{g kg}^{-1}$ [72].

2.2. Bacterial Toxins

Bacterial toxins have a significant impact on public health, with over 90 percent of food poisoning cases each year attributed to *Staphylococcus aureus*, *Salmonella*, *Clostridium perfringens*, *Campylobacter*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Bacillus cereus*, and Enteropathogenic *Escherichia coli* [73]. Despite bacterial food poisoning being the primary cause in more than 80% of cases, there are only a few recent HPLC-based techniques available for detecting bacterial toxins [74]. *Staphylococcus aureus*-induced food poisoning is a major global foodborne disease, particularly in milk and dairy products due to the production of one or more enterotoxins [75]. *Clostridium botulinum* and *Staphylococcus aureus* are the primary bacteria responsible for food intoxication, causing botulism and staphylococcal intoxication, respectively, while *Clostridium perfringens* and *Bacillus cereus* are also common bacteria responsible for food poisoning [76]. Additionally, fatal bacterial toxins such as bongkrekic acid and isobongkrekic acid are produced by the aerobic Gram-negative bacteria *Burkholderia gladioli* pathovar *coccovenans*, posing a serious health risk [77]. Unlike mycotoxins and algal toxins, there is currently a lack of recent research utilizing HPLC for the detection of bacterial toxins in food samples, presenting an opportunity for the development of new HPLC-based methodologies. Although there are no regulations for the maximum permissible limit of many bacterial toxins, the fatal dose of bongkrekic acid for humans, which has been established at a range between 1.0 to 1.5 mg orally, could serve as a reference value [78].

It is worth noting that bacterial toxins are typically assessed using advanced and effective detection methods. These methods encompass cytotoxicity assays in Vero or HeLa cell tissue cultures, enzyme-linked immunosorbent assays (ELISA), reverse passive latex agglutination (RPLA), genetic probes, and PCR assays [79]. However, as shown in Table 2, most studies on bacterial toxins detection have focused on chromatographic techniques, all of which utilize C-18 columns. These columns have proven their ability to separate various toxins using various mobile phases and mixtures, with formic acid, water, methanol, and acetonitrile being the most used solvents. Almost all the methods described in Table 2 have been validated, and all the validated methods have an LOD lower than the permissible limits, making them suitable for detecting bacterial toxins in food samples. Detailed analytical parameters are described below. Notably, the recoveries ranged from 70 to 110%, an acceptable range for the validation of organic analysis [80]. The American Society of Chemistry considers a recovery a range from 80 to 120% and standard deviations less than or equal to $\pm 20\%$ at each fortification level as good [49]. Therefore, the validated methods are suitable for analyzing toxins in food samples (see Table 2).

Table 2. HPLC-based techniques for detecting bacterial toxins in food and their analytical parameters.

Bacterial Toxins	Sample Type and Preparation	HPLC-Based Technique and Chromatographic Conditions	Analytical Parameters	Permissible Limits
Toxoflavin and ferverulin [81]	Rice bran oil, sweet potato starch, distiller's yeast, <i>Tremella fuciformis</i> Berk, rice noodles, and fermented corn flour 1.0 g of rice bran oil was extracted twice with 6 mL of methanol. The mixture was then centrifuged at 10,000 rpm for 5 min. The other samples (were weighed and extracted in an ultrasonic bath for 10 min with 10 mL of extraction solvent. For <i>Tremella fuciformis</i> Berk and distiller's yeast, methanol was used as the extraction solvent, while sweet potato starch, rice noodle, and fermented corn flour were extracted using 0.1:99.9 formic acid–water. These mixtures were then centrifuged at 10,000 rpm for 10 min. Subsequently, all samples were filtered and loaded into SPE cartridges. 6 different SPE cartridges and 6 solvents were tried, with the Oasis HLB having the best recovery, using methanol.	UHPLC-MS/MS A C18 column (150 × 2.1 mm; 5 µm) was used at 35 °C with a mobile phase consisting of 0.1% formic acid (<i>v/v</i>, solvent A) and methanol (solvent B). The flow rate was 0.4 mL/min. Gradient elution was programmed as follows: 0 min, 12% B; 1.00 min, 12% B; 2.50 min, 90% B; 5.00 min, 90% B; 5.01 min, 12% B; 10.00 min, 12% B. The injection volume was 5 µL. An Agilent 6460 triple quadrupole mass spectrometry (Yishun, Singapore) was used. The mass spectrometry was operated in the multiple reaction monitoring (MRM) mode with positive electrospray ionization (ESI+). Ion source conditions were as follows: N₂ as drying gas, temperature of 350 °C; drying gas (N₂) flow rate of 11 L min^{−1}; nebulizer gas pressure, 3.4 × 10⁵ Pa; capillary voltage, 4000 V.	Validated method LOD: 12–60 µg kg^{−1} LOQ: 40–200 µg kg^{−1} Linearity for toxoflavin: 20–1000 µg L^{−1} Linearity for ferverulin: 40–1000 µg L^{−1}	Not specified.
	Rice noodles A 2.0 g sample was mixed with 20 mL of acidic water (pH 4.4) and sonicated for 20 min. The sample solution was then filtered. Subsequently, 81 mg of magnetic nanotubes were added to the filtrate and vortex-mixed for 4.2 min. The magnetic nanotubes were isolated using a strong magnet, and the supernatant was discarded. The magnetic sorbent was eluted thrice with 0.5 mL of acetonitrile containing 1% formic acid. The eluate was collected and dried using a nitrogen stream at 40 °C. The residue was diluted to 1.0 mL with a 50% acetonitrile aqueous solution and filtered using a 0.22 µm filter.	HPLC-Orbitrap HRMS technology with magnetic halloysite nanotubes A C18 column (150 × 2.1 mm; 2.6 µm) was used at 35 °C. The mobile phase was used in a gradient elution program consisting of a 0.1% formic acid water solution (A) and acetonitrile (B). The gradient elution program was as follows: 0–3 min, 50% B to 70% B; 3–3.6 min, 70% B to 95% B; 3.6–5.5 min, 95% B; 5.5–5.6 min, 95% B to 50% B; 5.6–7.0 min, 50% B. The flow rate was 0.3 mL min^{−1}, and the injection volume was 5 µL. An HPLC-Orbitrap HRMS (Thermo Fisher Scientific, Germany) was used. Flow rates of sheath gas, auxiliary gas, and sweep gas were set at 45, 8, and 0 arbitrary units, respectively. The spray voltage was set at −3.0 kV. Mass range (<i>m/z</i>) was 100–600. The capillary and auxiliary gas heater temperatures were 320 °C and 350 °C, respectively.	Validated method LOD: 0.3 µg kg^{−1} LOQ: 1.0 µg kg^{−1} Linearity: 2–200 µg L^{−1}	Bongkreik acid: fatal for humans at 1.0–1.5 mg orally [78]

Acronyms used in Table 2: **UHPLC-MS/MS**: ultra-high-performance liquid chromatography with tandem mass spectrometry detection; **HPLC-HMRS**: high-performance liquid chromatography with high-resolution mass spectrometry detection; **LOD**: limit of detection; **LOQ**: limit of quantification.

2.2.1. HPLC-UV and HPLC-HRMS

Liquid chromatography with UV detection (LC-UV) has been reported for quantifying heat-labile enterotoxins from *E. coli* cultures. This method exhibited a limit of detection (LOD) of $15 \mu\text{g L}^{-1}$ and a limit of quantification (LOQ) of $45 \mu\text{g L}^{-1}$ [82]. Meanwhile, in a study by Liang et al. [77], rice noodle samples were analyzed to detect bongkrekic and isobongkrekic acids (mitochondrial toxins) using HPLC-Orbitrap HRMS (High-Resolution Mass Spectrometry) technology with magnetic halloysite nanotubes. The method demonstrated linearity in the concentration range of 2 to $200 \mu\text{g L}^{-1}$, with a correlation coefficient more significant than 0.998, LOD and LOQ of $0.3 \mu\text{g}$ and $1.0 \mu\text{g kg}^{-1}$, respectively [77].

2.2.2. UHPLC-MS/MS and Nano-LC-MS/MS

Ultra-High Performance Liquid Chromatography-tandem Mass Spectrometry (UHPLC-MS/MS) has been utilized to identify toxoflavin and fervenulin in various food products such as rice bran oil, sweet potato starch, distiller's yeast, *Tremella fuciformis* Berk, rice noodles, and fermented corn flour. Wang et al. [81] established the LOD for toxoflavin to be $12 \mu\text{g kg}^{-1}$ and for fervenulin, $24 \mu\text{g kg}^{-1}$. The recoveries ranged from 70.1 to 108.7%, while the intra-day and inter-day RSDs ranged from 0.9 to 9.5% [81]. It is important to note that technology in this field is constantly advancing. The Health Protection Agency (HPA) and Thermo Fisher Scientific worked together to develop a new mass spectrometry technique for simultaneously characterizing microorganism proteomes using nano-LC-MS/MS. This technique provides high resolution, even better than MALDI-TOF-MS detection, allowing for the differentiation of similar peptide sequences among closely related strains. This research successfully identified specific markers of the *E. coli* strain O104:H4 in clinical samples from patients during an outbreak [83].

Both UHPLC-MS/MS and HPLC-HRMS techniques demonstrated LODs and LOQs in the order of $\mu\text{g kg}^{-1}$, significantly lower than the fatal dose of bongkrekic acid for humans, which has been established at a range between 1.0 to 1.5 mg orally [50]. Therefore, these techniques could be effectively employed to detect bacterial toxins with high accuracy.

2.3. Cyanotoxins and Harmful Algal Blooms Toxins

Certain species of cyanobacteria can produce marine toxins [21]. These cyanotoxins can be classified as (a) hepatotoxins (cylindrospermopsins, microcystins, and nodularins), (b) neurotoxins (anatoxins and saxitoxins), (c) dermatotoxins, (d) cytotoxins, and (e) irritant toxins (lipopolysaccharide) [84]. According to regulations, the maximum permissible limit for the cyanobacterial toxin microcystin is $1 \mu\text{g g}^{-1}$ [43]. Sánchez-Parra et al. [85] analyzed food supplements containing microalgae using HPLC-MS to identify the presence of cyanotoxins such as microcystin-LR and anatoxin-A. They observed that the concentration of microcystin-LR in *Chlorella* samples was under the LOD (not specified), while in Klamath and *Spirulina* samples, it was $0.008 \mu\text{g g}^{-1}$, and in Upper Klamath Algae it was $0.002 \mu\text{g g}^{-1}$. As for anatoxin-A, the concentration was $0.034 \mu\text{g g}^{-1}$ for *Chlorella*, $0.025 \mu\text{g g}^{-1}$ for Klamath and *Spirulina*, and $0.002 \mu\text{g g}^{-1}$ for Upper Klamath Algae [85]. Another study by Fontaine et al. [86] involved the analysis of 37 samples of algal dietary supplements (*Spirulina*, *Aphanizomenon flos-aquae*, *Chlorella*, and kelp) to detect 27 cyanotoxins using HPLC-MS and HPLC-HRMS. They detected microcystins in all 8 *Aphanizomenon* samples, with levels reaching up to 1000 ng g^{-1} of dried weight, as well as 2, 4-diaminobutyric acid in all samples, with values ranging from 3 to 1600 ng g^{-1} . Additionally, they did not detect anatoxin-A nor β -amino-N-methylalanine [86]. The analytical parameters of the HPLC-MS techniques suggest that their LODs and LOQs are significantly lower than the reference values set by regulations, making them suitable for the detection of cyanobacterial toxins, such as microcystin, in food samples.

On the other hand, algal biotoxins are natural toxic metabolites that are typically produced during harmful algal blooms. These toxins accumulate in marine organisms and move up the food chain. They are produced as secondary metabolites to give their producers a competitive advantage against similar species or to defend against preda-

tors [16]. Additionally, these algae can be filtered by marine organisms, such as mussels, clams, oysters, and scallops, causing an accumulation of the so-called shellfish toxins, with most being produced by marine dinoflagellates and some by marine diatoms [87]. Primary contact with algal biotoxins can be through contact with seawater, inhalation of airborne substances, or consumption of toxins accumulated in the food chain. These toxins can cause intoxication with neurological and gastrointestinal effects, along with other diseases. However, seafood poisoning can be categorized into diarrhetic, neurotoxic, amnesic, and paralyzing pathologies [88]. Some of the marine toxins produced by algae include okadaic acid, dinophysistoxins, azaspiracid 1, 2, and 3, pectenotoxin 2, and yessotoxin [89]. It is important to have reliable methodologies for monitoring these compounds, and research has shown that HPLC has been used for this purpose.

In the past, the detection of marine toxins was carried out using mousse assays, but now there are more alternatives such as immunoassays, liquid chromatography (LC), HPLC, surface plasmon resonance biosensors, electrochemical, planar waveguide, and LFD cassette reader [90]. As presented in this review, the HPLC technique is versatile and can be combined with many other techniques. There are regulations for various algal biotoxins. For example, the permissible limit for okadaic acid, dinophysis toxins, and pecteno-toxins combined is 160 mg of okadaic acid equivalents per kg. For yessotoxins, the limit is 1 mg of YTX equivalents per kg. For azaspiracid, the maximum limit is 160 µg per kg. The maximum permissible limit for domoic acid is 20 µg per g of tissue [91], and for paralytic shellfish toxins, it is 80 mg STX eq/100 g [92]. Table 3 summarizes the HPLC-based techniques for detecting algal and cyanobacterial toxins (cyanotoxins) in food along with their analytical parameters.

Table 3. HPLC-based techniques for detecting algal and cyanobacterial toxins (cyanotoxins) in food and their analytical parameters.

Algal and Cyanobacterial Toxins (Cyanotoxins)	Sample Type and Preparation	HPLC-Based Technique and Chromatographic Conditions	Analytical Parameters	Permissible Limits
Cyanobacteria Microcystin-LR and anatoxin-A [85]	Food supplements containing microalgae The samples were extracted three times using 2.5 mL of a 75:25 methanol-water mixture at 60 °C. After extraction, the samples were dried in a Speedvac and reconstituted in 900 mL of methanol. Then, the reconstituted samples were transferred to 2 mL Eppendorf vials equipped with a cellulose-acetate filter (Corning Costar Spin-X centrifuge tube filters) and centrifuged for 5 min at 16,000× g.	HPLC-MS 20 mL of sample were loaded onto a C18 column (150 × 4.6 mm; 5 mm). The mobile phase consisted of water with 0.1% formic acid as eluent A and acetonitrile with 0.1% formic acid as eluent B. The elution program was as follows: 0–2 min at 30% B, a linear increase of B between 2 and 6 min, 6–12 min at 90% B, and a 5-min post-run at 30% B. The flow rate was maintained at 0.5 mL min ^{−1} , and the procedure was conducted at 40 °C. A hybrid mass spectrophotometer Agilent Q-TOF 6550, with an ionization source JetStream electrospray + i-Funnel. MS operated in the positive mode and N ₂ was used as the drying and collision gas. The quadrupole was operated in the unit mode and four spectra/s were recorded.	Not validated method Concentrations of toxins in samples from 0.002 ± 0.0001 µg g ^{−1} – 0.034 ± 0.002 µg g ^{−1}	Myrocistin: 1 µg g ^{−1} [93]

Table 3. Cont.

Algal and Cyanobacterial Toxins (Cyanotoxins)	Sample Type and Preparation	HPLC-Based Technique and Chromatographic Conditions	Analytical Parameters	Permissible Limits
Cyanobacteria 27 cyanotoxins [86]	<u>Spirulina, Aphanizomenon flos-aquae, Chlorella, and kelp algal dietary supplement samples</u> The samples were extracted twice with 5 mL of methanol + 0.1 M formic acid. The mixture was vortexed for 30 s and then placed in an ultrasonic bath for 15 min. After that, the supernatant was collected following centrifugation at 6000 rpm for 10 min. The supernatants were then treated with 100 mg of graphitized carbon black for clean-up. The resulting mixture was filtered using PES (0.2 µm) and evaporated at 45 °C. Subsequently, the samples were homogenized in 3 mL of water for 5 min in an ultrasonic bath. An aliquot was filtered using PES (0.2 µm). Finally, an oxidation step was performed using 340 µL potassium permanganate 0.4 M, 380 µL sodium periodate 0.35 M, and potassium carbonate 1 M to adjust the pH to 9 [86]	<u>HPLC-MS and HPLC-HRMS</u> 1 mL of sample was applied to a C18 online SPE column (20 × 2.1 mm; 12 µm), and then transferred to a Hypersil TM Gold C18 HPLC column (100 × 2.1 mm; 1.9 µm). For individual microcystins: UHPLC-HRMS, Thermo Q-Exactive Orbitrap was used. A heated electrospray ionization interface (HESI-II) operated in positive mode was used for analyte ionization. The ionization spray voltage was set at +3500 V; capillary temperature was set at 350 °C; the vaporizer temperature was set at 250 °C; sheath gas and auxiliary gas flow were set at 60 and 15 arbitrary units, respectively. For total microcystins: UHPLC-MS/MS, Thermo TSQ Quantiva was used. An SPE column Hypersyl Gold aQ C18 (20 × 2.1 mm; 12 µm) was used, and the analytical column Hypersil Gold C18 (50 mm × 2.1 mm, 1.9 µm particle size)	<u>Validated method</u> LOD: 0.1–35 ng g ^{−1} LOQ: 0.3–105 ng g ^{−1} Linearity: 10–1000 ng g ^{−1}	Microcystin: 1 µg g ^{−1} [93]
Algae Paralytic shellfish toxins [94]	<u>Stomach content and liver samples from dead kelp gulls, Magellanic penguins, Papua penguins, and imperial cormorants, along with zooplankton, squat lobsters, Fuegian sprat, and seabirds</u> Samples from DGL TM ** were extracted with cold 0.04 M acetic acid and sonicated 1 min at 4 s intervals. Samples were pre-treated with C18 SPE columns activated with methanol. [94]	<u>HPLC-UV</u> A C18 column (25 cm × 4.6 mm; 5–10 µm), at 40 °C, rate flow of 1.0–1.5 mL min ^{−1} . The mobile phase was a mixture of 100:899:1 acetonitrile-water-trifluoroacetic acid.	<u>Not validated method</u> LOD: 400 µg Kg ^{−1} LOQ: 3–8 pmol mL ^{−1}	For paralytic shellfish toxins: 80 mg STX eq/100 g [92]
Algae Okadaic acid [95]	<u>Shellfish</u> 0.9 mL of methanol was added to the samples, vortex-mixed, and sonicated. The resulting supernatants were centrifuged at 8000 rpm for 5 min. The extracts were then adjusted to a volume of 2 mL. Next, 2.5 M sodium hydroxide was added, and the mixture was incubated at 70 °C for 1 h. After cooling, an equivalent amount of hydrochloric acid was added, and the solution was filtered using a 0.22 µm filter. Finally, 6 mL of methanol and 6 mL of water were added.	<u>HPLC-UV</u> A C18 column with a UV-vis detector was used at 200 nm, with a flow rate of 2 mL min ^{−1} , an injection volume of 20 µL, and a mobile phase 35:65 (water-acetonitrile).	<u>Validated method</u> LOD: 4.55 × 10 ^{−3} ng mL ^{−1} [95]	Okadaic acid, dinophys toxins, and pectenotoxins together: 160 mg OA equivalents kg ^{−1} [92]

Table 3. Cont.

Algal and Cyanobacterial Toxins (Cyanotoxins)	Sample Type and Preparation	HPLC-Based Technique and Chromatographic Conditions	Analytical Parameters	Permissible Limits
Algae Domoic acid [96]	Mollusks 4 g of shredded and homogenized samples were mixed with 16 mL of solvent extraction (1:1 methanol-water solution), homogenized for 3 min at 10,000 rpm, then centrifuged for 10 min at 4000 rpm.	HPLC-UV A C18 column (250 × 4.6 mm; 5 µm) was used with a flow rate of 1 mL min ⁻¹ , a detection wavelength of 242 nm, an injection volume of 20 µL, and an oven temperature for the column of 40 °C.	Validated method LOD: 0.05 mg kg ⁻¹	Domoic acid: 20 µg g ⁻¹ of tissue [91]
Algae Okadaic acid, yessotoxin, pectenotoxin, and azaspiracid [97]	Processed food Deionized water was added (30% of the weighted sample), and 5 mL of the sample was diluted with 5 mL of ultrapure water.	HPLC-MS and UHPLC-MS The sample was loaded into an SPE cartridge and eluted with 2 mL of methanol and 3% ammonium hydroxide. Later, it was passed through a C18 column (100 × 2.1 mm; 1.7 µm) with detection by MS/MS at 40 °C. The injection volume was 5 µL with a flow rate of 0.2 mL min ⁻¹ . Different gradients of solution A (water) and B (acetonitrile), both with 0.04% v/v of ammonium hydroxide, were used, starting at B 20%, then B 85%, B 98%, and finally B 20%. A triple quadrupole mass spectrometer TSQ-Endura (Thermo Fisher Scientific, Waltham, MA, USA), equipped with a heated electrospray source (H-ESI II) operating in both positive (ESI+) and negative mode (ESI−). N ₂ was used as sheath and auxiliary gas, while Argon was used as collision gas. The optimized parameters were: capillary voltage (3500 V in ESI+ and 2700 V in ESI−), sheath gas flow rate (30 arbitrary units), auxiliary gas flow rate (10 arbitrary units), ion transfer tube temperature (270 °C), vaporizer temperature (240 °C) and collision gas pressure (2.5 mTorr).	Validated method LOQ: 3–8 µg kg ⁻¹	Okadaic acid, dinophysis toxins and pectenotoxins together: 160 mg Okadaic Acid (OA) equivalents kg ⁻¹ [92] Yessotoxins: 1 mg YTX equivalents kg ⁻¹ [92] Azaspiracid: 160 µg kg ⁻¹ [92]

Table 3. Cont.

Algal and Cyanobacterial Toxins (Cyanotoxins)	Sample Type and Preparation	HPLC-Based Technique and Chromatographic Conditions	Analytical Parameters	Permissible Limits
Algae Cyclic imine analogues, spirolide, gymnodimine, and pinnatoxin groups [98]	Mussel and oysters 1 g samples were extracted using 9 mL of methanol, vortex-mixed for 3 min, and centrifuged at $3400\times g$ for 8 min at 20 °C. The resulting supernatants were then filtered through a 0.2 µm nylon filter.	HPLC-MS and UHPLC-MS A C18 column (50×2.1 mm; 1.7 µm) was used at 30 °C with an injection volume of 2 µL and flow rates of 0.4–0.6 mL min ^{−1} . Various mobile phases were used, and 1 mM ammonium fluoride in methanol had the best signal intensity. A Xevo-TQ-S triple quadrupole mass spectrometer (MS/MS) with electrospray ionization was used, with a 150 °C source temperature, 600 °C desolvation temperature, 1100 L/Hr desolvation gas flow, 150 L/Hr cone gas flow and a 1.0 kV capillary voltage.	Validated method LOD: 0.01–16.5 µg kg ^{−1} LOQ: 0.03–55 µg kg ^{−1} Linearity: 0.1–80 µg kg ^{−1} , 0.1–40 µg kg ^{−1} , 0.4–40 µg kg ^{−1} , 0.1–20 µg kg ^{−1} , and 1–320 µg kg ^{−1} for PnTx-E and PnTx-F, PnTx-G and 20-Me-SPX-G, GYM-A and 13-desMe-SPX-C, and 12-Me-GYM toxins, respectively	Not regulated [99]
Algae: Phycotoxins and cyanotoxins [100]	Fresh and salt waters: 500 mL samples were acidified to pH 3 with formic acid.	HPLC-MS and UHPLC-MS: Samples were loaded onto an SPE cartridge at a flow rate of 5 mL min ^{−1} . The analytes were eluted using methanol with 1% formic acid. Subsequently, a C18 column (150×2.1 mm; 3.5 µm) preceding a pre-column C18, both maintained at 30 °C, was employed. A gradient was performed using solvent A (water) and solvent B (acetonitrile), containing 0.5% formic acid. The gradient involved a change from 95% A to 2% A with corresponding changes in B, at a flow rate of 0.2 mL min ^{−1} and an injection volume of 10 µL.	LOD: 0.3–29 ng L ^{−1} LOQ: 1–88 ng L ^{−1} Linearity: 2.5–250 µg L ^{−1}	For phycotoxins (domoic and okadaic acids): Okadaic acid, dinophysis toxins and pectenotoxins together: 160 mg OA equivalents kg ^{−1} [92] Domoic acid: 20 µg g ^{−1} of tissue [91] For cyanotoxins (microcystin): 1 µg g ^{−1} [93]
Algae: Azaspiracids, brevetoxins, okadaic acid group, and domoic acid [101]	Mussels: Homogenized samples were extracted with different acetic acid and methanol concentrations. Then, the extracts were loaded in C18 SPE cartridges.	HPLC-MS and UHPLC-MS: An ODS-3 C18 column (150×2.1 mm; 3 µm) was used at 40 °C, with a 2 µL injection volume and a flow rate of 0.2 mL min ^{−1} . The mobile phase comprised 0.1% aqueous formic acid (A) and acetonitrile (B). A linear gradient of 5–60% B, 60–70% B, and 90% B was used to separate lipophilic biotoxins. A 10% solution of B was utilized to analyze domoic acid.	LOD: 0.002–0.017 mg kg ^{−1} LOQ: 0.007–0.058 mg kg ^{−1} Detection range: 0.008–0.2 mg kg ^{−1} to 0.06–6 mg kg ^{−1}	For azaspiracid: 160 µg kg ^{−1} [92] Brevetoxins: not regulated [99] Okadaic acid, dinophysis toxins and pectenotoxins together: 160 mg OA equivalents kg ^{−1} [92] Domoic acid: 20 µg g ^{−1} of tissue [91]

Acronyms used in Table 3: **HPLC-UV**: high-performance liquid chromatography with ultraviolet detection; **HPLC-MS**: high-performance liquid chromatography with mass spectrometry detection; **HPLC-HMRS**: high-performance liquid chromatography with high-resolution mass spectrometry detection; **UHPLC-MS**: ultra-high-performance liquid chromatography with mass spectrometry detection; **LOD**: limit of detection; **LOQ**: limit of quantification.

As presented in Table 3, all authors consistently achieved LODs and LOQs significantly lower than the reference concentrations, typically in the parts per billion and parts per trillion range, making them suitable for detecting algal toxins in food and water samples. It is noteworthy that all the reported studies utilized a column C-18 and employed various mobile phases and combinations, such as acetonitrile, water, trifluoroacetic acid, ammonium fluoride, methanol, and formic acid for separation. Moreover, the validated methods

demonstrated acceptable percent recoveries, following the American Society of Chemistry guidelines, with recovery rates falling within the 80 to 120% range and standard deviations less than or equal to $\pm 20\%$ at each fortification level [49].

2.3.1. HPLC-UV

The HPLC-UV method has proven to be a reliable technique for detecting algal toxins, like domoic acid, with many methods based on the approach developed by Quilliam, several decades ago [102]. For example, the EU-Harmonised Standard Operating Procedure is founded on Quilliam's methodology [103]. Currently, HPLC-UV remains a crucial technique for marine toxin analysis. For instance, Cadaillon et al. examined stomach content and liver samples from deceased kelp gulls, Magellanic and Papua penguins, and imperial cormorants. They found varying levels of paralytic shellfish toxins up to $3427 \mu\text{g kg}^{-1}$ saxitoxins equivalent (STXeq). Additionally, they investigated phytoplankton, zooplankton, squat lobsters, Fuegian sprat, and seabirds, revealing potential toxin transformation across the food web and identifying possible transfer vectors. Concentrations of up to $2707 \mu\text{g STXeq kg}^{-1}$ were observed [94]. In a different study, Qin et al. [95] utilized an immunoassay to analyze shellfish samples for okadaic acid and validated the method using HPLC-UV with a C18 column and detection wavelength of 200 nm. Using this method, they analyzed spiked samples with concentrations of 0.5, 10, and 50 ng mL^{-1} [95]. Likewise, Aboulaalaa et al. [96] analyzed mollusks for domoic acid using HPLC-UV, achieving a detection limit of 0.05 mg kg^{-1} [96]. Like other HPLC techniques, the limits of detection and quantification for HPLC-UV-based methods are considerably lower than the maximum permissible limits for regulated algal biotoxins (refer to Table 1).

2.3.2. HPLC-MS and UHPLC-MS

The HPLC-MS method has proven to be effective in detecting marine toxins produced by algae. For instance, D'Amore et al. utilized ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) to identify biotoxins like okadaic acid, yessotoxin, pectenotoxin, and azaspiracid. They achieved precise results with RSD below 11.8% for each compound, recoveries ranging from 73 to 101%, and LOQs between 3 and $8 \mu\text{g kg}^{-1}$. The method's relative uncertainty ranged from 12 to 20.3% [97]. In another study, O'Neill et al. [98] identified cyclic imine analogs from the spirolide, gymnodimine, and pinnatoxin groups in mussel and oyster samples using a rapid six-minute UHPLC-MS/MS analysis. The LOD ranged from 0.01 to $16.5 \mu\text{g kg}^{-1}$, and the LOQ from $0.03 \mu\text{g kg}^{-1}$ to $55 \mu\text{g kg}^{-1}$ [98]. Similarly, Ochi and Suzuki [101] developed a solid-phase extraction and reversed-phase HPLC-MS methodology to determine azaspiracids, brevetoxins, okadaic acid, and domoic acid in mussels. They obtained good recoveries from 79.0 to 97.6% at three spiking levels for all analytes except brevetoxin-2, which ranged from 43.8 to 49.8% [101]. HPLC-MS/MS has also been used to monitor the water quality, which can be affected by food contamination. In this sense, Merlo et al. [100] determined phycotoxins and cyanotoxins in freshwaters and seawaters using simultaneous pre-concentration and quantification, achieving recoveries of 70 to 118% [100]. As shown in Table 1, all the authors achieved LODs and LOQs much lower than the reference concentrations.

3. Conclusions

HPLC has demonstrated remarkable adaptability to various methodologies and instrumental techniques, making it suitable for detecting a wide range of analytes based on the chosen detector. While bacterial toxins are a significant cause of food poisoning, HPLC techniques primarily focus on aflatoxins and algal toxins, leaving many other toxins unexplored. HPLC-MS has emerged as the primary technique for detecting toxins from algae, bacteria, cyanobacteria, and fungi, while HPLC-FD closely follows. Developing multi-toxin detection methods can be challenging due to the need to select an appropriate extraction solvent and overcome potential matrix effects caused by the complex composition of the samples. Nonetheless, multi-toxin detection methods can be cost-effective and time-saving

when analyzing multiple analytes simultaneously. When combined with techniques such as immunoassays or extraction processes, HPLC exhibits high sensitivity and selectivity, allowing for the analysis and differentiation of various analytes in complex matrices. These techniques have demonstrated LODs and LOQs well below regulated microbial toxin limits, making them suitable for analyzing food samples. It is essential to note that despite the vast array of microbial toxins, only a small fraction has been analyzed using HPLC, indicating the unexploited potential of HPLC for detecting numerous analytes.

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