Chromatographia (2017) 80:9–22
DOI 10.1007/s10337-016-3186-x

REVIEW

Analytical Methods for the Determination of *Alternaria* Mycotoxins

Yan Man[1,2,3] · **Gang Liang**[1,2,3] · **An Li**[1,2,3] · **Ligang Pan**[1,2,3]

Received: 7 April 2016 / Revised: 29 September 2016 / Accepted: 14 October 2016 / Published online: 28 October 2016

Abstract The genus *Alternaria* comprises ubiquitous pathogens and saprophytes. They can even grow at low temperature, so they are the main fungi responsible for the spoilage of various fruits, vegetables, grains and their products during long-distance transport and refrigerated storage. *Alternaria* mycotoxins are the secondary metabolite of the genus *Alternaria*. They can be divided into five main classes according to their chemical structures, including dibenzopyrone derivatives, tetramic acid derivatives, perylene derivatives, AAL toxins and miscellaneous structures. *Alternaria* mycotoxins are associated with many health effects because of their mutagenicity, teratogenicity and carcinogenicity, which can cause economic losses to agriculture and serious diseases in humans and animals. So far, there is still a lack of monitoring data on these contaminants of *Alternaria* mycotoxins. Moreover, there are still no statutory or guideline limits set for *Alternaria* mycotoxins in food and feed by regulatory authorities worldwide. Until now, many analytical methods have been developed for the detection and quantification of *Alternaria* mycotoxins. On the basis of briefly introducing the chemical structures and toxicities of *Alternaria* mycotoxins, this article provides an overview of the progress achieved in the detection techniques for *Alternaria* mycotoxins, focusing on the analytical methods of thin layer chromatography (TLC), gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), liquid chromatography (LC), liquid chromatography-mass spectrometry (LC-MS), enzyme-linked immunosorbent assay (ELISA) and so on. Finally, the problems of these analytical methods and future development trends are discussed.

Keywords *Alternaria* mycotoxins · Toxicity · Alternariol · Alternariol monomethyl ether · Tenuazonic acid · Detection

Introduction

Alternaria toxins belong to mycotoxins produced by fungi of the genus *Alternaria* (about 300 species), which can infect various crops and foodstuffs, such as wheat, sorghum, barley, tomato, citrus fruits, apple, olive and so on [1–3]. It is noteworthy that *Alternaria* species can grow at low temperature, so they are the main fungi responsible for the spoilage of these fruits, vegetables, grains and their products during long-distance transport and refrigerated storage [4]. Alternariol (AOH), altenariol monomethyl ether (AME), altenuene (ALT), tenuazonic acid (TeA), altertoxins I, II and III (ATX-I, ATX-II, ATX-III) and tentoxin (TEN) are considered to be some of the important *Alternaria* mycotoxins [5]. These mycotoxins are mutagenic, teratogenic, carcinogenic and so on, which could cause a threat to the health of humans and animals. So far, there are still no statutory or guideline limits set for *Alternaria* mycotoxins in food and feed by regulatory authorities worldwide. However, fortunately the scientific opinion about the health risk of *Alternaria* mycotoxins in food and feed for humans and animals has been published by the European Food Safety Authority (EFSA); moreover, the European Standing Committee has

✉ Ligang Pan
panlg@brcast.org.cn

[1] Beijing Research Center for Agricultural Standards and Testing, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China

[2] Risk Assessment Lab for Agro-products (Beijing), Ministry of Agriculture, Beijing, China

[3] Beijing Municipal Key Laboratory of Agriculture Environment Monitoring, Beijing, China

recommended that EU member states should collect data about the occurrence of *Alternaria* mycotoxins in food commodities [6]. Currently, scientists are surveying the occurrence of *Alternaria* mycotoxins in various crops and foodstuffs. For example, AOH, AME and TeA were verified to be the most common *Alternaria* mycotoxins in Argentinean wheat [5]. Moreover, the occurrence of *Alternaria* mycotoxins (AOH, AME, TeA, TEN and ALT) in foodstuffs in the Netherlands was also surveyed [4, 7], and the results showed that AOH, AME, TeA and TEN existed in one or more food commodities, TeA was found in 27% of samples, and relatively high concentrations (up to 2345 $\mu g\ kg^{-1}$) could be found in sunflower seeds, tomato sauces and dried figs. ALT was not found in any of the samples, but appeared frequently in cereals, tomato sauces, figs, wine and sunflower seeds. Additionally, the origin, occurrence and risks and the ecophysiology, mycotoxin production and toxicology of *Alternaria* mycotoxins were also reviewed by Logrieco et al. [8] and Lee et al. [9].

At present, a variety of analytical methods mainly focused on thin layer chromatography (TLC), gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), liquid chromatography (LC), liquid chromatography-mass spectrometry (LC-MS) and especially LC tandem mass spectrometry (LC-MS/MS) or LC multi-stage mass spectrometry ($LC\text{-}MS^n$) are being developed for the detection and quantitation of *Alternaria* mycotoxins. In this article, the analytical methods and their application for *Alternaria* mycotoxins are mainly discussed and summarized. Besides, the chemical structures and toxicity of *Alternaria* toxins are also introduced. Finally, the problems of these analytical methods and future development trends are discussed.

Chemical Structures and Toxicity of *Alternaria* Toxins

Alternaria mycotoxins can be divided into five different classes according to their chemical structures (see Fig. 1): (1) dibenzopyrone derivatives, which include AOH, AME and ALT; (2) tetramic acid derivatives, TeA and *iso*-tenuazonic acid (*iso-TeA*); (3) perylene derivatives, altertoxins I, II and III (ATX-I, ATX-II and ATX-III); (4) *Alternaria alternate* f. sp. *Lycopersici* TA1, TA2, TB1 and TB2 toxin (AAL TA1, TA2, TB1 and TB2); (5) miscellaneous structures, such as tentoxin (TEN), *iso*-tentoxin (*iso*-TEN) and dihydrotentoxin (DHT), which are a cyclic tetrapeptide [6, 10].

AOH and AME are widely distributed worldwide and show no acute toxic effects to human and animal health. However, they possess the property of carcinogenicity and exhibit a high incidence of esophageal cancer [11].

Fig. 1 The chemical structure of *Alternaria* mycotoxins. *AOH* alternariol, *AME* altenariol monomethyl ether, *ALT* altenuene, *TeA* tenuazonic acid, *iso-TeA iso*-tenuazonic acid, *ATX-I, ATX-II, ATX-III* altertoxins I, II and III, *AAL TA1, TA2, TB1 and TB2 toxin* alternaria alternate f. sp. *Lycopersici* TA1, TA2, TB1 and TB2 toxin, *TEN* tentoxin, *iso-TEN iso*-tentoxin, *DHT* dihydrotentoxin

AOH also shows mutagenicity and genotoxicity. It could induce hypoxanthine–guanine phosphoribosyltransferase and thymidine kinase mutations in Chinese hamster V79 and mouse lymphoma L5178Y $tk^{+/-}$ (MLC) cells, respectively [12], and induce single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) breaks [13–15]. Moreover, AOH also could inhibit DNA relaxation and stimulate DNA cleavage activity of topoisomerase in cell-free assays, so it was characterized as a powerful inhibitor of topoisomerase activity, contributing to its genotoxicity [9, 16, 17]. In addition, AOH has been reported to possess cytotoxicity in vitro [18]. ALT is the most toxic dibenzopyrone derivative when given in a single dose to mice, and the LD_{50} value is lower with 1/3 mice dying at 50,000 $\mu g\ kg^{-1}$ b.w. [6].

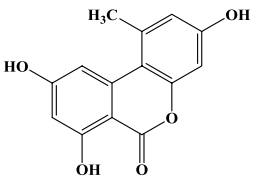
TeA as the only *Alternaria* mycotoxin was listed in the Food and Drug Administration (FAD) toxic chemical register [10]. It has acute toxicity effects on human and animal health [19], and the mechanism is that TeA can inhibit the release of the proteins generated in the cell from the intracellular area to the cytoplasm. TeA is also a strong chelating agent. It can chelate with calcium, magnesium, copper and other metals, and then the chelation combines with the active center of transpeptidase, so the peptide bond formation of protein synthesis is inhibited [20]. In addition, TeA demonstrates cytotoxicity [18, 21], carcinogenicity and a synergistic effect [22].

ATXs have mutagenic activity [23, 24], and the mutagenicity is higher than for AOH and AME in the *Salmonella* Ames test [25]. ATX-I is acutely toxic in mice and mutagenic in the mammalian cell line. Besides, ATX-I and ATX-III play a potential role in cell transformation [26]. AAL toxins mainly show phytotoxic effects [27] and have been confirmed to affect the viability of mammalian cells, especially in the dog kidney, rat liver hepatoma and mouse fibroblast cell lines according to Abbas et al. [28]. TEN was produced by *Alternaria* fungi along with DHT and *iso*-TEN [29]; they were all considered phytotoxins inhibiting photophosphorylation and inducing chlorosis [30].

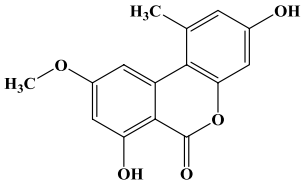
Analytical Methods for *Alternaria* Mycotoxins

TLC

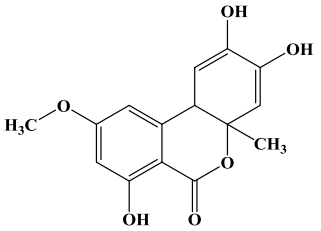
TLC as a simple and rapid qualitative analysis method has been used in various analysis fields. It also has been applied for the determination of *Alternaria* mycotoxins. For



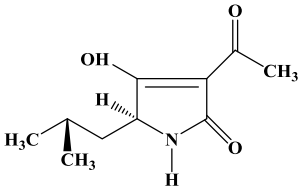
AOH



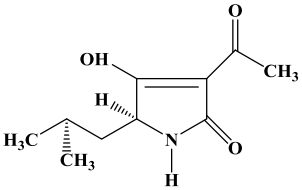
AME



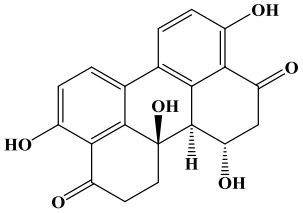
ALT



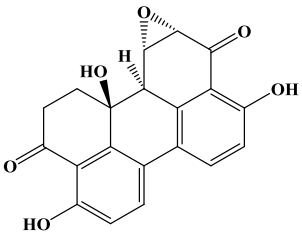
TeA



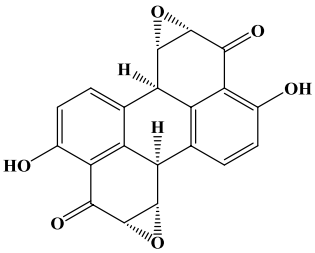
***iso*-TeA**



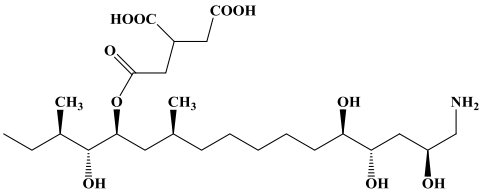
ATX-I



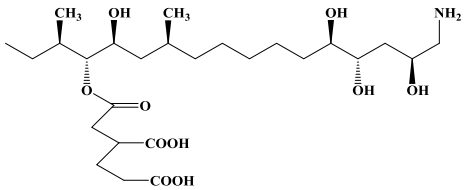
ATX-II



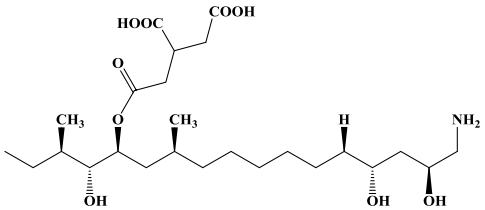
ATX-III



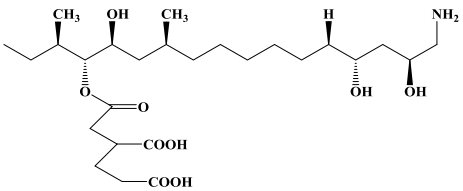
AAL TA1



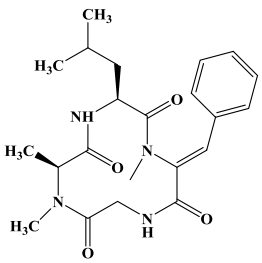
AAL TA2



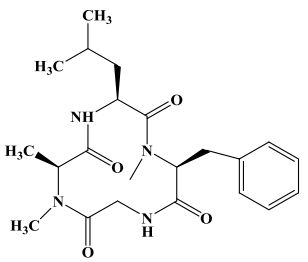
AAL TB1



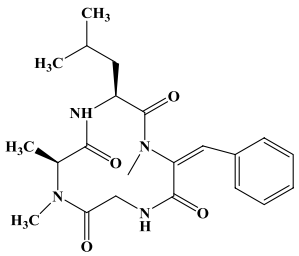
AAL TB2



TEN



DHT



***iso*-TEN**

example, Hasan et al. [31] used chloroform/acetone (97:3, v:v) as a solvent system for detecting *Alternaria* mycotoxins (AOH, AME, TeA, ATX-I and ATX-II) from tomato by TLC-UV. From the results, we can see that the main mycotoxins in rotted tomato were AOH, AME and TeA. The limits of detection (LODs) of AOH, AME and TeA were 100, 100 and 700 μg kg^{-1}, respectively. Fàbrega et al. [32] also detected AOH, AME, ALT, ATX-I and TEN in the cultures of *Alternaria alternata* IMI 354942 by means of TLC-UV. The LODs of AOH, AME, ALT, ATX-I and TEN were 250, 125, 250, 250 and 5000 μg L^{-1}, respectively.

Compared with TLC, high-performance thin-layer chromatography (HPTLC) has higher separation efficiency and detection sensitivity. Matysik and Giryn [33] combined gradient HPTLC with densitometry for the detection of AOH and AME from raspberry, tomato, wheat and oat samples; the LOD was about 60 μg kg^{-1}. Moreover, AOH, AME, ALT and TeA in fresh grape juice, must and wine were also quantified by HPTLC; the limits of quantitation (LOQs) were 1.5 μg L^{-1} of AOH and AME and 7.5 μg L^{-1} of TeA [34].

The analytical methods of TLC and HPTLC used for the detection and quantification of *Alternaria* mycotoxins are listed in Table 1. Although the separation efficiency and detection sensitivity of TLC are lower than those of HPLC and GC, TLC is still an indispensable analytical tool for

Table 1 TLC for *Alternaria* mycotoxin detection

Targets	Sample	Analytical methods	LODs	QODs	References
AOH	Tomatoes	TLC-UV	100 μg kg^{-1}	–	[31]
AME			100 μg kg^{-1}	–	
TeA			700 μg kg^{-1}	–	
AOH	Cultures of *Alternaria alternata* IMI 354942	TLC-UV	250 μg L^{-1}	–	[32]
AME			125 μg L^{-1}	–	
ALT			250 μg L^{-1}	–	
ATX-I			250 μg L^{-1}	–	
TEN			500 μg L^{-1}	–	
TeA	Sunflower seeds	TLC	200 μg kg^{-1}	–	[37]
AOH	Cultures of the fungus *Alternaria tenuis*	TLC	–	–	[38]
AME					
ALT					
AOH	Fruit and vegetable products	TLC-UV	3 μg kg^{-1}	–	[39]
AME					
AOH	Raspberries, tomatoes, wheat and oats	HPTLC-densitometry	60 μg kg^{-1}	–	[33]
AME					
AOH	Grape juice, must and wine	HPTLC	–	1.5 μg L^{-1}	[34]
AME					
ALT				–	
TeA				7.5 μg L^{-1}	
AOH	Lentils	HPTLC	–	–	[40]
AME			15 μg kg^{-1}	–	
ALT			15 μg kg^{-1}	–	
TeA			75 μg kg^{-1}	–	
AOH	Wheat grain, rape seed	HPTLC	2 μg kg^{-1}	5 μg kg^{-1}	[41]
AME			2 μg kg^{-1}	5 μg kg^{-1}	
ALT			2 μg kg^{-1}	5 μg kg^{-1}	
TeA			10 μg kg^{-1}	25 μg kg^{-1}	
AOH	Olives, olive husks, olive oil	HPTLC	50 μg kg^{-1}	–	[42]
AME			30 μg kg^{-1}	–	
ALT			100 μg kg^{-1}	–	
ATX-I			200 μg kg^{-1}	–	
TeA			100 μg kg^{-1}	–	

AOH alternariol, *AME* altenariol monomethyl ether, *ALT* altenuene, *TeA* tenuazonic acid, *ATX-I* altertoxins I, *TEN* tentoxin, *TLC* thin-layer chromatography, *HPTLC* high-performance thin-layer chromatography

the detection of mycotoxins in various matrices because of its advantages, such as simplicity of operation and sample pretreatment, rapidity, cost effectiveness, not having any memory effects and consuming smaller amounts of solvents than LC. Therefore, TLC is more environment-friendly and so on [35, 36]. Moreover, HPTLC with a densitometric detector has higher detection sensitivity, comparable to GC and HPLC.

GC

GC coupled to different detection techniques has also been applied for the detection of *Alternaria* mycotoxins. GC, especially GC-MS, not only has high sensitivity and selectivity, but also can detect certain substances in the mixture. It is suitable for the detection of non- and semi-polar, volatile and semi-volatile compounds, but most *Alternaria* mycotoxins are small, non-volatile and polar molecules [43], so the *Alternaria* mycotoxins usually need to be derivatized prior to GC/GC-MS analysis. Harvan et al. [44] derivatized TeA using a mixture of acetyltrimethylsilane, trimethylsilane and pyridine (6:2:9, v:v:v) and detected the TeA using GC with a flame ionization detector (FID); the LOD of TeA was 100 $\mu g\ kg^{-1}$. In addition, Scott et al. [45] first derivatized *Alternaria* mycotoxins using heptafluorobutyrate (HFB) and trimethylsilyl (TMS), respectively, and followed this using GC-MS for the detection of AOH, AME, ALT, ALTX-I and TeA in apple juice. The results showed that both HFB and TMS derivatives are appropriate for the separation of *Alternaria* mycotoxins by GC prior to MS detection, and LODs of AOH and AME of 1 $\mu g\ kg^{-1}$ were reported in apple juice.

Although the above GC/GC-MS methods demonstrated excellent sensitivity, these methods have not been widely used for the detection of *Alternaria* mycotoxins. The main reason is that the sample preparation of *Alternaria* mycotoxins mostly requires derivatization. The derivatization causes the disadvantages of matrix interference, poor repeatability, being time-consuming, using expensive derivatization reagents and complex operation in the GC-MS detection process. In addition, GC-MS also has memory effects from the injection of the previous sample. Therefore, applications of GC for the determination of *Alternaria* mycotoxins are limited because of the laborious derivatization reactions needed.

LC Coupled with Classical Detectors

LC, commonly reversed-phase LC coupled with classical detectors, such as an ultraviolet detector (UVD), diode-array detector (DAD), fluorescence detector (FLD), electrochemical detector (ECD), evaporative light-scattering detector (ELSD) and mass spectrum (MS), has largely superseded GC and TLC for the detection of *Alternaria* mycotoxins in recent years (see Tables 2, 3).

LC Coupled with a UV/DAD/FLD/ECD Detector

LC-UV LC coupled with a UV detector (LC-UV) has been widely used for the detection of *Alternaria* mycotoxins because most organic molecules and some inorganic molecules have the properties of ultraviolet absorption. The contents of TeA and AME in tomatoes and tomato products were determined by reverse phase LC-UV, and the LODs of TeA and AME were 25 and 3 $\mu g\ kg^{-1}$, respectively [46]. Solfrizzo et al. [47] used reversed-phase LC with a UV diode array detector (LC-UV/DAD) for the detection of ATX-I, AOH, AME and TeA in carrots, and solid phase extraction (SPE) was used as the pretreatment. The results showed that the LODs of TeA, ATX-I, AME and AOH were 20, 20, 10 and 5 $\mu g\ kg^{-1}$, respectively. Moreover, 64 wheat samples harvested in Argentina in 2004 and 2005 were detected by HPLC-UV [5]. The results showed that 23% of wheat samples contain AME, 6% contain AOH and 19% contain TeA. The mean concentrations of AME, AOH and TeA in the positive sample were 2118, 1054 and 2313 $\mu g\ kg^{-1}$, respectively.

LC-DAD AOH in Estonian grain [48] and TeA in Canadian ice wines [49] were quantified by LC-DAD; the LODs were 100 $\mu g\ kg^{-1}$ and 70 $\mu g\ L^{-1}$, respectively. In addition, TeA is a strong chelating agent. As mentioned earlier, it could form complexes with metal ions, so when detecting TeA, a metal ion chelating agent, such as zinc sulfate ($ZnSO_4$), was usually added to the mobile phase. For example, TeA in tomato products was detected by the HPLC-DAD method using the methanol–water (90:10, v:v) mixture as the mobile phase containing 300 mg $ZnSO_4{\cdot}H_2O$. The LOQ of TeA was 11 $\mu g\ kg^{-1}$, and the average recovery was 78% [50]. Compared with the UV detector, LC-DAD is rarely used for the detection of *Alternaria* mycotoxins because of the poor sensitivity. However, the sensitivity of diode array UV detection (LC-UV/DAD) is high. Aresta et al. [51] purified TeA by solid-phase microextraction (SPME) and detected it using the LC-UV/DAD method. The LOD of TeA was $25 \pm 6\ \mu g\ kg^{-1}$.

In addition to the above SPE and SPME techniques, a quick, simple and effective sampling preparation technique, called QuEChERS for short, which stands for quick, easy, cheap, effective, rugged and safe, has recently been developed for pesticide detection in fruits and vegetables [52, 53]. The QuEChERS extraction technique was also coupled with HPLC-DAD for the simultaneous determination of AOH, AME and TEN in pomegranate fruit and juice; the LODs were from 15 to 20 $\mu g\ kg^{-1}$, while the LOQs were between 50 and 66 $\mu g\ kg^{-1}$ [54].

Table 2 LC-UV/DAD/FLD/ECD/ELSD for *Alternaria* mycotoxin detection

Toxins	Sample	Analytical methods	Extraction/clean-up	LOD	References
AOH	Wheat grain	HPLC-UV	–	50 μg kg^{-1}	[5]
AME				50 μg kg^{-1}	
TeA				80 μg kg^{-1}	
AME	Tomatoes and tomato products	LC-UV	SPE	3 μg kg^{-1}	[46]
TeA				25 μg kg^{-1}	
AOH	Tomatoes and tomato products	LC-UV	SPE	5 μg kg^{-1}	[47]
AME				10 μg kg^{-1}	
TeA				20 μg kg^{-1}	
ATX-I				20 μg kg^{-1}	
AOH	Oats grain, wheat grain, barley grain	HPLC-DAD	Acetonitrile/4% KCl (9:1, v:v)	100 μg kg^{-1}	[48]
TeA	Ice wine	LC-DAD	SPME	70 μg L^{-1}	[49]
TeA	Tomato products	HPLC-DAD	–	–	[50]
TeA	Cornflakes	HPLC–UV/DAD	SPME	25 ± 6 μg kg^{-1}	[51]
AOH	Pomegranate fruit and juice	HPLC-DAD	QuEChERS	15–20 μg kg^{-1}	[54]
AME					
TeA					
AOH	Tomato paste	HPLC-FLD	SPE	1.93 μg L^{-1}	[55]
AOH	Fiber flax seeds, linseed seeds, pea seeds	HPLC-FLD	Methanol	3 μg kg^{-1}	[56]
AME				2 μg kg^{-1}	
ALT				1 μg kg^{-1}	
AOH	Wheat grain	HPLC-FLD	Acetonitrile/4% KCl (9:1, v:v)	50 μg kg^{-1}	[57]
AME				50 μg kg^{-1}	
ALT				100 μg kg^{-1}	
ATX-I	Wheat grain	HPLC-UV		200 μg kg^{-1}	
TeA				100 μg kg^{-1}	
AOH	Fungal cultures, maize and rice	LC-ECD	Methanol and aqueous ammonium sulfate	Sub-nanogram	[58]
AME					
ATX-I					
ATX-II					
ATX-I	Maize, rice and tomatoes	HPLC-ECD	Methanol and aqueous ammonium sulfate	Sub-parts per million	[59]
ATX-II					
AAL	Fungal culture	HPLC-ELSD	Syringe-filter	6000 μg L^{-1}	[60]
TEN	Fermentation of *Alternaria porri* (Ellis) Ciferri	LC-UV	*n*-Hexane	100 μg L^{-1}	[61]
AOH	Tomato pulp	HPLC–DAD	Methanol	5.0 μg kg^{-1}	[62]
AME				2.0 μg kg^{-1}	
TeA				8.0 μg kg^{-1}	
AME	Sorghum-based mixed feed (swine)	HPLC-FLD	Chloroform/water	10 μg kg^{-1}	[63]
AOH	Oilseed rape meal	HPLC-FLD/UV	Acetonitrile/4% aqueous potassium chloride solution (9:1)	50 μg kg^{-1}	[64]
AME				40 μg kg^{-1}	
ALT				40 μg kg^{-1}	
ATX-I				200 μg kg^{-1}	
TeA				350 μg kg^{-1}	

AOH alternariol, *AME* altenariol monomethyl ether, *ALT* altenuene, *TeA* tenuazonic acid, *iso-TeA iso*-tenuazonic acid, *ATX-I, ATX-II* Altertoxins I, II, *AAL toxin Alternaria alternate* f. sp. *Lycopersici* toxin, *TEN* tentoxin, *LC* liquid chromatography, *HPLC* high-performance liquid chromatography, *UV/DAD* UV diode array detector, *SPE* solid phase extraction, *SPME* solid-phase microextraction, *FLD* fluorescence detector, *ECD* electrochemical detector, *ELSD* evaporative light-scattering detector

Table 3 LC–MS for *Alternaria* mycotoxin detection

Toxins	Sample	Analytical methods	Extraction/clean-up	LODs (μg kg^{-1})	QODs (μg kg^{-1})	References
AOH	Wines, grape juices and cranberry juices	LC-ESI-MS/MS	SPE	0.01–0.8	–	[3]
AME				0.01–0.5	–	
AOH	Wine, apple, apple juices, tomato, tomato sauces	LC-ES-MS/MS	Acetonitrile/water/formic acid (84/16/1, v/v/v)	–	1.5–5.0	[4]
AME						
ALT						
TeA						
TEN						
AOH	Flavedo	HPLC–MS/MS ESI	SPE	<0.13	<0.50	[65]
AME						
AOH	Soy, St John's wort, garlic, Ginkgo biloba, black radish	HPLC-ESI-MS/MS	SPE	8	–	[66]
AME				30	–	
ALT				2	–	
AOH	Sweet pepper	HPLC-ESI-MS/MS	SPE	3.3	–	[67]
AME				12	–	
ALT				0.6	–	
AOH	Maize silage	LC-ESI-MS/MS	QuEChERS	10	–	[68]
AME				6	–	
AOH	Tomato products	UPLC–MS/MS	QuEChERS	3.0–18.3	9.8–61.5	[69]
AME	Fruit and vegetable juices			–	1.1–5.7	
ALT						
TEN						
ATX-I						
AOH	Barley	HPLC-Orbitrap® MS	QuEChERS	–	–	[70]
ALT						
AOH	Feed matrices	U-HPLC–MS/MS	QuEChERS	–	1250	[71]
AME						
ALT						
TEN						
TeA	Cereals	HPLC-ESI-IT-MS^2	–	10	–	[75]
TeA	Bottled Beers	LC-ESI-IT MS^2	–	2	–	[76]
AAL-TA	Maize silage	HPLC-ESI-MS	Methanol/water (3:1, v/v)	20	–	[77]
AAL-TB				20	–	
TEN	Culture liquid of *A. porri.*	LC/APCI-MS	Acetonitrile/water (3:7, v/v)	–	–	[78]
*iso*TEN						
DHT						
AOH	Tomato, bakery products, sunflower seeds, fruit juices, vegetable oils	HPLC-ESI-MS/MS	Methanol/water/formic acid (49:50:1, v/v/v)	0.2–2.8	0.6–9.3	[79]
AME				0.04–0.4	0.1–1.2	
ALT				0.8–24	2.5–81	
*iso*ALT				1.3–19	4.4–62	
TeA				3.6–34	12–110	
ATX-I				2.1–14	6.9–48	
TEN				0.1–2.0	0.5–6.6	
TA1				2.8–5.4	9.3–18	
TA2				1.2–17	3.8–55	
AOH	Apple juices, beers, tomato products, olives and dried basil	LC-APCI-MS/MS	SPE	0.16–12.31	0.54–41.04	[80]
AME						
ALT						
TeA						
TEN						

Table 3 continued

Toxins	Sample	Analytical methods	Extraction/clean-up	LODs (μg kg^{-1})	QODs (μg kg^{-1})	References
AOH	Beer	UHPLC-orbitrap MS	Acetonitrile	–	–	[85]
AME						
ALT						
AOH	Tomato products	UPLC–MS/MS	QuEChERS	3.0–18.3	9.8–61.5	[69]
AME	Fruit and vegetable juices			–	1.1–5.7	
ALT						
ATX-I						
TEN						
AOH	Apple, orange, sweet cherry and tomato fruits	UPLC–MS/MS	SPE	–	1–2	[86]
AME						
ALT						
TeA						
TEN						
AOH	Tomato products	UPLC-ESI-MS/MS	SPE	4	–	[81]
AME				1	–	
ALT				2	–	
TeA				2	–	
ATX-I				2	–	
TEN				2	–	
AOH	Maize, groundnuts, sorghum, millet, rice, wheat, soy, dried fruits	LC–MS/MS ESI	Acetonitrile/water/acetic acid, 79:20:1, v/v/v	5	–	[82]
AME				8	–	
ATX-I				3	–	
TEN				0.4	–	
AOH	Almonds, hazelnuts, peanuts and pistachios	UHPLC-MS/MS ESI	Acetonitrile/water/acetic acid, 79:20:1, v/v/v	3.0	–	[83]
AME				0.8	–	
ATX-I				13	–	
TEN				1.2	–	
AOH	Bread, fruits, vegetables, jam, cheese, chestnuts and red wine	UPLC/ESI-MS/MS	Acetonitrile/water/acetic acid 79:20:1, v/v/v	2	–	[84]
AME				0.1	–	
ALT				6	–	
TEN				0.5	–	
AOH	Microbial metabolites in indoor matrices	HPLC-ESI-MS/MS	SPE	3	–	[87]
AME				1	–	
ALT				390	–	
ATX-I				44	–	
AAL				440	–	
TEN				3	–	

AOH alternariol, *AME* altenariol monomethyl ether, *ALT* altenuene, *TeA* tenuazonic acid, *iso-TeA iso*-tenuazonic acid, *ATX-I* altertoxins I, *AAL toxin TA and TB Alternaria alternate* f. sp. *Lycopersici* toxin TA1, TA2, TB1 and TB2 toxin, *TEN* tentoxin, *iso-TEN iso*-tentoxin, *DHT* dihydrotentoxin, *LC* liquid chromatography, *HPLC* high-performance liquid chromatography, *SPE* solid phase extraction, *ESI* electrospray ionization, *APCI* atmospheric pressure chemical ionization, *UPLC* ultra-performance liquid chromatography, *UHPLC* ultra-high-performance liquid chromatography or ultra-high-pressure liquid chromatography

LC-FLD FLD is commonly used in HPLC. Its selectivity and sensitivity are higher than those of UV and DAD detectors; the LOD could reach to μg L^{-1}. AOH in tomato paste was determined by HPLC-FLD. The sample was extracted via SPE cartridges. The LOD of AOH was 1.93 μg L^{-1}, and the range of linearity was 5.2–196 μg L^{-1} [55]. AOH, AME and ALT in fiber flax, linseed and peas were detected by HPLC-FLD, and the LODs of AOH, AME and ALT were 3, 2 and 1 μg kg^{-1}, respectively [56]. Moreover, these mycotoxins were also detected in wheat grain by HPLC-FLD; the LODs of AOH, AME and ALT were 50, 50 and 100 μg kg^{-1}, respectively [57]. Although the FLD detector has high detection sensitivity, it has some limitations because only few mycotoxins have the fluorescence

property. Take TeA, for example: it could not be detected by the FLD detector because it has no fluorescent functional groups. Therefore, LC-UV and LC-DAD are much more widespread than LC-FLD for detecting *Alternaria* mycotoxins.

LC-ECD ECD has been widely used in the analysis of trace samples. It has extreme detection sensitivity, and the LOD can reach 10^{-6} μg L^{-1}, but this ECD is only used to detect electroactive molecules that can be easily oxidized or reduced. Among *Alternaria* mycotoxins, AOH, AME, ATX-I and ATX-II are all electroactive molecules. They have been detected by dual-electrode coulometric and single-electrode amperometric detection techniques, respectively, and the mycotoxins could be detected at sub-nanogram levels [58]. Moreover, an HPLC method coupled with dual in-series electrodes in the "redox" mode improves the detection sensitivity of ATX [59]. Samples containing ATX-I and ATX-II were extracted from artificially infected maize, rice and tomatoes, and the LOD reached sub-ppm levels.

LC-ELSD The detection of AAL toxins mainly relied on tedious derivatization or immunoassay procedures because of the lack of a UV chromophore. Here, Xu and Du [60] developed a direct, fast and sensitive analytical method by coupling a C_{18} reverse phase HPLC to an ELSD for the quantitative detection of AAL toxins in the fungal culture, and the LOD was about 6000 μg L^{-1}. The analytical method of ELSD provides a mean for the study of host-specific mycotoxins, but it needs a signal transducer when coupled with LC and needs to configure the high-pressure nitrogen or air. Moreover, it produces a harmful exhaust gas in the detection process.

LC-MS

The MS detection techniques have higher sensitivity compared with UV, DAD, FLD and ECD detectors. LC-MS, especially LC-MS/MS or LC-MS^n based on electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) interfaces, have played an important role in the simultaneous detection and quantitation of *Alternaria* mycotoxins in various samples without derivatization over the last years. An ESI source was the most widely used for the detection of *Alternaria* mycotoxins because it has higher sensitivity than APCI. The analytical methods of LC-MS used for the detection and quantification of *Alternaria* mycotoxins are listed in Table 3.

Dibenzopyrone derivative detection For the detection of AOH, AME and ALT toxins, Magnani et al. [65] employed SPE as the pretreatment and used HPLC-MS/MS for the detection of AOH and AME on flavedo. The results were that the linearity range was 0.50–20.0 μg kg^{-1}; the LOD and LOQ were less than 0.13 and 0.50 μg kg^{-1}, respectively, with the relative standard deviations (RSD) ≤14.4%. Then, a multi-mycotoxin HPLC-MS/MS method was developed for the detection of 23 mycotoxins in 6 different food supplements [66]. The analysis sample was first extracted using ethyl acetate/formic acid (95:5, v/v), followed by using an OASIS HLB™ SPE column for purification. Among the 23 mycotoxins, the LODs of ALT, AOH and AME were 2, 8 and 30 μg kg^{-1}, respectively, whereas the LOQs were about three times higher. Moreover, 23 mycotoxins in sweet pepper were also detected by HPLC-MS/MS, but the sample preparation was different [67]. This developed multi-mycotoxin LC-MS/MS method fulfilled the method performance criteria required by Commission Regulation (EC) no. 401/2006.

The QuEChERS extraction technique could not only be coupled with LC-DAD, as mentioned above, but could also couple with LC-MS for the simultaneous determination of *Alternaria* mycotoxins. The extraction of multi-mycotoxin containing AOH and AME from silage samples was performed by QuEChERS, and LC-ESI-MS/MS was applied for the detection. The LODs of AOH and AME were 10 and 6 μg kg^{-1}, respectively [68]. In addition to the above-described LC or HPLC, ultra-performance liquid chromatography (UPLC), which is also termed ultra-high-performance liquid chromatography or ultra-high-pressure liquid chromatography (UHPLC), has also been widely applied for the analysis of these mycotoxins. For example, the AOH, AME, TEN, ALT and ATX-I in tomato products, fruit and vegetable juices were extracted by the QuEChERS method and detected by UPLC-MS/MS; the results showed that the LOD of these toxins in tomato products was 3.0–8.3 μg kg^{-1}, the LOQ was 9.8–61.5 μg kg^{-1}, and the LOQ of these toxins in fruit and vegetable juices was 1.1–5.7 μg kg^{-1} [69]. In addition, the ALT, AOH in barley [70] and AOH, AME, TEN and ALT in feed matrices [71] were also extracted by the QuEChERS-based method and analyzed by ultra-high-resolution mass spectrometry (HPLC-Orbitrap® MS) and ultra-high-performance liquid chromatography coupled with sensitive tandem mass spectrometry (UHPLC–MS/MS), respectively.

Tetramic acid derivative detection The derivatization in the column [72] or ion-pairing techniques have to be used when detecting TeA due to the fact that TeA can cause a poor peak shape in HPLC because of the properties of high acidity and metal chelating. In ion-pairing techniques, $ZnSO_4$ is widely used in the HPLC elution buffer [73, 74], but it is not compatible with MS ion sources. To solve the above-mentioned problems, Siegel et al. [75, 76] modified the TeA by derivatization with 2,4-dinitrophenylhydrazine (DNPH) and quantified TeA in cereals and beer by HPLC-IT–MS^2. The results showed that the DNPH-TeA derivative can give a high response in (+) ESI-IT–MS^2. The LODs of TeA in cereals and beer were 10 and 2 μg kg^{-1} without

sample preconcentration, respectively, and the ranges of linearity were 50–5000 and 8–500 μg kg^{-1}, respectively. TeA was detected in 13 and quantified in 3 out of 27 cereal samples; the concentration was up to 851 μg kg^{-1}.

Detection of other toxins For the detection of AAL toxins, TA_1 and TB_1 were detected in maize silage by HPLC-ESI-MS with a LOD of 20 μg kg^{-1} [77]. Besides, TEN, isotentoxin (*iso*-TEN) and dihydrotentoxin (DHT) toxins from *Alternaria porri* were determined simultaneously by LC-MS based on APCI, ESI and FAB (fast atom bombardment), respectively [78]. The results showed that LC-APCI-MS was the most sensitive and selective method for the detection of TENs (TEN, *iso*-TEN, DHT), and a linearity range of TENs from 100 ng/injection to 10 μg/injection was received within 15 min. This method can be used for the quantification of TENs in different samples.

For the simultaneous detection *Alternaria* mycotoxins of different chemical structures, Hickert et al. [79] used the HPLC-ESI-MS/MS approach for the quantification of nine *Alternaria* toxins (AOH, AME, TeA, ALT, *iso*ALT, TEN, ATX-I and TA_1 and TA_2) in food from a German market. The LODs and LOQs were 2.8–5.4 and 9.3–8 μg kg^{-1} for TA_1, respectively, and were 1.2–7 and 3.8–55 μg kg^{-1} for TA_2, respectively. Moreover, five *Alternaria* mycotoxins of AOH, AME, TeA, ALT and TEN in apple juices, beers, tomato products, olives and dried basil were detected by LC-APCI-MS/MS. The results showed that the LOD and LOQ were in the range of 0.16–12.31 and 0.54–41.04 μg kg^{-1}, respectively, and the most common *Alternaria* mycotoxin was AOH, followed by ALT [80]. Recently, the levels of the above five *Alternaria* mycotoxins from food products in the Netherlands were also quantified by LC-ESI-MS/MS [4]. Six *Alternaria* mycotoxins of TeA, AOH, AME, TEN, ATX-I and ALT in tomato products were studied by UPLC-ESI-MS/MS. The results showed that TeA was found most frequently (81 out of 85 samples), with its highest concentration up to 790 μg kg^{-1}; AOH and AME were found in lower concentrations, ranging from <1 to 33 μg kg^{-1} for AOH and <5 to 9 μg kg^{-1} for AME. Moreover, ALT and TEN were only found in a few samples, and ATX-I was never detected in any samples [81]. In addition, AOH, AME, ATX-I and TEN mixed with the other mycotoxins in different samples were also detected by LC-ESI-MS/MS, respectively [82–84].

LC-MS with Stable Isotope Dilution Assays (SIDA)

LC-MS suffers significantly from ion suppression; thus, the quantitative results have to be corrected by special techniques of suitable internal standards that compensate for this effect [88]. In addition, *Alternaria* infests a variety of analytical samples requiring different sample preparation and separation methods. Here, SIDA as a perfect tool can not only significantly decrease the ion suppression in the ESI interface, but also compensate for analyte losses during sample preparation [89]. Moreover, SIDA is also a useful tool in analytical applications; for instance, it can offer significant benefits for trace analysis, render the quantitative results more accurate and enhance the specificity of the determination [90]. So far, the principle applications of SIDA for mycotoxin analysis have been critically reviewed by Rychlik [90] and Asam [91], respectively. The methods of LC-MS with SIDA used for the detection of *Alternaria* toxins are listed in Table 4.

AOH, AME and ATX analysis $[^2H_4]$-AOH and $[^2H_4]$-AME as the internal standards. $[^2H_4]$-AOH and $[^2H_4]$-AME as the internal standards were synthesized by palladium-catalyzed protium–deuterium exchange. They were applied for the determination of AOH and AME in fruit juices by HPLC-MS/MS. The method has a high sensitivity; the LODs of AOH and AME were 0.03 and 0.01 μg kg^{-1}, respectively, and the LOQs were 0.09 and 0.03 μg kg^{-1}, respectively; the reproducibility from spiked apple juice was 100.5 ± 3.4 and 107.3 ± 1.6%, respectively [89]. After 2 years, the same research group precisely quantified AOH and AME in cereal, fruit and vegetable products using SIDA; an AOH of 13–250 μg kg^{-1} and AME of 3–100 μg kg^{-1} were found in cereals, and an AOH of 2.6–25 μg kg^{-1} and AME of 0.1–5 μg kg^{-1} were found in vegetable products [92].

$[^{13}C_{20}]$-ATXs, $[^{13}C_{14}]$-AOH and $[^{13}C_{15}]$-AME as internal standards. Alternative labelings of $[^{13}C_{20}]$-ATXs, $[^{13}C_{14}]$-AOH and $[^{13}C_{15}]$-AME were applied as internal standards in a stable isotope dilution LC-MS/MS method [93]. The LODs of AOH, AME, ATX-I and ATX-II were 0.36, 0.09, 0.36 and 0.53 μg kg^{-1}, respectively, and the LOQs were 1.1, 0.27, 1.1 and 1.6 μg kg^{-1}, respectively. The inter-/intra-day relative standard deviations (RSDs) were below 13%, and the recoveries ranged from 96 to 109%. Moreover, they found that if the samples were contaminated by ATX, they may also be contaminated by the other *Alternaria* toxins, such as AOH, AME and TEN, but not necessarily vice versa.

TeA analysis Two different internal standards of $[^{13}C_6, {}^{15}N]$-TeA and $[^{13}C_2]$-TeA were applied in the previous paper.

$[^{13}C_6, {}^{15}N]$-TeA as internal standard. Asam et al. [94] first synthesized the stable-isotope-labeled $[^{13}C_6, {}^{15}N]$-TeA by Dieckmann intramolecular cyclization after acetoacetylation with diketene and applied $[^{13}C_6, {}^{15}N]$-TeA as the internal standard for the quantification of TeA in tomato by LC-MS/MS. The LOD was 0.1 μg kg^{-1}, and the LOQ was 0.3 μg kg^{-1}. Then, the same research group quantified the TeA content by SIDA with LODs of 0.15 μg kg^{-1} in fruit juices, 1.0 μg kg^{-1} in cereals and 17 μg kg^{-1} in spices [95]. Moreover, they also analyzed the content of

Table 4 LC–MS with SIDA for *Alternaria* mycotoxin detection

Toxins	Sample	Analytical methods	Extraction/clean-up	LODs	QODs	References
AOH	Wine, vegetable juices	SIDA, HPLC-MS/MS	RP-18 SPE	0.03 μg kg^{-1}	0.09 μg kg^{-1}	[89]
AME				0.01 μg kg^{-1}	0.03 μg kg^{-1}	
AOH	Cereal	SIDA, HPLC-MS/MS		13–250 μg kg^{-1}	–	[92]
	Fruit, vegetable products			2.6–25 μg kg^{-1}	–	
AME	Cereal			3–100 μg kg^{-1}	–	
	Fruit, vegetable products			0.1–5 μg kg^{-1}	–	
AOH	Commercial food, such as potatoes, cereals, bread, sorghum feed	SIDA, LC-MS-MS	–	0.36 μg kg^{-1}	1.1 μg kg^{-1}	[93]
AME				0.09 μg kg^{-1}	0.27 μg kg^{-1}	
ATX-I				0.36 μg kg^{-1}	1.1 μg kg^{-1}	
ATX-II				0.53 μg kg^{-1}	1.6 μg kg^{-1}	
TeA	Tomato	SIDA, LC-MS/MS	–	0.1 μg kg^{-1}	0.3 μg kg^{-1}	[94]
TeA	Fruit juices	SIDA, LC-MS/MS	C_{18}-SPE	0.15 μg kg^{-1}	0.5 μg kg^{-1}	[95]
	Cereals			1.0 μg kg^{-1}	3 μg kg^{-1}	
	Spices			17 μg kg^{-1}	50 μg kg^{-1}	
TeA	Infant foods and beverages	SIDA, LC-MS/MS	C_{18}-SPE	–	–	[96]
TeA	Human urine		C_{18}-SPE	0.2 μg L^{-1}	0.6 μg L^{-1}	[97]
TeA	Pig plasma	SIDA, LC-ESI-MS/MS	Water/MeOH (80:20 and 95:5, v/v);	0.01 μg L^{-1}	5.0 μg L^{-1}	[98]
	Broiler chicken plasma			0.22 μg L^{-1}	5.0 μg L^{-1}	
TeA	Rice, oat flakes and barley	SIDA, LC–MS/MS	–	0.93 μg kg^{-1}	1.86 μg kg^{-1}	[99]
AME				0.77 μg kg^{-1}	1.53 μg kg^{-1}	
TeA	Tomato, pepper	SIDA, HPLC-MS/MS	RP18-SPE	0.86 μg kg^{-1}	2.89 μg kg^{-1}	[100]
TEN	Bread, cereals, chips, juice, nuts, oil, sauce, seeds, and spices	SIDA, LC-MS/MS	C_{18}-phenyl SPE	0.10–0.99 μg kg^{-1}	–	[101]
DHT						
isoTEN						

AOH alternariol, *AME* altenariol monomethyl ether, *TeA* tenuazonic acid, *ATX-I, ATX-II* altertoxins I and II, *TEN* tentoxin; *iso-TEN iso*-tentoxin, *DHT* dihydrotentoxin, *SIDA* stable isotope dilution assays, *SPE* solid phase extraction, *LC* liquid chromatography, *HPLC* high-performance liquid chromatography, *SPE* solid-phase extraction, *ESI* electrospray ionization, *LC-MS* liquid chromatography-mass spectrometry, *LC-MS/MS* LC tandem mass spectrometry

TeA in infant foods and beverages [96]. The study indicated that the median content of TeA in infant tea infusions was 2 μg L^{-1}, and the values of TeA in fennel tea infusions reached 20 μg L^{-1}. The median content of TeA in pureed baby food in jars was 7 μg kg^{-1}. Higher values were found in tomato (25 μg kg^{-1}), banana and cherry (80 μg kg^{-1}), and sorghum (20 μg kg^{-1}). Additionally, the TeA content in human urine was also analyzed using SIDA for further study [97]. TeA was detected in the urine of six volunteers in this study, and the results showed that TeA can be rapidly absorbed from food and nearly completely excreted via the urine. The linearity of the response curve was 0.02–100 (n/n = labeled standard/analyte); the LOD and LOQ were 0.2 and 0.6 μg L^{-1}, respectively. Moreover, the content of TeA in pig and broiler chicken plasma was also quantified using LC-ESI-MS/MS using [$^{13}C_6$, ^{15}N]-TeA as internal standard, and its comparative toxicokinetics were surveyed by Fraeyman et al. [98]. The LOD and LOQ of TeA were 0.01 and 5.0 μg L^{-1} for pig plasma, respectively, and 0.22 and 5.0 μg L^{-1} for broiler chicken plasma, respectively. After oral administration, the TeA in pigs and broiler chickens remained completely bioavailable; however, the toxicokinetics showed significant differences. The absorption and elimination of TeA in broiler chickens are slower than in pigs. Additionally, Walravens et al. [99] developed an UPLC-$ES^{+/-}$-MS/MS method and applied isotopically labeled internal standards [$^2H_4$]-AME and [$^{13}C_6$, ^{15}N]-TeA for simultaneous detection of free (AOH, AME, ALT, TeA, TEN, ATX-I) and conjugated (sulfates and glucosides of AOH and AME) *Alternaria* toxins in rice, oat flakes and barley products. The values of recovery (>95%) and precision (<10%) were obtained.

[$^{13}C_2$]-TeA as internal standard. Lohrey [100] synthesized isotopically labeled [$^{13}C_2$]-TeA by a new efficient and economical three-step procedure starting from unlabeled *tert*-butyloxycarbonyl-protected isoleucine and followed by condensation with Meldrum's acid, thermal cyclization and decarboxylation, and finally labeled carbons via 3-C acetylation with [$^{13}C_2$] acetyl chloride. The [$^{13}C_2$]-TeA was used as internal standard for the detection of TeA in tomato

and pepper by HPLC-MS/MS. The LOD of TeA was 0.86 μg kg^{-1}, and the LOQ was 2.89 μg kg^{-1}. The recovery was 91.0 ± 1.2% for pepper paste and 102 ± 4.4% for tomato products. The concentration of TeA from 3 to 2330 μg kg^{-1} was detected in different samples from the German market.

TEN, DHT and isoTEN analysis TEN, DHT and *iso*TEN in 103 food samples were also quantified by stable isotope dilution LC-MS/MS, which developed by Liu and Rychlik [101]. The results showed that the samples contaminated by TEN and DHT were 55 and 85%, respectively, and the highest concentrations of TEN and DHT found in paprika were 52.4 and 36.3 μg kg^{-1}, respectively. The LODs of the three toxins ranged from 0.1 to 0.99 μg kg^{-1}; the inter-/intraday RSDs were below 8.8%.

ELISA

Instrumental analytical methods of GC-MS and LC-MS require expensive equipment and highly qualified technicians. Nevertheless, enzyme-linked immunosorbent assay (ELISA) is characterized by simplicity of operation, miniaturization, rapidity and portability, which could offset the weaknesses of above instrumental analytical methods. It has become a research focus for both quantitative and semi-quantitative detection of mycotoxin, of course including *Alternaria* toxin. Until now, the ELISA method has been used for detecting AAL, AOH and TeA, and it can be helpful for assessment of the occurrence of *Alternaria* mycotoxin in foods and animal feeds.

AAL toxin TA was the first *Alternaria* mycotoxin detected by ELISA with high sensitivity and selectivity [102, 103]. TA toxin was derivatized with protein bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH), respectively, and the LOD of AAL toxin TA was in the low parts per billion range, with no significant cross-reactivity with some structurally similar compounds, such as fumonisin B1 and sphinganine. Additionally, Yu et al. [104] used direct competitive ELISA for the analysis of AAL toxin TA and the other five mycotoxins in hay, silage and mixed feed with an LOD of 50 μg kg^{-1}.

In 2011, Ackermann et al. [105] not only screened and obtained the polyclonal antibody of AOH, but also obtained the monoclonal antibody for the first time. Monoclonal and polyclonal antibodies were applied in ELISA for detecting AOH in the food, respectively, and the LODs of AOH were 35 ± 6.9 and 59 ± 16 ng L^{-1}, respectively. Then, the two methods based on monoclonal antibody and polyclonal antibodies, respectively, were also used for detecting AOH in a variety foods in the German market, and the detection results were compared with those of HPLC. Additionally, Burkin and Kononenko [106] also obtained the polyclonal antibody of AOH and applied it in an indirect competitive ELISA for detecting the content of AOH in corn, animal feed and natural ingredients, and the LOD of AOH was 0.4 μg L^{-1}. So far, ELISA has not been used for AME detection.

For the detection of TeA, Gross et al. [107] derivatized TeA using succinic anhydride and coupled it with KLH. The KLH conjugate was used for polyclonal antibody screening, and then the obtained polyclonal antibody was used for the development of competitive ELISA. The sensitivity of ELISA for TeA acetate was better than that of TeA. The average standard curve detection limit of TeA acetate was 5.4 ± 2.0 μg L^{-1}, and the LOD of TeA in apple and tomato was 25–50 μg kg^{-1}.

Electrochemical Method

So far, the electrochemical method used for the detection of *Alternaria* mycotoxin has been reported only once. The authors used a carbon paste electrode modified with mushroom tyrosinase for the quantitative detection of AOH and AME by the electrochemical method [108]. The results showed that both AME and AOH are the substrates of mushroom tyrosinase; moreover, the LODs of AOH and AME were 2.4 × 10^{-5} and 1.9 × 10^{-5} M, respectively, and the linear range up to 1.8 × 10^{-4} and 2.0 × 10^{-4} M, respectively. In addition, the method has low noise and a small background current, and it has easy and rapid electrode surface renewal.

Conclusions and Discussion

Alternaria species could produce more than 70 phytotoxins, but the toxicity and chemical structure of only a few of them have been characterized and reported to act as *Alternaria* mycotoxins to humans and animals. Currently, the EFSA has published their scientific opinion on the risks for animal and public health related to *Alternaria* toxins in feed and food, but there are still no statutory or guideline limits set by regulatory authorities for *Alternaria* mycotoxins. The detection methods of *Alternaria* mycotoxins, such as TLC, GC and GC-MS, LC and LC-MS, ELISA and electrochemical methods, have been reviewed in the text. Among them, TLC has the lowest detection sensitivity; GC/GC-MS has the high sensitivity, but *Alternaria* toxins generally need laborious derivatization, which will lead to poor repeatability of the detection results. Therefore, TLC and GC/GC-MS have been replaced by LC and LC-MS. LC, LC-MS, and especially LC-MS/MS or LC-MS^n have been the mainstay for *Alternaria* mycotoxin detection. Nevertheless, LC and LC-MS require large-scale equipment and highly qualified technicians, so they cannot meet the food safety field's requirement of real-time, rapid or

portable detection. The ELISA method could compensate for the disadvantages of large-scale equipment because of its characteristic of simplicity of operation, miniaturization and portability, and it has been used for the detection of AOH, TeA and AAL toxin TA. With further developments, the ELISA method and other varieties of rapid detection techniques will be established for the detection of *Alternaria* mycotoxins.

Acknowledgements This work was supported by the Special Projects of Construction of Science and Technology Innovation Ability of Beijing Academy of Agriculture and Forestry Sciences (KJCX20150408) and China Postdoctoral Science Foundation (2015M581020).

Compliance with Ethical Standards

Conflict of interest The authors have declared no conflict of interest.

References

1. Scott PM (2001) J AOAC Int 84:1809–1817
2. Terminiello L, Patriarca A, Pose G, Pinto VF (2006) Mycotoxin Res 22:236–240
3. Scott P, Lawrence G, Lau B (2006) Mycotoxin Res 22:142–147
4. López P, Venema D, de Rijk T, de Kok A, Scholten JM, Mol HG, de Nijs M (2016) Food Control 60:196–204
5. Azcarate M, Patriarca A, Terminiello L, Pinto VF (2008) J Food Protect® 71:1262–1265
6. Alexander J, Benford D, Boobis A, Ceccatelli S, Cottrill B, Cravedi J, Di Domenico A, Doerge D, Dogliotti E, Edler L (2011) EFSA J 9:2407–2504
7. López P, Venema D, de Rijk T, de Kok A, Scholten J, Mol H, de Nijs M (2014) Occurrence of Alternaria mycotoxins in food products in the Netherlands. In: World Mycotoxin Forum, Vienna, Austria
8. Logrieco A, Moretti A, Solfrizzo M (2009) World Mycotoxin J 2:129–140
9. Lee HB, Patriarca A, Magan N (2015) Mycobiology 43:93–106
10. Ostry V (2008) World Mycotoxin J 1:175–188
11. Liu G, Qian Y, Zhang P, Dong W, Qi Y, Guo H (1992) Chin Med J Peking 105:394–400
12. Brugger E-M, Wagner J, Schumacher DM, Koch K, Podlech J, Metzler M, Lehmann L (2006) Toxicol Lett 164:221–230
13. Pfeiffer E, Schmit C, Burkhardt B, Altemöller M, Podlech J, Metzler M (2009) Mycotoxin Res 25:3–10
14. Solhaug A, Holme J, Haglund K, Dendele B, Sergent O, Pestka J, Lagadic-Gossmann D, Eriksen G (2013) Toxicol Lett 219:8–17
15. Solhaug A, Torgersen M, Holme J, Lagadic-Gossmann D, Eriksen G (2014) Toxicology 326:119–129
16. Fleck SC, Sauter F, Pfeiffer E, Metzler M, Hartwig A, Koberle B (2016) Mutat Res Gen Tox En 798–799:27–34
17. Pahlke G, Tiessen C, Domnanich K, Kahle N, Groh IA, Schreck I, Weiss C, Marko D (2016) Toxicol Lett 240:93–104
18. Vejdovszky K, Warth B, Sulyok M, Marko D (2016) Toxicol Lett 241:1–8
19. Schrader T, Cherry W, Soper K, Langlois I (2006) Mutat Res Gen Tox En 606:61–71
20. Carrasco L, Vazquez D (1973) BBA-Nucleic Acids and Protein Synthesis 319:209–215
21. Zhou B, Qiang S (2008) Afr J Biotechnol 7:1151–1156
22. Sauer DB, Seitz LM, Burroughs R, Mohr HE, West JL, Milleret RJ, Anthony HD (1978) J Agric Food Chem 26:1380–1383
23. Stack ME, Mazzola EP, Page SW, Pohland AE, Highet RJ, Tempesta MS, Corley DG (1986) J Nat Prod 49:866–871
24. Stack ME, Prival MJ (1986) Appl Environ Microbiol 52:718–722
25. Schrader T, Cherry W, Soper K, Langlois I, Vijay H (2001) Teratogen Carcin Mut 21:261–274
26. Osborne L, Jones V, Peeler J, Larkin E (1988) Toxicol In Vitro 2:97–102
27. Abbas HK, Vesonder R, Boyette C, Peterson S (1993) Can J Bot 71:155–160
28. Abbas H, Tanaka T, Shier W (1995) Phytochemistry 40:1681–1689
29. Kono Y, Gardner J, Takeuchi S (1986) Agric Biol Chem 50:2401–2403
30. Arntzen C (1972) BBA Bioenergetics 283:539–542
31. Hasan H (1995) Mycopathologia 130:171–177
32. Fabrega A, Agut M, Calvo M (2002) J Food Sci 67:802–806
33. Matysik G, Giryn H (1996) Chromatographia 42:555–558
34. Ostrý V, Škarkova J, Prochazkova I, Kubatova A, Malíř F, Ruprich J (2007) J Czech Mycol 59:241–254
35. Fuchs B, Süß R, Nimptsch A, Schiller J (2008) Chromatographia 69:95–105
36. Monteiro MLG, Máralém ET, Lázaro CA, Conte-Júnior CA (2016) J Anal Chem 71:459–470
37. Chulze SN, Torres AM, Dalcero AM, Etcheverry MG, Ramírez ML, Farnochi MC (1995) J Food Protect® 58:1133–1135
38. Pero RW, Owens RG, Harvan D (1971) Anal Biochem 43:80–88
39. Giryn H, Szteke B (1994) Rocz Panstw Zakl Hig 46:129–133
40. Ostry V, Skarkova J, Ruprich J (2004) Occurrence of *Alternaria mycotoxins* and *Alternaria* spp. in lentils and human health. In: Abstracts 26th Mykotoxin-Workshop, Herrsching, Germany
41. Skarkova J, Ostry V, Prochazkova I (2005) Planar chromatographic determination of Alternaria toxins in selected foodstuffs. In: Proceedings of the international symposium on planar separations, planar chromatography, milestones in instrumental TLC, Siofok, Hungary
42. Visconti A, Logrieco A, Bottalico A (1986) Food Addit Contam 3:323–330
43. Köppen R, Koch M, Siegel D, Merkel S, Maul R, Nehls I (2010) Appl Microbiol Biot 86:1595–1612
44. Harvan D, Pero R (1974) J Chromatogr A 101:222–224
45. Scott P, Weber D, Kanhere S (1997) J Chromatogr A 765:255–263
46. Stack ME, Mislivec PB, Roach J, Pohland AE (1984) J Assoc Off Anal Chem 68:640–642
47. Solfrizzo M, De Girolamo A, Vitti C, Visconti A, van den Bulk R (2004) J AOAC Int 87:101–106
48. Kütt M, Lõiveke H, Tanner R (2010) Agron Res 8:317–322
49. Abramson D, Delaquis P, Smith D (2007) Mycotoxin Res 23:147–151
50. Scott P (1991) Prikl Biokhim Mikrobiol 28:819–827
51. Aresta A, Cioffi N, Palmisano F, Zambonin CG (2003) J Agric Food Chem 51:5232–5237
52. Lehotay SJ, Tully J, Garca AV, Contreras M, Mol H, Heinke V, Anspach T, Lach G, Fussell R, Mastovska K (2007) J AOAC Int 90:485–520
53. Lehotay SJ (2005) Kok Ad, Hiemstra M, Bodegraven Pv. J AOAC Int 88:595–614
54. Myresiotis CK, Testempasis S, Vryzas Z, Karaoglanidis GS, Papadopoulou-Mourkidou E (2015) Food Chem 182:81–88
55. Fente C, Jaimez J, Vázquez B, Franco C, Cepeda A (1998) Analyst 123:2277–2280
56. Kralova J, Hajslova J, Poustka J, Hochman M, Bjelkova M, Odstrcilova L (2006) Czech J Food Sci 24:288

57. F-q Li (2000) Yoshizawa T. J Agric Food Chem 48:2920–2924
58. Palmisano F, Zambonin P, Visconti A, Bottalico A (1989) Chromatographia 27:425–430
59. Visconti A, Sibilia A, Palmisano F (1991) J Chromatogr A 540:376–382
60. Xu L, Du L (2006) J Microbiol Meth 64:398–405
61. Suemitsu R, Horiuchi K, Ohnishi K, Hidaka T, Horiuchi M (1991) J Chromatogr A 539:211–214
62. da Motta S, Valente Soares LM (2001) Food Addit Contam 18:630–634
63. Sydenham EW, Thiel PG, Marasas WF (1988) J Agric Food Chem 36:621–625
64. Nawaz S, Scudamore KA, Rainbird SC (1997) Food Addit Contam 14:249–262
65. Magnani RF, De Souza GD, Rodrigues-Filho E (2007) J Agric Food Chem 55:4980–4986
66. Di Mavungu JD, Monbaliu S, Scippo M-L, Maghuin-Rogister G, Schneider Y-J, Larondelle Y, Callebaut A, Robbens J, Van Peteghem C, De Saeger S (2009) Food Addit Contam 26:885–895
67. Monbaliu S, Van Poucke C, Van Peteghem C, Van Poucke K, Heungens K, De Saeger S (2009) Rapid Commun Mass Sp 23:3–11
68. Rasmussen RR, Storm IMLD, Rasmussen PH, Smedsgaard J, Nielsen KF (2010) Anal Bioanal Chem 397:765–776
69. Walravens J, Mikula H, Rychlik M, Asam S, Devos T, Njumbe Ediage E, Di Mavungu JD, Jacxsens L, Van Landschoot A, Vanhaecke L, De Saeger S (2016) J Agric Food Chem 64:5101–5109
70. Rubert J, Dzuman Z, Vaclavikova M, Zachariasova M, Soler C, Hajslova J (2012) Talanta 99:712–719
71. Dzuman Z, Zachariasova M, Lacina O, Veprikova Z, Slavikova P, Hajslova J (2014) Talanta 121:263–272
72. Shephard G, Thiel P, Sydenham E, Vleggaar R, Marasas W (1991) J Chromatogr B Biomed Sci Appl 566:195–205
73. da Motta S, Soares LV (2000) Food Chem 71:111–116
74. Lebrun M, Dutfoy F, Gaudemer F, Kunesch G, Gaudemer A (1990) Phytochemistry 29:3777–3783
75. Siegel D, Rasenko T, Koch M, Nehls I (2009) J Chromatogr A 1216:4582–4588
76. Siegel D, Merkel S, Koch M, Nehls I (2010) Food Chem 120:902–906
77. Mansfield MA, Archibald DD, Jones AD, Kuldau GA (2007) Phytopathology 97:504–511
78. Horiuchi M, Akimoto N, Ohnishi K, Yamashita M, Maoka T (2003) Chromatogr 24:109–116
79. Hickert S, Bergmann M, Ersen S, Cramer B, Humpf H-U (2015) Mycotoxin Res 32:1–12
80. Prelle A, Spadaro D, Garibaldi A, Gullino ML (2013) Food Chem 140:161–167
81. Noser J, Schneider P, Rother M, Schmutz H (2011) Mycotoxin Res 27:265–271
82. Warth B, Parich A, Atehnkeng J, Bandyopadhyay R, Schuhmacher R, Sulyok M, Krska R (2012) J Agric Food Chem 60:9352–9363
83. Varga E, Glauner T, Berthiller F, Krska R, Schuhmacher R, Sulyok M (2013) Anal Bioanal Chem 405:5087–5104
84. Sulyok M, Krska R, Schuhmacher R (2007) Anal Bioanal Chem 389:1505–1523
85. Zachariasova M, Cajka T, Godula M, Malachova A, Veprikova Z, Hajslova J (2010) Rapid Commun Mass Spectrom 24:3357–3367
86. Wang M, Jiang N, Xian H, Wei D, Shi L, Feng X (2016) J Chromatogr A 1429:22–29
87. Vishwanath V, Sulyok M, Labuda R, Bicker W, Krska R (2009) Anal Bioanal Chem 395:1355–1372
88. Jessome LL, Volmer DA (2006) Lc Gc North America 24:498
89. Asam S, Konitzer K, Schieberle P, Rychlik M (2009) J Agric Food Chem 57:5152–5160
90. Rychlik M, Asam S (2008) Anal Bioanal Chem 390:617–628
91. Asam S, Rychlik M (2015) Anal Bioanal Chem 407:7563–7577
92. Asam S, Konitzer K, Rychlik M (2011) Mycotoxin Res 27:23–28
93. Liu Y, Rychlik M (2015) Anal Bioanal Chem 407:1357–1369
94. Asam S, Liu Y, Konitzer K, Rychlik M (2011) J Agric Food Chem 59:2980–2987
95. Asam S, Lichtenegger M, Liu Y, Rychlik M (2012) Mycotoxin Res 28:9–15
96. Asam S, Rychlik M (2013) Eur Food Res and Technol 236:491–497
97. Asam S, Habler K, Rychlik M (2013) Anal Bioanal Chem 405:4149–4158
98. Fraeyman S, Devreese M, Broekaert N, De Mil T, Antonissen G, De Baere S, De Backer P, Rychlik M, Croubels S (2015) J Agric Food Chem 63:8560–8567
99. Walravens J, Mikula H, Rychlik M, Asam S, Ediage EN, Di Mavungu JD, Van Landschoot A, Vanhaecke L, De Saeger S (2014) J Chromatogr A 1372:91–101
100. Lohrey L, Marschik S, Cramer B, Humpf H-U (2012) J Agric Food Chem 61:114–120
101. Liu Y, Rychlik M (2013) J Agric Food Chem 61:2970–2978
102. Szurdoki F, Trousdale E, Gee SJ, Ward B, Hammock BD, Gilchrist DG, Beier R, Stanker L (1996) Immunoass Residue Anal Food Saf 621:330–340
103. Szurdoki F, Trousdale E, Ward B, Gee SJ, Hammock BD, Gilchrist DG (1996) J Agric Food Chem 44:1796–1803
104. Yu W, Yu F-Y, Undersander DJ, Chu FS (1999) Food Agric Immunol 11:307–319
105. Ackermann Y, Curtui V, Dietrich R, Gross M, Latif H, Märtlbauer E, Usleber E (2011) J Agric Food Chem 59:6360–6368
106. Burkin A, Kononenko G (2011) Appl Biochem Micro 47:72–76
107. Gross M, Curtui V, Ackermann Y, Latif H, Usleber E (2011) J Agric Food Chem 59:12317–12322
108. Moressi M, Zon A, Fernandez H, Rivas G, Solis V (1999) Electrochem Commun 1:472–476