

质谱技术在黄曲霉毒素检测中的研究进展

Recent advances of mass spectrometry in the detection of aflatoxin

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摘要:文章对黄曲霉毒素的主要类型进行了分析,对黄曲霉毒素的质谱检测方法和质谱检测黄曲霉毒素在食品中的应用进行了综述,并对其未来发展方向进行了展望。

关键词:质谱技术;黄曲霉毒素;类型;检测方法

Abstract: In this paper, the main types of aflatoxins were analyzed, the mass spectrometry detection methods of aflatoxins and the application of mass spectrometry detection of aflatoxins in food were reviewed, and the future development direction of aflatoxins was prospected.

Keywords: mass spectrometry technology; aflatoxin; type; detection method

黄曲霉毒素(Aflatoxins, AFT)存在于天然污染的食物中,能抵抗热降解^[1],是具有遗传毒性、致突变性和免疫毒性的真菌毒素。不良的储存条件、加工过程有可能会引入 AFT,并导致花生、面粉、香料和坚果等食品污染。而摄入这些被污染的食品会导致各种疾病,如癌症、出生缺陷、消化系统紊乱、生殖功能障碍和免疫抑制^[2]。在发展中国家以及湿热地区,肝癌的发病率与 AFT 的污染密切相关。AFT 有很好的耐热性,需加热至 280~300 °C 才能被分解,因此,在正常的食品加工过程中,这些毒素很难被去除掉。此外,修饰或掩蔽性的霉菌毒素(通常以与糖等极性物质共轭的方式存在,进而转化为极性更强的结合态代谢产物)的毒性可能与原霉菌毒素相同,甚至更高^[3]。

目前,常用的 AFT 分析方法有高效液相色谱法(HPLC)^[4]、酶联免疫吸附法(ELISA)^[5]、荧光光谱法^[6]、

薄层色谱法(TLC)^[7]等,虽然这些方法均被广泛应用,但普遍存在各种问题。例如 ELISA 的检测范围较窄,荧光分光光度法的回收率、精密度以及检测范围较小^[8],且变异系数较大;而便宜且方便的 TLC 技术的主要目的是定性,并不能够精确定量。为了更加准确、快速和高通量地检测食品中 AFT 含量是否超标,质谱技术作为一种独特的定性、定量及分离待测物的有力工具而逐渐被大量采用,其特点是检测限较低,线性关系好,与其他方法可相互兼容,能用于日常生活中的不同食品基质及环境土壤中 AFT 的检测。文章拟对质谱技术检测 AFT 的方法进行综述,为进一步开发有关质谱的 AFT 的分析研究方法提供依据。

1 黄曲霉毒素的主要类型

1.1 黄曲霉毒素 B 类

AFT(图 1)为二氢呋喃香豆素的衍生物,极性小,形成无色至淡黄色的晶体,这些晶体如果大量存在,则会在紫外光下呈强烈的荧光,发出蓝光的为 AFB₁ 类化合物,其中 AFB₁ 是 AF 家族的标志性真菌毒素,毒性最强,其肝脏毒性和遗传毒性已在动物研究中被证实^[9],AFB₁ 及其代谢物可通过食物链在肉鸡肝脏中积聚,人类摄入后会引起慢性或急性肝损伤,甚至肝癌^[10]。几乎所有粮食谷物在适宜条件下均会成为此种化合物的寄生基质。流行病学证据^[11-12]表明,人类接触 AFB₁ 与包括非洲部分地区在内的许多中低收入国家的肝癌发病率之间存在密切联系。而在中国,AFB₁ 一直是被重点控制的对象。

1.2 黄曲霉毒素 M 类

AFM₁ 是潜在致癌物 AFB₁ 的主要氧化衍生物,由肝脏细胞色素 P450 相关酶产生,主要存在于牛乳及乳制品中,其毒性和致癌性与 AFB₁ 基本相似^[13-14]。AFM₁ 可引起生鲜乳安全问题^[15],因此通常被认为是“乳毒素”^[16]。巴氏消毒法无法破坏 AFM₁ 的结构,而牛乳及乳制品作为婴幼儿的主要食物,AFM₁ 的存在对婴幼儿的身体健康构成了极大威胁。目前,已报道的 AFM₁ 的

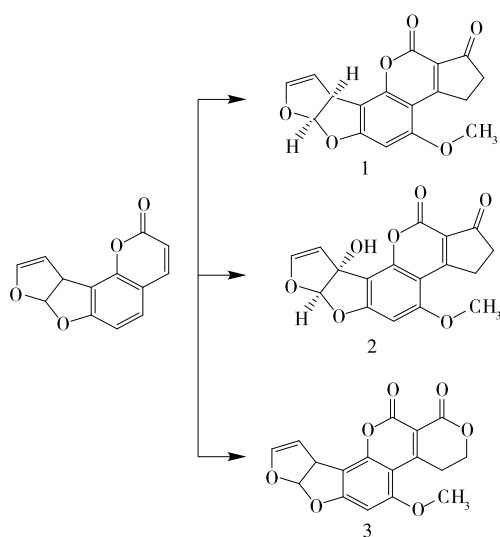
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1. 黄曲霉毒素 B₁ 2. 黄曲霉毒素 M₁ 3. 黄曲霉毒素 G₁

图 1 AFT 的主要化学结构式

Figure 1 The main chemical formula of AFT

分析方法有 ELISA 法^[17-18]、LC 法^[19]等,这些方法虽具有高度特异性和一定的选择性等优点,但耗时、操作繁琐和价格昂贵。质谱技术作为一种高通量、高选择性、普适性的方法来用于准确测定 AFM₁ 是非常适合的。

1.3 黄曲霉毒素 G 类

AFG 类主要包括 AFG₁ 和 AFG₂,其中 AFG₁ 是仅次于 AFB₁ 的第二大毒性 AFT^[20]。动物试验^[21]表明,AFG₁ 会诱导小鼠的肺增生性病变和肺腺癌,也会导致试验动物肝脏肿瘤的发生。文献^[22]报道了 AFG₁ 诱导的肺炎是肿瘤坏死因子(TNF)所依赖的,其增强了炎症肺组织 AT-II 细胞的 DNA 损伤以及细胞色素 P450 (CYP2A13)的表达,同时可诱导与肺泡上皮细胞氧化应激相关的慢性肺部炎症^[23]。在检测 AFG 类化合物时,大多数情况下可采用 HPLC-FLD 和 HPLC-MS 法。如果用 FLD 检测器检测分离的组分,则需要柱后衍生化以增强 AFG₁ 的天然荧光性质^[24]。

2 黄曲霉毒素的质谱检测分析方法

2.1 液质联用法

近年来,液相色谱串联质谱(LC-MS/MS)技术因其可同时监测多种真菌毒素而日益受到重视。其中液相部分从最初的常规 LC,逐渐发展为超高压液相色谱(UHPLC)^[25]、二维液相色谱(2D-LC)、纳升液相色谱(Nano-LC)^[26]等,极大地提升了液质联用技术的检测效率。质谱部分可供选择的离子源包括电喷雾离子源(ESI)、大气压化学电离源(APCI)、大气压光电离源(APPI)、基质辅助激光解吸/电离(MALDI)、实时直接分析质谱离子源(DART)^[27]等。与离子源搭配的质量分析器包括三重四极杆分析器(QQQ)、飞行时间分析器(TOF)^[28]、轨道离

子阱分析器(Orbitrap)^[29]、傅里叶变换离子回旋共振(FT-ICR)^[30]等。MALDI 的广泛应用主要与其电离极性分子有关,该过程几乎完全产生单电荷离子,近 10 年来,该技术作为一种快速、可靠的鉴定方法得到了广泛应用,与通过喷洒样品溶液使分析物分子电离的 ESI 不同, MALDI 分析的样品需要在靶板上与过量的小分子基质(通常是可吸收紫外线的有机酸)共结晶,然后通过激光辐射产生样品离子,其与高质量范围的分析器 TOF 可以很好地结合在一起,只需一次电离就可以观察到高分子量的生物分子。Ramos 等^[31]采用 MALDI-TOF-MS 对 AFB₁、B₂、G₁ 和 G₂ 进行分析,获得了无基质质谱,并加入 NaCl,通过 Na⁺增强灵敏度,从而实现了 AFB₁、B₂、G₁ 和 G₂ 的有效检测。该技术速度快、样品制备少,不需要衍生化或色谱分离,因此适合于 AFT 的高通量筛选。LC 与 UPLC 联用时,其中的 UPLC-Q-TOF-MS^[32]作为机械分离科学中的一个全新类别,借助于 HPLC 的色谱理论及原理,涵盖了小颗粒填料、超低系统体积及快速检测手段等全新技术,增加了分析的通量、灵敏度及色谱峰容量。姚婷等^[33]采用 UPLC-Q-TOF-MS 来分析发酵黑茶中 AFB₁ 的含量,与传统的检测方法相比,其样品前处理过程简单、时间短,有机溶剂用量少,且目标分析物无需衍生即可直接测定,能够显著提高结果的准确性。

2.2 荧光分析法结合质谱法

随着分析技术的发展,生物分析中出现了荧光分析^[34]、比色分析^[35]、化学发光分析^[36]、电化学分析^[37]等新方法。其中,荧光分析(FLD)因其背景强度低、操作简单、检测灵敏度高而备受关注^[38]。但由于 AFT 天然荧光较弱,许多方法在进行前需对样品柱前或柱后衍生化^[39]。基于质谱技术的分析方法具有较高的特异性和灵敏度,并且随着 MS 仪器的广泛使用^[40],该技术的应用变得越来越普遍,其能与 LC-FLD^[41-42]兼容,可用于单一或多种 AFT 的测定,而无需额外的样品处理。Trucksess 等^[41]采用 LC-FLD 法结合三重四极杆质谱联用仪,对玉米、花生和花生酱中的 AFT 总量和单个 AFT 进行测定,其回收率比单独使用 FLD 法高,此种方法适用于植物来源的食品中 AFT 的检测。此外,Hao 等^[43]基于 T7 核酸内切酶辅助的级联循环扩增策略和 DNA 模板银纳米簇(DNA-AgNC)的方法,建立了 AFB₁ 的无标记 FLD 检测新方法。即在靶标 AFB₁ 存在的情况下,通过夹夹 H1 特异性地捕捉靶标,形成复合体 H1-AFB₁,这一步为复合体 H1-AFB₁-H2 的形成创造了条件,此过程会逐步循环放大,并产生大量的序列,这些序列即 AgNC 的合成模板。其显示的荧光强度与 AFB₁ 浓度呈良好的线性关系。该方法与质谱技术联用,已被成功应用于食品样品中 AFB₁ 的测定,检出限可达 0.89 pg/mL。其中所用到的以 DNA

为模板的银纳米(DNA-AgNC)作为一种新型的荧光载体受到了特别的关注,其性能优于有机染料和量子点^[44]。更重要的是,作为 AgNC 合成模板的 DNA 序列可以灵活地编程到探针^[45-46]中,避免了用荧光团的繁琐和麻烦,既节省了试验成本,又节省了人力和时间,是整个质谱检测体系中的新步骤。

2.3 同位素稀释质谱法

自 Hevesy 等^[47]提出同位素稀释法以来,已经按照这种“稀释”原理创建了多种分析方法,在此基础上,同位素稀释质谱法(IDMS)已被广泛地应用于多种有机物和无机物的测定,受到了极大的关注和发展。LC-MS 未使用同位素内标,样品前处理步骤繁琐,目标物损失严重,检出限高,回收率偏低。IDMS 优化了影响试验结果的一系列参数,能够得到最佳的试验条件,可用于谷物及其制品、豆类及其制品、坚果及籽类、油脂及其制品、调味品、婴幼儿食品中 AFB 族和 G 族的测定^[48]。罗兰等^[49]使用同位素内标,并采用 UPLC-MS 的方法来检测婴儿辅食中 AFM₁ 含量,得到的检出限为 0.01 $\mu\text{g}/\text{kg}$,RSD 值均 < 4%。魏敏等^[50]用液相色谱—同位素稀释质谱法来测定婴幼儿配方奶粉中 AFM₁ 基体标准物质研制的定值,其检出限为 0.01 $\mu\text{g}/\text{kg}$,定量限为 0.05 $\mu\text{g}/\text{kg}$ 。Henrion^[51]提出了精确匹配双同位素稀释质谱(EMD-IDMS)的概念。EMD-IDMS 是一种通过有效地消除仪器分析步骤中的偏差,并通过直接连续多次测量两种混合物来减少不确定度的方法。通过尖峰加强要分析的物质,以便产生样品混合物和校准混合物,标准物质的数量使得样品和校准混合物中的同位素比率在双重 IDMS 中“精确”匹配,在这种情况下,所有与仪器有关的偏差均变得可以忽略不计^[52]。与 LC-MS 结合使用时,由于大气压电离源抑制了分析物的电离,快速分析时间和高进样量可能会对 LC-MS 的信号强度产生不利影响^[53-54],但同位素比率在一定程度上不受影响^[55],这既补偿了基质效应的影响,也可有效抵消基质效应和前处理的操作损失,而提高色谱分辨率也是减少基质效应的一种较好的方法,因为在离子抑制的情况下,信号强度有所提高^[56]。稳定同位素稀释法(SIDA)同样是在 IDMS 的基础上发展起来的,Zhang 等^[57]采用 SIDA 与 LC-MS/MS 法同时测定食品中的多种真菌毒素,样品用乙腈/水提取,浓度为 1.0 ~ 1 000.0 ng/g ,回收率的 RSD 值 < 10%,整个过程不仅简化了样品的制备,也实现了样品的同时鉴定和定量分析。

2.4 电感耦合等离子体质谱

2001 年,Zhang 等^[58]提出了基于电感耦合等离子体质谱(ICP-MS)检测的免疫分析方法。随后,ICP-MS 在生物分子分析中的重要性呈指数增长^[59]。与其他成熟的方法相比,ICP-MS 具有谱线简单、稳定性高、特异性强、灵敏度高、易于与多种分离技术耦合的特点。最初,分析

仅限于那些含有 ICP-MS 能检测到的杂原子的物种,容易受到光谱干扰,分析优值系数也会受到影响^[60-61]。为了提高分析优值,以及解决不含杂原子生物分子的测定问题,生物分子可以通过与杂原子或有机金属化合物的化学反应进行衍生化^[62-63],但这种方法的选择性较低。目标分析物也可以通过使用杂原子标记的抗体免疫反应来标记^[64-65]。由于抗原—抗体反应的高度特异性,可以在复杂的混合物中如奶制品中测定 AFT。此过程使用的杂原子标记物包括过渡金属和金属纳米颗粒等,而 Pérez 等^[66]认为,使用金属纳米颗粒是提高分析优值(如灵敏度等)的最有利方法,并通过使用二级生物素化的抗体与链霉素和素结合的金属纳米颗粒的竞争性免疫反应,建立了 ICP-MS 对超痕量牛奶样品中的 AFM₁ 进行检测,在最佳条件下,该免疫分析方法的检出限可低至 0.005 $\mu\text{g}/\text{kg}$ 。以 ICP-MS 为基础发展起来的氢化物—电感耦合等离子体质谱法(HG-ICP-MS)^[67]、气相色谱—电感耦合等离子质谱联用(GC-ICP-MS)^[68]、在线电感耦合等离子体质谱(Electrochemical On-line-ICP-MS)^[69]、四极杆 ICP-MS^[70]、激光消融-ICP-MS(LA-ICP-MS)^[71]、毛细管电泳—电感耦合等离子体质谱(CE-ICP-MS)^[72]结合的方法得到了广泛关注,可为检测 AFT 技术的发展提供参考,尤其是在检测 AFM₁ 时,由于其具有较高的健康风险和限制性的法律要求,可用半抗原模型来评估以上不同方法的优缺点。

3 应用质谱技术检测 AFT 在食品中的应用

3.1 谷物

质谱技术是检测谷物、粮食中 AFT 是否超标的重要步骤。郭芳芳等^[73]采用 LC-MS 对小麦粉中的 AFB₁ 进行测定,样品用甲醇—水提取并用免疫亲和柱净化富集,串联质谱检测后得到的检测限为 0.03 $\mu\text{g}/\text{kg}$,可为检测其他食品中的真菌毒素提供参考。谷叶等^[74]采用 TSQ-MS 检测花生、玉米、燕麦片中的 AFT,其对 AFB₁、B₂、G₁、G₂ 的检出限分别为 0.110,0.009,0.012,0.008 $\mu\text{g}/\text{kg}$,且回收率与精密度较好,可为其在谷物和粮食检测方面提供依据。

3.2 奶制品

奶制品中污染最大的真菌毒素主要是 AFM₁,通过奶牛的尿液和奶汁排出。近年来,液质联用法用于检测奶制品中 AFT 含量具有很大的应用价值,张俊等^[75]采用 LC-MS/MS 检测生鲜乳中 AFM₁ 含量,通过优化质谱参数以达到最大的离子强度,其回收率为 80% ~ 110%,RSD 在 8.4% 以内,重复性良好,此种方法在检测奶制品方面值得推广应用。此外,Campone 等^[76]采用在线固相萃取-UHPLC-MS 对 69 种牛奶样品进行分析,以评估

AFM₁ 的发生率。结果表明,巴氏杀菌牛奶中 AFM₁ 的检出率为 72.7%,其中在线 SPE 清除具有高度的选择性,能为检测奶制品中 AFT 提供新方法。

3.3 调味品

酱油、食用醋等调味品生产中主要使用的是曲霉菌,一定程度上给 AFT 的产生提供了条件^[77],污染严重不仅会对人体产生严重的危害,也会造成巨大的经济损失。为了检测传统调味品中 AFT 含量是否超标,杨春林等^[78]将豆瓣等样品先通过免疫亲和柱净化后再用 UPLC-

TSQ-MS 进行检测,通过调整信噪比,最终得到的检出限为 0.017~0.051 μg/L,提示此方法较为灵敏,能同时测定调味品中的多种 AFT。除 UPLC-TSQ-MS 外,新型质谱联用技术如 QuEChERS 萃取-UPLC-MS/MS 也被广泛应用,梁剑锋等^[79]为了测定花生酱中 AFB₁ 含量,先采用 QuEChERS 净化样品,再用 UPLC-MS/MS 扫描检测,该方法检出限较低,回收率较高,精密度高,作为检测调味品中 AFT 的技术具有较大的推广价值。

表 1 为 AFT 质谱检测技术在食品中的应用。

表 1 AFT 质谱检测技术的分类及应用

Table 1 Classification and application of AFT mass spectrometry detection technology

质谱检测 类型	应用技术	最低检测限(LOD)/ (μg · kg ⁻¹)	线性范围/ (ng · mL ⁻¹)	食品应用	文献
LC-MS	LC-QQQ-MS	0.008 0	0.308~32.450	花生、玉米、燕麦片、 面粉、饲料、生鲜乳	[74-75,80-81]
	Online-SPE UHPLC-MS/MS	0.000 7	0.500~50,000	牛奶,饲料	[76,82-83]
	LC-Orbitrap-MS	0.100 0	0.200~60,000	花生、玉米、大米	[84-85]
	LC-DART-MS	0.100 0	4.000~1 000,000	玉米、牛奶	[86-87]
	UPLC-Q-TOF-MS	0.060 0	0.000~200,000	茶叶	[28,33]
	基于磁珠-适配体的 LC-MS/MS	0.080 0	0.390~50,000	谷物、豆瓣	[88-89]
FLD-MS	SPE-HPLC/FLD 结合 MS	0.250 0	5,000~100,000	高粱	[41,90]
	DNA-AgNC 为模板结合 MS	0.000 9	100,000~1 000,000	花生、玉米、小麦	[43]
ID-MS	IDMS 结合 UPLC-MS	0.010 0	0.050~5,000	婴儿配方米粉、面条	[49-50]
	SIDA 结合 LC-MS/MS	0.005 0	1,000~1 000,000	玉米、花生酱、小麦粉	[57]
ICP-MS	免疫分析法结合 ICP-MS	0.005 0	0,300~50,000	牛奶	[66]

4 结语与展望

近年来,各种质谱新技术不断涌现,已成为推动现代分析技术进步的重要支撑,其高通量、高选择性、高灵敏度、高精密度的特性,可对复杂样品进行实时分析等特点,使其在黄曲霉毒素检测方面的应用越来越广泛。将新型质谱检测技术应用于饲料、烟草、粮食谷物及奶制品中,并扩大到其他领域,可以在最大程度上节省检测时间,提高准确度。虽然传统的检测技术因特异性差、选择性低、灵敏度差、检测限高等缺点应用逐渐减少,但仍不失为较简易的方法。质谱技术在黄曲霉毒素的检测领域虽然具有较多自身优势,但同样存在一些如设备成本较高,对仪器操作人员要求高等缺点。因此如何能够针对不同种类的食品基质,通过优化前处理条件,利用不同种类的质谱技术对样品进行专门检测将成为未来一段时间内较为热门的话题。此外,进一步开发原位、快速、实时、操作简单、高通量的质谱方法,并将其做到便携化、小型化,将极大地推动质谱技术在现场食品黄曲霉毒素检测中的广泛应用。

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