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基于液相色谱-串联高分辨质谱技术的食品中污染物检测技术研究进展

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摘 要: 食品中污染物种类繁多, 且暴露涉及食品原料、加工、运输、储藏、包装和销售等多个环节, 随着人们对食品健康营养安全需求的提高, 食品中的污染物问题受到越来越多的关注。液相色谱-串联高分辨质谱(liquid chromatography-high resolution mass spectrometry, LC-HRMS)技术结合了液相色谱的分离能力和高分辨质谱的质量范围宽、扫描速度快、灵敏度高等优点, 成为食品安全分析领域的重要手段。本文综述了四极杆/飞行时间质谱、四极杆/静电场轨道阱质谱技术的特点以及近 5 年来 LC-HRMS 技术在食品中污染物检测方面的研究进展, 重点讨论了 LC-HRMS 靶向、非靶向测定食品中农药残留、兽药及其代谢物、非法添加物、食品添加剂、毒素等方面的应用, 并对其未来发展趋势进行了展望, 旨在为今后食品中污染物的检测提供参考。
关键词: 液相色谱-串联高分辨质谱技术; 农药残留; 兽药及其代谢物; 非法添加物; 食品添加剂; 毒素

Research progress on determination of pollutants in food by liquid chromatography-high resolution mass spectrometry

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ABSTRACT: There are many types of pollutants in food, and exposure involves multiple stages such as raw material, processing, transportation, storage, packaging, and sales. With the improvement of people's requirements for the healthy, nutritive and safe condiments, the pollutants in food have received more and more attention. Liquid chromatography-high resolution mass spectrometry (LC-HRMS) combines the separation ability of liquid chromatography with the advantages of high resolution mass spectrometry, such as wide mass range, fast scanning speed, and high sensitivity, making it an important means in the field of food safety analysis. This review summarized the technical characteristics of quadrupole-time of flight mass spectrometry and quadrupole-orbitrap mass spectrometry, as well as the research progress in the detection of pollutants in food using LC-HRMS technology over

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the past 5 years. The article focused on the application of LC-HRMS in the targeted and non-targeted determination of pesticides residues, veterinary drugs and their metabolites, food additives, illegal additives and mycotoxins, and prospected the development trend. Aiming to provide reference for the detection of pollutants in food in the future.

KEY WORDS: liquid chromatography-high resolution mass spectrometry; pesticide residues; veterinary drugs and their metabolites; illegal additive; food additives; mycotoxin

0　引　言

随着社会经济的快速发展及人民生活水平的不断提高, 食品的新鲜度、安全性越来越受到大众的关注。食品中污染物种类繁多, 主要分为化学性和生物性两类, 具有积累性、生物毒性、来源广泛性、风险隐蔽性等特点, 其暴露涉及食品原料、加工、运输、储藏、包装和销售等多个环节, 是危害食品安全的主要因素[1–3]。研究表明, 这些污染物可能通过食物链累积对人体健康带来不良影响, 包括损伤免疫系统、干扰内分泌等[4–6]。受污染物种类、前处理方法、基质干扰等因素影响, 食品污染物检测仍面临诸多挑战[7–8], 目前关于污染物的研究正从单一组分检测转向多组分分析, 从已知污染物定量检测转向未知物的非靶向筛查。

目前食品中污染物的检测多以色谱、低分辨质谱为主, 低分辨质谱虽在定量检测上具有突出优势, 但受限于灵敏度和分辨率的不足, 难以实现对不同类型不同结构的污染物进行全面的、精确的鉴别。液相色谱-串联高分辨质谱技术(liquid chromatography-high resolution mass spectrometry, LC-HRMS)能通过高分辨率全扫描获得目标物精确质量数, 将复杂基质中干扰物与目标分析物区分开[9–10], 同时能降低对标准品的依赖, 实现对复杂基质中痕量化合物高通量定量分析及未知物的非靶向筛查[11–13]。目前以液相色谱-四极杆串联飞行时间质谱法(liquid chromatography-quadrupole-time of flight mass spectrometry, LC-Q-TOF)和液相色谱-四极杆串联静电场轨道阱质谱法(liquid chromatography-quadrupole-orbitrap mass spectrometry, LC-Q-Orbitrap)为代表的 LC-HRMS 在食品污染物的检测呈逐年上升趋势[14–16], 因此本研究对近 5 年来 LC-HRMS 技术在食品中农药残留、兽药残留、天然或加工产生的毒素、添加剂和非法添加物等方面的研究进展进行了综述, 旨在为食品安全检测技术的开发和研究提供理论参考。

1　HRMS 概述

目前在食品污染物检测领域应用最多的为 Q-TOF 与 Q-Orbitrap, 同三重四极杆串联质谱法(triple quadrupole tandem mass spectrometry, QqQ)相比, Q-TOF 是将四极杆与飞行时间质谱仪串联起来的高分辨质谱法, Q-Orbitrap 是将四极杆与傅里叶变换静电场轨道阱质谱仪串联起来的高分辨质谱法, 如图 1 所示[17]。Q-TOF 的扫描速度非常快, 在高通量筛查方面具有突出优势, 多用于大分子和复杂样品的检测分析。Q-Orbitrap 的质量精度较 Q-TOF 更高, 设备整体尺寸较小, 但其扫描速度相对较慢。因其分辨率高、稳定性好的特点, 更适用于化合物化学结构的鉴定, 近年来多用于非靶向未知物筛查。

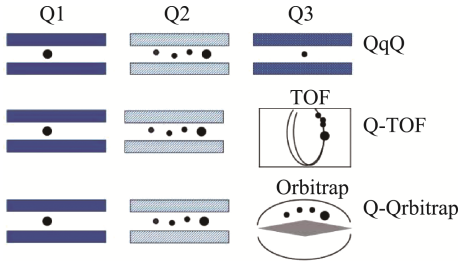

图 1　质谱结构图

Fig.1　Structure diagram of mass spectrum

仪器分辨率取决于质荷比范围和扫描速度[18]。同为高分辨质谱, Q-TOF 与 Q-Orbitrap 存在一定的区别, 如表 1 所示 Q-TOF 在高 *m*/*z* 时具有最佳质量分辨率, Q-Orbitrap 则在低 *m*/*z* 时具有最佳质量分辨率; 其次, 两者的扫描速率不同, Q-TOF 的扫描速率较高(大于 20 Hz), 最快的显示扫描速率≥100 Hz, 但通常以 20~50 Hz 使用更为频繁, Q-Orbitrap 扫描速率取决于所选的分辨率, 在 *R*=140000、70000、35000 和 17500 (*m*/*z*=200)时, 扫描速率分别为 1.5、3.0、6.0 和 12.0。

表 1　Q-TOF 与 Q-Orbitrap 参数区别

Table 1　Differences in Q-TOF and Q-orbitrap parameters

仪器	适宜质量范围	典型分辨率	扫描速率/Hz
Q-TOF	高 *m*/*z*	15000~50000	>20
Q-Orbitrap	低 *m*/*z*	35000~140000	1.5、3.0、6.0、12.0

2　在食品污染物检测方面的应用

2.1　在农药残留检测方面的应用

农药在农产品虫害防治方面起到重要的作用, 但部分农药会经水、空气、土壤等途径被植物吸收, 经过食物链的逐级富集, 导致最终产品的农药残留超标, 人类长期食用农药残留超标的食物可能会引起慢性中毒。目前, 国

内外常用的农药高达千余种，主要分为有机磷类、有机氯类、拟除虫菊酯类等。GB 2763—2021《食品安全国家标准 食品中农药最大残留限量》对 548 种农药制定了限量要求，农业农村部《禁限农药名录》中有 56 种农药禁止使用，12 种农药在部分范围内禁止使用。研究表明，同类型不同名称的农药会产生毒性积累[19]，因此食品中农药残留的高通量靶向、非靶向检测显得尤为重要。

2.1.1 农药残留靶向检测

近年来，LC-HRMS 在农药残留靶向检测方面的应用逐渐增加，基质涉及果蔬、豆类、畜禽、水产、巴氏杀菌乳等，前处理多采用 QuEChERS 法，表 2 为 LC-HRMS 在食品中农药残留的应用实例。韩梅等[33]分别考察了未净化法、QuEChERS 法、氨基柱固相萃取法(solid-phaseextraction, SPE) 3 种不同前处理方法对 125 种农药残留提取效率，该实验证明了 SPE 法净化效果最佳，能极大地降低基质效应，但毒死蜱等 11 种化合物无回收，未净化法和 QuEChERS 法适用大部分农药的检测，此实验对不同农药残留的检测具有一定指导意义。杨明等[34]采用 QuEChERS-高效液相色谱法-Q-TOF/MS (QuEChERS-high performance liquid chromatography-Q-TOF/MS, QuEChERS-HPLC-Q-TOF/MS)快速筛查蔬菜中 32 种农药残留，该方法较国家标准方法极大的缩短了前处理时间，降低了吡虫啉、虫酰肼等 9 种农药的检出限。罗丽娟等[35]构建了 418 种农药高分辨质谱数据库，该方法可对粮食作物历史采集数据进行回溯定性及半定量筛查，无需重新采集样品，极大地缩短了粮食中农药残留风险评估的时间。

2.1.2 农药残留非靶向检测

目前针对农药残留的非靶向检测研究主要集中在方法学的探究上，如前处理的改进、高分辨质谱方法开发等，对于化学计量学方法的优化以及基于非靶向检测的实际应用较少。唐雪妹等[36]开发了一种基于 UPLC-HRMS 的农药残留识别与确认的非靶向筛选策略，建立了 651 种农药高分辨二级碎片谱图数据库。该方法在原有商品化高分辨二级碎片谱图库的基础上，引入保留时间校正策略，拓宽了外部数据库适用度，提高定性筛查准确性，适用于未知农药残留的快速筛查与定量分析。徐红斌等[37]采用 QuEChERS 法进行前处理，结合自建的农药数据库对上海市流通环节 200 批次蔬菜进行了农药残留的快速非靶向筛查。可能由于该研究农药覆盖较广，发现 200 批次样品均存在农药多残留的情况，且检出率略高于其他报道的上海蔬菜农药残留检出率。此方法的成功应用对食品安全抽检及膳食摄入风险评估等工作提供了支撑。LIANG 等[38]采用 HPLC-Q-TOF/MS 技术，筛查了 2012—2018 年期间 37462 份果蔬样品中禁用农药残留，结果表明 66.62%的样品中至少检出 1 种农药，风险评估显示，11.71%的阳性样品超过安全限值，构成不可接受的风险，而 37.29%的阳性样品构成可接受的风险，评估了与禁用农药残留相关的膳食暴露风险。

2.2 在兽药及其代谢物检测方面的应用

兽药可预防和治疗动物疾病，在水产、畜禽养殖过程中起到了重要的作用。常见的兽药有喹诺酮类、磺胺类、激素类、大环内酯类、硝基咪唑类等。动物源性食品基质较为复杂、兽药类别多样，虽现有兽药残留的检测标准众多，但通常一类化合物一种检测方法且前处理复杂。随着 LC-HRMS 的不断发展，兽药残留有望打破技术壁垒。目前，LC-HRMS 在兽药残留检测方面的应用较多，提取溶剂多采用乙腈或酸化乙腈，对于四环素类药物常加入乙二胺四乙酸(ethylene diamine tetraacetic acid, EDTA)缓冲溶液以与基质中的金属离子发生络合反应提高提取效率。净化方式多采用 PRiME HLB，也有部分报道采用 QuEChERS，详见表 3。

表 2 LC-HRMS 在食品中农药残留的应用

Table 2 Application of LC-HRMS to pesticide residues in food

序号	仪器	前处理	基质	检测项目	定量限/(μg/kg)	文献
1	UPLC-Q-Orbitrap	QuEChERS	蔬菜	例行监测的 61 种农药	1~10	[19]
2	UPLC-Q-Orbitrap	QuEChERS	豆类杂粮	甲拌磷等 53 种农药	1~10	[20]
3	UPLC-Q-Orbitrap	QuEChERS	水产	甲胺磷等 62 种农药	2~10	[21]
4	UPLC-Q-Orbitrap	改良 QuEChERS	鸡蛋	除草剂、杀菌剂等 5 类 94 种农药	1~10	[22]
5	UPLC-Q-Orbitrap	改良 QuEChERS	鸡肉	双酚胺类和双酚肼类 13 种农药	0.2~1.0	[23]
6	UPLC-Q-Orbitrap	改良 QuEChERS	茶叶	166 种农药	10~25	[24]
7	UPLC-Q-Orbitrap	改良 QuEChERS	蓝莓	亚砜磷等 90 种农药	0.2~20.2	[25]
8	UPLC-Q-Orbitrap	QuEChERS	猪肉、鸡肉	157 种农药	0.2~8.8	[26]
9	LC-Q-TOF	改良 QuEChERS	芒果、香蕉、释迦和莲雾	8 种新烟碱类杀虫剂、25 种杀菌剂 33 种农药	0.5~50	[27]
10	LC-Q-Orbitrap	QuEChERS-冷冻诱导液液萃取	蔬菜、水果	77 种农药	0.05~0.5	[28]
11	LC-Q-TOF	改良 QuEChERS	牛肉	129 种农药残留	0.003~11.37	[29]
12	UPLC-Q-Orbitrap	PRiME HLB	杨梅	29 种农药残留	6	[30]
13	UPLC-Q-TOF	改良 QuEChERS	蔬菜、水果	420 种农药残留	0.1~5	[31]
14	UPLC-Q-TOF	改良 QuEChERS	青菜	214 种农药残留	0.206~18.125	[32]

注：超高效液相色谱(ultra performance liquid chromatography, UPLC)。

表 3　LC-HRMS 在食品中兽药残留的应用

Table 3　Application of LC-HRMS to veterinary drugs in food

序号	仪器	前处理	基质	检测项目	定量限/(μg/kg)	文献
1	LC-Q-Orbitrap	提取: 80%乙腈-水溶液(含 0.2%甲酸) 净化: Oasis PRiME HLB	畜禽肉	108 种兽药残留	0.2~100	[39]
2	UPLC-Q-Orbitrap	提取: 乙腈、乙酸乙酯 净化: PSA	鱼	112 种兽药残留	2.0	[40]
3	UPLC-Q-TOF	提取: 乙腈、0.1 mol/L EDTA	牛奶	89 种抗生素	0.1~9	[41]
4	UPLC-Q-Orbitrap	液液微萃取	养殖鱼	52 种兽药残留	2~50	[42]
5	LC-Q-Orbitrap	提取: 80%乙腈-水溶液(含 0.2%甲酸) 净化: Oasis PRiME HLB	猪、鸡、鱼	14 类 160 种兽药残留	0.5~50	[43]
6	UPLC-Q-Orbitrap	提取: 10 mmol/L EDTA-2Na 溶液+乙腈:甲醇:水(3:1:1, 含 1%乙酸) 净化: Oasis HLB	鱼、虾、贝	11 类 79 种兽药残留	0.2~5.0	[44]
7	LC-Q-Orbitrap	提取: 乙腈:水(8: 2) 净化: /	水产品	15 类 123 种兽药残留	0.1~50	[45]
8	LC-Q-TOF	提取: 甲醇, 0.1%甲酸, 0.1 mol/L EDTA 净化: /	猪肉	65 种兽药残留	/	[46]
9	UPLC-Q-Orbitrap	提取: 80%乙腈-水溶液(含 0.2%甲酸) 净化: Oasis PRiME HLB	动物源性食品	21 类 155 种兽药残留	0.1~10	[47]
10	UPLC-Q-TOF	提取: 1%甲酸乙腈 净化: Oasis PRiME HLB	猪肉、牛肉	30 种兴奋剂类	0.2~4.0	[48]
11	LC-Q-TOF	提取: 乙腈、乙酸乙酯 净化: QuEChERS(增强型脂质吸附剂)	牛肉、鸡肉、猪肝	32 种激素类	3.0~30	[49]
12	UPLC-Q-Orbitrap	提取: 0.2%甲酸乙腈 净化: Oasis PRiME HLB	乳制品	60 种抗生素	5.0~10.0	[50]
13	UPLC-Q-Orbitrap	提取: 乙腈 净化: EMR-Lipid 净化柱	猪肉	阿托品、普鲁卡因	0.10	[51]
14	UPLC-Q-Orbitrap	提取: 乙腈 净化: 正己烷	猪肉	9 种大环内酯类	0.1~0.4	[52]
15	UPLC-Q-Orbitrap	提取: 1%甲酸乙腈 净化: BONDESIL-SAX、C_{18}	鸡蛋	磺胺喹恶啉、二甲氧苄啶	0.3~0.6	[53]

注: N-丙基乙二胺(N-propyl ethylenediamine, PSA); /代表文献中无相关表述。

近年来国家市场监督管理总局关于兽药残留不合格的通报中样品多为水产品, 其中以恩诺沙星检出率最高。DAI 等[54]应用 UPLC-Q-Orbitrap 结合 Compound Discoverer 软件分析, 首次检测并鉴定出了除环丙沙星以外的另一种恩诺沙星代谢物 deethylene-ENR, 同时在 14 份鱼肉样品中检测出 12 份有环丙沙星及 deethylene-ENR。目前恩诺沙星的含量是以恩诺沙星与环丙沙星之和计, DAI 的发现为恩诺沙星的判定提供了新的参考。LI 等[40]以 PSA 为吸附剂开发了一种可一步净化的新型净化装置, 用于鱼肉样品中 112 种兽药的筛查和定量, 并对多个城市鱼肉样品中兽药残留进行了评估, 该方法可用于食品安全管理中兽药残留的多残留监测。欧阳少伦等[45]建立了在线净化/LC-Q-Orbitrap 快速筛查水产品中 123 种药物残留, 方法最低筛查浓度水平能满足水产品中药物残留的限量要求, 有效提高了水产品中兽药残留的检测效率。潘晓东等[55]通过对猪肉空白基质加标的形式, 分别比较了数据依赖分析(dd-MS2)、全离子碎片分析和数据独立分析(data independent analysis, DIA) 3 种数据采集模式检测结果, 该方法表明 3 种质谱采集方式在低水平的兽药残留筛查时均存在遗漏, 相对而言 DIA 模式能获得较多的产物离子。ZHU 等[56]开发了一种基于 HRMS, 用于可疑和未知外源性物质的回顾性分析的策略, 鉴定出猪肉样品中 48 种“未知”兽药为外源性药物。LIANG 等[57]构建了 3710 种兽药及其代谢物的数据库, 通过总结其质谱裂解特征, 建立了基于碎片特征的非靶向程序, 可对未知化合物及其代谢物进行筛查, 并将该程序应用到 33 份鸡蛋样品中, 鉴定出 4 种兽药 3 种代谢产物。

2.3　在非法添加物检测方面的应用

食品中的非法添加物包括食品中可能违法添加的非食用物质以及食品中可能滥用的食品添加剂。自 2008 年以来, 全国打击违法添加非食用物质和滥用食品添加剂专项整治领导小组发布了 5 批《食品中可能违法添加的非食用

物质和易滥用的食品添加剂名单》，包括工业染料、罂粟壳、荧光增白物质、吊白块等。

2.3.1 违法添加的非食用物质检测

违法添加的非食用物质多为人工合成化合物，随着监管力度不断加大，新的违法添加物也在不断增多，近年来 LC-HRMS 在违法添加的非食用物质应用逐渐增多。食品基质较为复杂，在 HRMS 全扫模式下，食品基质中许多天然物质会对目标物造成一定的干扰，饶雅琨等[58]先通过比对紫外光谱发现了一种未知新型伐地那非类似物，然后采用 UPLC-Q-Orbitrap 对其结构进行质谱解析，最后通过标准品比对确定该物质为 O-丙基伐地那非，该研究为层出不穷的新型那非类非法添加物的筛查提供了重要解题思路。林佳曼等[59]用乙腈润湿的一次性碳纤维束取样头直接对样品进行吸附取样，建立了食品中 8 种非法染料的快速筛查方法，该方法几秒钟即可实现对单个样品的分析，为食品安全检测提供了更精准、高效的检测手段。徐红斌等[60]将研究聚焦在非法添加物的衍生物上，建立了减肥抗衰保健食品中 32 种非法添加药物的快速筛查和鉴定方法，同时采用 Trace Finder 软件建立高分辨数据库，总结了数据库应用过程的要点，并将该方法应用到了实际检测工作中。何嘉雯等[61]建立了凉茶中如解热镇痛药、糖皮质激素药等 167 种非法添加物的筛查方法及准确的质谱数据库，突破检测局限，实现了非法添加药物的高通量、高精度筛查，为打击凉茶非法添加提供了新的技术支撑。宁霄等[62]采用 QuEChERS 净化结合 SWATH 全息式数据非依赖型采集定量的 UPLC-Q-TOF-MS 分析方法对老年乳粉中 300 种非法添加化学药物进行快速测定，同时采用应用电子激活解离裂解技术建立了未知物的非靶向筛查及鉴定策略，该方法在 60 份样品中检出苯乙双胍、西地那非 2 种非法添加药物，阳性样品检出率为 5%，并鉴定出乙非他明这种已撤出市场的减肥药品。

2.3.2 滥用的食品添加剂检测

食品添加剂可改善食品的色、香、味被广泛应用在食品中，但摄入过量食品添加剂会对身体造成一定影响。目前国家标准中大多食品添加剂采用液相色谱检测，LC-HRMS 在食品添加剂的应用较少。冯峰等[63]采用亲水作用 LC-Q-Orbitrap 建立了白酒和饮料中天然甜味剂甜菊糖苷的分析方法，并通过研究甜菊糖苷类化合物的裂解规律，发现此类化合物的 2 个共性碎片离子，实现了对样品中未知甜菊糖苷类化合物的非靶标筛查。刘松等[64]采用 UPLC-Orbitrap 建立了白酒中 16 种甜味剂的快速筛查方法，并对市售白酒进行检测，发现 3 款白酒检出蔗糖，1 款检出甜菊苷。何强等[65]采用 UPLC-Orbitrap 建立了奶粉中 10 种 N-亚硝胺类化合物残留快速检测方法，并在 16 批奶粉中发现 14 批奶粉含有 N-亚硝基-N-乙基苯胺，11 批奶粉含有 N-亚硝基哌啶，对于奶粉中 N-亚硝胺类化合物的质量安全控制具有积极作用。

2.4 在毒素检测方面的应用

目前采用 LC-HRMS 法在食品中毒素检测方面的应用，多为真菌毒素和蘑菇毒素。由于 α-鹅膏毒肽的同位素峰会影响 β-鹅膏毒肽的检测，因此无法通过低分辨质谱进行区分，贺丽迎等[66]建立了一种 UPLC-Orbitrap 方法测定蘑菇中 5 种鹅膏肽类毒素，并将 γ-鹅膏毒肽的异构体鉴定为鹅膏酰胺，该方法简便、准确、特异、灵敏，可在公共卫生紧急情况下对化合物进行快速定性、定量检测。王昌钊等[67]以番茄为研究对象，建立了 UPLC-Orbitrap 法测定 11 种真菌毒素，该方法利用一级质谱精确质量数定性筛查，二级碎片离子定量检测，实现了番茄中真菌毒素的快速筛查和准确定量，同时在 20 份番茄样品检出 2 份含有交链孢酚、链孢酚单甲醚。何卓霖等[68]将乳制品经酸化乙腈提取后，采用 Captiva-EMR Lipid 净化，C_{18} 色谱柱色谱分离，质谱采用全扫描/数据依赖的二级扫描模式，外标法定量检测 21 种真菌毒素，该方法具有较高的适用性和推广性。韦环等[69]应用 UPLC-Orbitrap 技术建立了蜂蜜中 20 种植物源毒性成分的快速筛查和测定方法，并建立了相应的质谱数据库，在实际样品中检出了东莨菪碱、倒千里光碱和 N-氧化千里光菲灵碱，食用蜂蜜存在一定的食品安全风险。

3 结束语

本文主要综述了 LC-HRMS 技术在食品中污染物检测方面的研究情况，包括农药残留、兽药残留、非法添加及毒素等。LC-HRMS 技术以其宽质量范围、高精密度及高分辨率等优势在定性筛查方面具有巨大优势，很好的弥补了低分辨质谱宽泛性不够、依赖标准品等不足。目前已报道的研究多集中在农兽药残留高通量定向方法开发，非靶向筛查及实际应用检测分析相对较少。LC-HRMS 非靶向筛查尚处于发展阶段，但距标准化仍有一段距离，主要原因为：高昂的设备费用和维护成本；对专业技术人员的高需求；数据量大，极度依赖数据库。HRMS 技术的推广中数据分析仍然是研究难点，未来需多个学科交叉融合，综合运用分析化学、统计学、信息科学技术等建立国际认可、统一通用的集成化数据库，以支持食品中污染物非靶向监管需求。

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