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色谱质谱技术在食品安全分析检测中的应用

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摘　要: 食品安全是关系国计民生的重要议题。准确、深入、高效的食品安全分析检测技术是防控食品安全事件发生及处理相关贸易纠纷、立法追责的基础和保障。色谱质谱技术作为高效的分离和检测手段是目前食品安全分析领域最重要、最主流的技术手段。近年来, 色谱质谱领域的新发展也推动了食品安全分析检测向更快速, 更有效, 更可靠, 更安全的目标迈进, 本综述就其中最重要并有望代表未来食品安全分析发展趋势的色谱质谱技术做了总结和点评。如用于食品样品预处理的固相微萃取技术; 气相色谱及气相色谱质谱联用技术中的快速气相色谱方法及其与质谱联用方法, 二维气相色谱方法及其与质谱联用方法; 液相色谱及液相色谱质谱联用技术中的超高效液相色谱及其与质谱联用方法, 毛细管液相色谱和纳流液相色谱及其与质谱联用方法, 超临界色谱及其与质谱联用方法, 二维液相色谱及其与质谱联用方法; 质谱分析技术中的超高分辨率质谱方法, 常压敞开式离子源质谱技术等。

关键词: 食品分析; 食品安全; 样品预处理; 气相色谱法; 液相色谱法; 质谱法

Application of chromatography and mass spectrometry in food safety analysis

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ABSTRACT: Food quality and safety have become one of the most concerns in recent years. Demand for the development and the applications of more efficient and more powerful tools to analyze food components and food contaminants have significantly increased. Chromatography and mass spectrometry are such tools that play major roles in food safety analysis. This review described the application of technology advances of chromatography and mass spectrometry in analysis of known and unknown compounds in food matrix. The summarized development included solid phase microextraction, high speed gas chromatography and its combination with mass spectrometry, two-dimensional gas chromatography and its combination with mass spectrometry, ultra-high pressure liquid chromatography and its combination with mass spectrometry, capillary-liquid chromatography and nano-liquid chromatography and their combination with mass spectrometry, supercritical fluid chromatography and its combination with mass spectrometry, two-dimensional liquid chromatography and its combination with mass spectrometry, high-resolution mass spectrometry and ambient mass spectrometry.

KEY WORDS: food analysis; food safety; sample pretreatment; gas chromatography; liquid chromatography; mass spectrometry

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1　引　言

食品安全是公共安全的重要组成部分, 是关系民生的重大议题。在全球化的时代, 食品安全也演变为一个国际化的问题, 食品安全对大众生命健康的影响会产生全球联动, 同时也有以食品安全问题为借口制造技术壁垒和贸易摩擦的现象。

威胁食品安全的有害物质主要分为内源性和外源性有害物质。内源性有害物质主要来源于自身携带或作为食品原料的动植物在生长过程中受到的农用化学品滥用以及环境污染的影响。外源性有害物质主要来源于食品添加剂的不当使用或在食品加工、包装、储存、运输和销售等环节所引入的有害物质。食品安全问题的解决涉及政治经济、政策法规等多方面因素, 而监督把控食品安全需要先进健全的食品分析检测方法做保障。威胁食品安全的有害物质具有多样性、多源性、微量性以及所处基质复杂等特性, 使得食品安全检测面临严苛考验, 也成为分析科学尤其是仪器分析科学的研究热点。和其他领域类似, 在食品安全分析领域, 虽然光谱、核磁共振波谱、电化学等分析方法也能针对特定的一类化合物实现高灵敏度的准确检测, 但最普适最强有力的分离分析手段依然是色谱质谱联用, 尤其是液相色谱质谱联用技术。本综述就近年来国际范围内色谱质谱技术在食品安全分析检测应用方面的主要进展进行详细调研。

2　食品安全分析中的样品预处理技术

同其他领域的分析工作一样, 食品安全分析当中样品预处理也是最耗时耗力的环节。食品形态多样基质复杂, 为其中的有害物质尤其是痕量物质的分析带来极大困难, 分离检测的第一步是要将目标分析物从食品基质中提取富集。最新的研究进展都致力于将样品预处理技术向更快速、更简便、更经济、更有效、更可靠、更安全(quick, easy, cheap, effective, rugged, and safe, QuEChERS)的目标发展[1]。故传统的溶剂萃取技术以及液液萃取的新形态如快速溶剂萃取和液液微萃取等将不再赘述, 本节侧重于固相微萃取技术的发展对食品安全分析的推动。

固相微萃取技术(solid phase microextraction, SPME)是近年来发展迅速的样品预处理手段, 能够一次性完成样品的采集、萃取、富集以及上样, 基本不消耗溶剂, 满足 QuEChERS 标准。SPME 的主要优势是它的装置极易小型化、自动化, 因此它更易与色谱分离装置联用。基于分配原理的 SPME 技术的核心是萃取固定相, 它决定了对于分析物的萃取选择性及萃取效率。因此, 同其他应用领域一样, SPME 在食品安全分析方面的发展也主要集中在 SPME 萃取固定相的改进创新。

针对食品安全分析领域中不同的目标分析物, 溶胶凝胶(sol-gel)技术是制备不同特性 SPME 萃取相的最有效手段[2-4]。溶胶凝胶法制备的 SPME 具有更好的热稳定性, 因此能够使得分析物在较高的温度下解吸附, 更好的与气相色谱(GC)兼容。同时, 溶胶凝胶法可以在基底上通过共价键合的方式制备萃取相, 从而大大提高萃取相的机械稳定性, 使得 SPME 能够和液相色谱(LC)更好地兼容[3-5]。这样就使 SPME 技术既适用于食品安全分析中的易挥发性物质又适用于难挥发性物质。在食品安全分析中, 溶胶凝胶法制备 SPME 萃取相涂层报道最多的是冠醚类涂层[4,6]。因为冠醚类涂层可通过调整环数来调控涂层极性, 通过调控环内杂原子来调控涂层电负性, 同时冠醚类化合物的 3D 结构也能够增加萃取相的比表面积。

除溶胶凝胶技术外, 离子液体也越来越多的被应用于 SPME 萃取相用作食品安全分析[7]。通过调节离子液体的阴阳离子可以根据特定目标分析物制备出具有特定选择性萃取富集的 SPME 涂层。如制备 1-vinyl-3-hexadecylimidazolium hexafluorophosphate 涂层能选择性萃取菊酯类农药[8]。食品基质中的杀虫剂检测也常用聚二甲基硅氧烷(PDMS)涂层的 SPME[9]。

在固相萃取中被广泛应用的分子印迹技术(molecular imprinting techniques, MIPs)同样被 SPME 所关注。在食品基质中, 目标分析物通常与基质组分结合紧密, 合成特定的分子印迹材料能够显著增强萃取的选择性和灵敏度。如将 MIPs-SPME 与气相色谱质谱(GC-MS)联用可实现对三嗪类除草剂的高灵敏度检测[10-12]。以 Sudan I 染料为模板制备的 MIPs-SPME 与高效液相色谱联用(HPLC)可高选择性高灵敏度检测辣椒粉及家禽饲料中的 Sudan I~IV 染料[13,14]。诸如碳纳米管(CNTs)这样的明星材料也被引入 SPME, 用于牛奶中己烯雌酚的预富集, 能够有效剔除食品基质中非常难处理的脂肪基质[15]。碳纳米管也被用于苹果所带痕量氨基甲酸酯农残的高效富集, 富集倍数达 49~308 倍[16]。

尽管 SPME 有诸多优势且发展迅速, 但该技术用于食品样品预处理依然有局限性, 主要是因为缺少相应的标准化流程, 使得实验室间的方法重现性难以保证, 无法将其发展为常规检测的标准方法[17]。所以在开发 SPME 萃取相材料的同时, 拓展现已标准化的 SPME 装置在食品安全分析领域的应用也非常关键。例如, 对酒精饮料中影响其风味、口感、品质的多种化合物的分离提取[18,19], 对封酒用木塞中的氯苯甲醚的萃取检测[20,21], 对腐败食材中腐臭味物质的富集提取[22,23], 对土豆中含硒类化合物的富集检测[24], 苹果中酚类物质的高通量提取[25], 蜂蜜来源地分析[26]等等都已在商品化 SPME 装置上实现。

3　食品安全分析中的色谱分离及色质联用技术

气相色谱和液相色谱是最为主流的高效分离技术。前

者发展较早更加稳定成熟，广泛应用于食品安全分析的各个分支领域，部分已成为标准检测方法，但气相色谱不能直接分析难挥发、热不稳定、强极性、较大分子量的物质，其应用只能涵盖食品安全分析对象的有限部分。相比较而言，液相色谱不仅能够弥补气相色谱的局限性，且从分离模式到仪器装置上的创新发展都更有生命力，已经占据了食品安全分析的绝大多数领域，更受科研工作者的关注。近年来，质谱作为一种高灵敏度、高兼容性的分子水平分析检测器已成为色谱分离最重要也最强大的检测器，相关串联技术也发展迅速，气相色谱-质谱联用(GC-MS)或液相色谱-质谱联用(LC-MS)已被广泛应用于食品安全分析，因此，本综述若非特殊说明，相关色谱分离方法均以质谱为检测器，属于色质联用的范畴。

3.1 气相色谱法(GC)及气质联用法(GC-MS)

3.1.1 快速气相色谱

常规气相色谱方法在食品安全分析领域的应用在此不再赘述，本节主要侧重于气相色谱方法开发的新趋势，主要是高速气相色谱在快速食品分析的应用以及多维气相色谱在复杂食品分析和高通量分析中的应用。

快速气相色谱技术主要是缩短了有效分离过程的时间，同时又保留和传统 GC 方法相当的分离度。快速 GC 方法所利用的色谱柱内径更小(0.1 mm id 代替 0.25 mm id)，长度更短(10 m 代替 30 m 或 60 m)，固定相涂层厚度更薄(0.1 μm 代替 0.25 μm)，使得固定相所带来的传质阻力更小。相应的，快速 GC 所需的载气速度也更高，可达 65~70 cm/s，且载气使用氢气，因为载气的分子量越小，溶质在流动相里的扩散系数越大，越有利于快速分离的实现[27]。理论研究发现，快速 GC 的程序升温速率应该控制在每个死时间单位不超过 10 ℃[28]，否则分析物没有充裕的时间在固定相中分配会降低分离效率。因此，快速 GC 方法的开发也要求有能够匹配分离速度的检测器。

对食品安全分析而言，即使是易挥发组分，其蒸汽压或极性的分布范围也较宽，快速 GC 方法目前不能全部涵盖。快速 GC 方法在食品安全分析的应用主要集中在脂肪酸酯类[29,30]、动植物油或精油类[31, 32]、农药类的检测[33-36]。

3.1.2 二维气相色谱法(GC × GC)

快速 GC 依然是一维气相色谱，增加分离维度的二维气相色谱(GC×GC)是扩大峰容量、提高检测灵敏度和获得更好分离度的有力手段，所以二维气相色谱尤其是全二维气相色谱在痕量分析、族组成分析和指纹分析方面独具优势。二维分离最理想的状况是正交分离，即两个维度的分离机制完全正交。实际所用的二维分离最起码是两个维度的分离机制不同。

GC × GC 技术中最关键的是连接两个维度色谱柱的接口。全二维 GC × GC 的接口既要保证全部样品能够经历两个分离维度，又要保证在第一维已实现的分离能够在第二维继续保持。所以 GC × GC 的接口要起到捕集、聚焦并快速释放分离组分的作用[37]。在应用中，常用的 GC × GC 是非极性色谱柱搭配极性色谱柱。GC × GC 方法已用于葡萄酒中吡嗪类物质的分离检测[38]，饮料中芳香类物质的检测[39]，酸奶中脂肪酸的检测(衍生化)[40]，白酒指纹组分分析[41]，咖啡指纹组分分析[42,43]，蜂蜜指纹组分分析[44]，橄榄油指纹组分分析[45]，以及水果中手性组分的分析[46]，都获得了比一维气相色谱更好的分离及更丰富的信息。

3.2 液相色谱法(LC)及液质联用法(LC-MS)

气相色谱作为一种更普及的色谱分离手段在食品安全分析领域占有重要席位，但气相色谱无法分析沸点较高、极性强、热不稳定的化合物，液相色谱及其与质谱联用方法因克服了气相色谱的固有局限性而占据了食品安全分析领域的主流地位。本节着重介绍液相色谱及液质联用应用于食品安全分析的新形式、新发展。

3.2.1 超高效液相色谱法(UHPLC)

超高效液相色谱(UHPLC)是在高效液相色谱(HPLC)的基础上以更高的压力、更小颗粒直径的柱填料获得更快速高效分离的液相色谱技术，近年来发展迅猛。更高效的分离使得超高效液相色谱所得色谱峰峰宽更窄，所以与 UHPLC 联用的质谱检测器多是快速扫描型质谱如时间飞行质谱(TOF MS)等。超高效液相色谱-质谱联用(UHPLC-MS)在食品安全领域的 ppb 级以下痕量分析中发挥重要作用[47]。有研究工作将 UHPLC-MS 方法与 HPLC-MS 方法进行了比较，如分析牛奶中 38 种驱虫药[48]，分析婴儿食品中的杀虫剂[49]等，都证明 UHPLC-MS 方法可以获得更好的分离度、更快的分析时间和更高的检测灵敏度。UHPLC-MS 方法在食品违禁物质的检测如类固醇激素类[50-52]、兴奋剂类[53-56]等，在食品限制类物质的检测如喹诺酮类[57,58]，磺胺类[59-61]，杀虫剂类[62-64,57,58]，以及以上化合物类型同时分析和多组分筛查方面[65-67]都表现出色。

3.2.2 毛细管液相色谱法(CLC)和纳流液相色谱法(nanao-LC)

毛细管液相色谱(CLC)和纳流液相色谱(nanao-LC)所采用的色谱柱是内径 10~320 μm 并填充了固定相填料的毛细管柱[68]，相比于传统高效液相色谱具有快速分离、极少样品及流动相消耗量、可直接与质谱联用的特点。由于采用小内径的毛细管柱，毛细管液相色谱和纳流液相色谱就必须在仪器设计上如进样阀、流通管路、检测池等方面注意将死体积降到最小以减小柱外效应。毛细管液相色谱和纳流液相色谱所用固定相分为填充型和整体材料型，填充型填料发展趋势同 HPLC 和 UHPLC，粒径从 3~5 μm 发展到 1.5~1.9 μm，整体固定相一般采用毛细管内原位合成的方法。在 HPLC 和 UPLC 尝试过的部分食品安全分析领域，毛细管液相色谱和纳流液相色谱也都表现出较好的分离分析特性，如果汁中花青素的分析[69]，金银花中绿原酸的分析[70]，核桃中黄酮类化合物的分析[71]，霉菌毒素[72,73]、杀

虫剂[74-76]、除草剂[77-79]等有害物质分析, 氨基酸[80-82]、多肽和蛋白质[83-86]、蛋白质[87-90]等与食品营养或食品毒性及过敏原有关的分析。在实际应用中, 由于所用样品量在 nL 级, 毛细管液相色谱和纳流液相色谱的检测灵敏度一直具有争议, 但通常它们都与质谱联用, 大部分报道结果都具备与 HPLC-MS 相当或更好的灵敏度。

3.2.3 超临界色谱法(SFC)

超临界色谱(SFC)是较为特殊的一类色谱技术, 所采用的流动相是超临界流体。虽然超临界色谱的应用并不广泛, 但因为其特定的选择性使得它在食品安全分析领域也占有一席之地。同时, 超临界色谱还具有有机溶剂用量小、绿色环保等优势。最常用的流动相是超临界二氧化碳, 在 31 ℃, 73 bar 条件下即可获得超临界态, 实际应用中会根据分离体系不同添加一定的有机共溶剂或添加剂。因为超临界色谱自身的特点, 其检测器可涵盖从 GC 到 LC 的检测器, 如火焰离子化检测器(FID)、紫外可见检测器(UV-Vis), 蒸发光散射检测器(ELSD), 质谱检测器等。尤其是实现与质谱联用后, SFC 的应用被进一步拓展, 如包括脂肪酸、三酰甘油酯、磷脂分析在内的脂质类分析[91]。尤其是超临界色谱在手性分离上有特殊优势, 可对食品安全中因手性异构物质产生的隐患进行分析[92]。

3.2.4 二维液相色谱法(LC×LC)

同二维气相色谱类似, 二维液相色谱相比于一维液相色谱也因多一维度的分离大大提高了峰容量、分离度及灵敏度。相比于气相色谱, 液相色谱具有更多种分离模式, 因此二维液相色谱也能够开发出更多的应用模式。同样, 两个维度之间的分离, 即两种不同分离模式之间的接口非常重要。LC×LC 两维之间的接口通常是配备了样品环的八通阀或十通阀[93]。为了保证不损失第一维的分离, 第二维常采取快速分离的模式。如对牛奶、植物油、大豆、玉米、花生等不同食品基质中的三酰甘油酯类物质的分析可用银离子色谱(第一维)和非水反相色谱(第二维)相结合的 LC×LC[94-99]。而第一维亲水色谱/正相色谱第二维反相色谱的 LC×LC 已是磷脂分析的有力手段[100-104]。当然, LC×LC 的最有力检测手段依然是质谱检测。

除了常规液相色谱之间相串联成为二维色谱, 上文所提到的气相色谱和超临界色谱也可以和液相色谱串联构成二维色谱并应用于食品安全分析。GC×LC 的仪器构造较为复杂, 用于分析食品中的三酰甘油酯和脂肪酸甲酯[105,106]。相比之下, SFC×LC 的优势更明显, 尤其是将 SFC 和反相液相色谱(RPLC)串联能够实现正交分离。接口设计通常是基于溶剂替换原则[93]。在完成第一维分离后, 超临界流动相通过背压阀回到常压, 通过 T 型三通与水互溶并被引入第二维反相色谱分离。SFC×LC 已被用于食品中三酰甘油酯类化合物和脂肪酸的直接分析[107,108]。

4 食品安全分析中的高分辨质谱技术

质谱无疑是目前进行分子水平分析的最有力检测器, 上文所述各种色谱分离技术也均与质谱联用方能发挥更大效能。食品安全分析中定量分析常用到三重四级杆串联质谱(QQQ-MS), 定性定量分析常用到四级杆时间飞行质谱(Q-TOF-MS)。其中飞行时间质谱(TOF-MS)已经是通常意义的高分辨质谱。本节所介绍的主要是分辨率达十万以上甚至到百万的高分辨质谱, 如静电场轨道阱质谱法(Orbitrap-MS)和傅里叶离子回旋共振质谱法(FT-ICR MS)。这类具有超高分辨率的质谱可以提供精确分子质量数, 用以获得分析物的精确分子组成, 尤其适合食品安全分析中的全组分分析或筛查分析[109,110]。有研究人员利用 Orbitrap-MS 建立了蜂蜜中化合物组分的数据库[111], 或是对面包加工进行质控[112], 或是对蔬菜及水果中的农残进行筛查[113]。类似的, FT-ICR MS 也被用于三酰甘油酯类化合物的筛查确认[114]。

超高分辨率质谱有力推动了食品安全筛查分析, 同时常温常压敞开式质谱离子源的快速发展也使食品安全筛查分析更加便利。这类质谱离子源的典型代表是解吸附电喷雾离子源(DESI), 实时直接分析离子源(DART), 萃取电喷雾离子源(EESI)。常温常压敞开式质谱离子源能够对样品进行实时直接分析, 受基质效应干扰较小, 最大限度地避免或缩减了样品预处理流程。如 DART 用于茶叶中咖啡因的快速分析[115], 用于茶叶或水果表皮的毒物分析[116,117]; DESI 用于食品农残的分析[118-120]; EESI 用于啤酒、食用油、蜂蜜、乳酪等食品基质的主成分及添加剂分析[121]。常温常压敞开式质谱离子源的优势在于快速直接分析, 但检测灵敏度有所损失, 故目前主要用于食品安全的筛查分析。

5 结 论

色谱质谱技术的发展, 包括量体裁衣式固定相材料的研发、分离模式的拓展、仪器设备的升级提质、质谱电离机制的创新、质谱检测分辨率及精度的提升都将大力推动食品安全分析技术的发展, 色谱质谱技术在未来几年依然会是食品安全分析领域的主流方法, 并有望全面标准化, 为监控食品安全提供可靠技术保障。

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“食品快速检测技术及智能装备”专题征稿函

近年来，随着经济和科学技术的高速发展，食品安全问题也越来越突出，受到了世界各国的广泛关注。原有的食品检测技术已不能满足目前经济市场和科学研究的需求。新的检测技术与智能装备已经成为一种需求，如何快速、高效、方便和快捷地检测食品已成为目前食品安全检测领域的研究热点并且具有重要的意义。

鉴于此，本刊特别策划了“**食品快速检测技术及智能装备**”专题，由江苏大学**黄星奕教授**担任专题主编，围绕**化学比色分析、酶联免疫法(ELISA)、免疫胶体金试纸检测、计算机视觉技术、生物芯片、生物传感器、便携式色谱质谱联用仪、生物化学发光检测仪**等食品安全快速检测技术及智能装备或**您认为本领域有意义的问题**进行论述，计划在2016年6月份出版。

鉴于您在此方面的杰出成就，**黄星奕教授**与本刊主编**吴永宁研究员**特邀请您撰稿，展示您的研究成果与学术发现，以期为食品安全快速检测领域的推广应用、科研发展提供理论和实践指导。请您请在2016年5月30日前通过网站或Email投稿。我们将快速处理并优先发表。

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