液质联用技术在天然产物结构鉴定中的应用进展*

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摘要: 天然产物的结构具有多样性与复杂性,因此寻找天然产物快速识别与高效分离的方法是亟待解决的关键问题。液质联用技术将高效液相色谱优秀的分离能力与质谱高灵敏度和高专属性的检测有机地结合在一起,在天然产物研究中发挥着越来越重要的作用。本文综述了液质联用技术近 10 年来在常见类型的天然产物结构鉴定中的应用情况,并对其发展趋势进行了展望。

关键词: 液质联用技术; 天然产物; 结构鉴定; 发展方向; 综述

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Progress on application of LC - MS technology in structural identification of the natural products*

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Abstract: Owing to the diversity and complexity of structures of natural products, it is an urgent task to establish rapid identification and effective separation methods as soon as possible. LC - MS technology has combined the excellent separation ability of HPLC, high sensitivity and specificity of mass spectrometer together, and has been playing a more and more important role in the research of natural products. In this paper, the applicability of mass spectrometry for the structural elucidation of ordinary natural products in the past decade was reviewed, and the development trend of LC - MS technology was prospected.

Key words: LC - MS technology; natural products; structural identification; development direction; summerize

天然药物化学成分结构类型变化繁多,加之传统的化学研究方法(提取→分离→结构鉴定)耗时耗力、重复性强,要将天然药物中的有效成分阐释清楚难度很大。因此,寻找天然产物快速识别和高效分离的方法,是天然药物化学领域亟待解决的关键问题。液质联用以其高分辨率、高灵敏度和专属性的检测方法,已广泛用于医药、化工、生物、环保等领域。近十多年来,随着 LC - MS 技术发展的日臻完善,其在天然产物结构鉴定研究中的应用也越来越广泛,展现出广阔的应用前景。本文对液质联用技术在常见类型的天然产物结构鉴定中的应用进行了综述。

1 酚类化合物

酚类化合物是自然界普遍存在的化合物,其中以黄酮、香豆素、木脂素、蒽醌和酚酸最为常见。近年来,采用 LC - MS 分析中药及天然药物中酚类化学成分的报道占半数以上。

黄芩 Scutellariae radix 临床上多用于治疗上呼吸道感染、肝炎、痢疾以及各种炎性疾病,主要含有黄酮类化合物。Han 等[1] 采用 HPLC - DAD - ESI - MS^n 鉴定了黄芩中的 26 个化合物,包括 5 个碳苷、12 个氧苷以及 9 个苷元,基本阐明了其物质基础。

麦冬 Ophiopogonis radix 中含有一类结构较为特殊的高异黄酮类化合物,其主要特征为连接 B 环和 C 环的碳链较普通黄酮多一个亚甲基。Ye 等[2] 研

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究了该类化合物的 ESI – MS^n裂解行为,并据此从麦冬的氯仿 – 甲醇提取物中鉴定了 18 个高异黄酮类化合物,其中 7 个为新化合物。Qi 等[3] 应用 HPLC – DAD – MS/MS 研究了 11 个麦冬高异黄酮的裂解行为,并从麦冬提取物中鉴定了 28 个该类化合物,其中 7 个为新化合物。Lin 等[4] 则研究了川麦冬和杭麦冬中的高异黄酮类成分,分别从其中鉴定了 17 个和 19 个该类化合物,并从化学成分组成上对 2 种麦冬进行了区分。

丹参 Salviae miltiorrhizae radix et rhizoma 中的酚酸类成分一直是研究的热点。Liu 等[5] 根据 11 个酚酸标品的 ESI – MS^n裂解途径,从药材中鉴定了 42 种酚酸化合物,其中 16 种为新的微量成分。另外,Zeng 等[6] 则用 HPLC – MS/MS 从丹参中鉴定了 28 种酚酸类成分。目前也有用 ESI – TOF – MS 分析丹参酚酸的报道[7-8],各种 LC – MS 技术从不同侧面获取的结果,更有利于全面了解丹参药材中的化学成分。

灯盏细辛 Erigerontis herba 有效成分为黄酮、香豆素、木脂素以及羟基桂皮酸类化合物。Zhang[9] 应用 Finnigan LCQ Deca XP plus IT 质谱仪对其化学成分进行了深入研究,最后鉴定了 53 个化合物,主要包括咖啡酰奎尼酸类、黄酮类、黄酮醇类和二氢黄酮类化合物,该结果对于灯盏细辛的质量控制具有很好的参考意义。

中药大黄临床上多用于治疗便秘、各种炎症以及癌症,其有效成分主要为酚类化合物。Jin 等[10] 采用 Q – TOF 质量分析器,从唐古特大黄中鉴定了 41 个化合物,包括 16 个蒽醌类,7 个苯基丁酮类,4 个芪类以及 14 个单宁类化合物,其中 9 个化合物通过与已知化合物对照而得到准确鉴定。Ye 等[11] 则采用 HPLC – DAD – ESI – MS 法研究了六种大黄属药材提取物中的化学成分,最后共鉴定了 107 种化合物,结果表明六种药材中酚类化合物的差异较大,并提出了大黄属药材在临床上应避免混用的建议。

近年来还有大量关于连翘[12]、蒲公英[13]、蜂胶[14-15]、生姜[16]、枳壳和枳实[17-20]、藤黄属植物[21]、柑橘[22-23]、石斛属植物[24-25]、千里香[26]、贯叶连翘[27]、白花蛇舌草[28]、白果[29]、降香黄檀[30]、万寿菊[31]、五味子[32]、Scandiceae tribe[33] 等药材中黄酮、酚酸、木脂素、环烯醚萜等的 LC – MS 研究报道,极大地促进了 LC – MS 技术在酚类成分结构鉴定中的推广应用。

2 皂苷类化合物

皂苷类化合物依据苷元连接糖链数目的不同可分为单糖链、双糖链以及三糖链皂苷,在某些皂苷的糖链上还可通过酯键连接其他基团。由于大多数皂苷仅存在末端吸收,难以采用紫外检测器进行分析;蒸发光散射检测器(ELSD)信号响应不强,噪音较高,很容易掩盖微量成分,限制了其应用范围;质谱检测器则比较完美地解决这些问题,成为当前研究皂苷类化合物的最佳检测器。

人参 Ginseng radix et rhizoma 和三七 Notoginseng radix et rhizoma 都是富含皂苷的有名药物。Leung 等[34] 采用 HPLC – APCI – MS/MS 建立了 3 种人参、三七和西洋参的鉴定方法,可以对 3 种药材加以区分。三七生品与蒸制品的功效截然相反,如何从化学成分的变化上阐明炮制方法对药效的影响便显得非常有意义。Chan 等[35] 应用 UPLC – TOF – MS 分别从其生品和熟品中鉴定了 74 个和 146 个化合物,表明蒸制法对皂苷的种类和含量影响很大,为三七生品与熟品不同的功效从化学成分层面提供了依据。

黄芪 Astragali radix 是常用的补气类中药,三萜皂苷、黄酮、多糖以及 γ – 酪氨酸是其主要的有效成分。Xu 等[36] 采用 HPLC – APCI – MS 和三重四极杆质量分析器从黄芪提取物中鉴定了 12 个皂苷类成分,但如何区分皂苷同分异构体的问题尚待解决。而 Xiao 等[37] 采用 LC – MS 技术以及核糖体 DNA 条形码技术从化学成分和遗传性能方面对多批次的黄芪药材进行了细致的研究。Tang 等[38] 则应用 HPLC – DAD – ESI – MS/MS 对黄芪药材抗病毒部位中的化学成分进行了系统的研究,取得了很好的效果。

白薇 Cynanchi atrati radix et rhizoma 和蔓剪草 Cynanchum chekiangense 是两种重要牛皮消属药材,主要的药效成分均为 C_{21} 甾类化合物。Zheng 等[39] 采用 ESI – MS^n正负模式对 C_{21} 甾类化合物的裂解方式进行了系统的研究,并根据这些化合物的裂解行为,从白薇 90% 甲醇提取物中鉴别了 10 个甾体皂苷类化合物,阐明了其中主要的化学成分。Tai 等[40] 则应用在线 HPLC – ESI – MS/MS 鉴定了蔓剪草氯仿提取物中的 9 个甾体皂苷类成分,随后又采用离线的 FTICR – MS/MS 和 NMR 法对研究结果进行了进一步的确证。

3 生物碱类化合物

生物碱是天然产物中类型最多的一类化合物,

多具有复杂的环状结构,其氮原子多具有孤对电子,易于失去一个电子或得到质子,故非常适合质谱检测。从上世纪 90 年代开始,随着大气压电离方式(API)尤其是电喷雾电离(ESI)的日臻成熟,相关的研究也越来越多。

乌头属植物附子 Aconiti lateralis radix praeparata、川乌 Aconiti radix 和草乌 Aconiti kusnezoffii radix 主要分布于亚洲北部和北美地区,临床应用广泛。鉴于该属植物大多具有较强的毒性,建立一种灵敏便捷的乌头生物碱检测方法对乌头属药材以及成方制剂的质量控制非常有必要。刘淑莹课题组近年来进行了大量此方面的研究工作[41-43]。如,采用 ESI-MS^n鉴定了草乌花提取物中的 70 余个乌头生物碱[41],采用 LC-MS 技术系统研究双酯型二萜乌头碱(DDA)在不同溶剂以及不同 pH 缓冲液中的稳定性[42]等。而 Wu 等[44]等则应用 MALDI-TOF-MS 技术快速鉴定和比较了附子、延胡索以及草乌中的化学成分,为下一步的定向分离新化合物以及质量控制提供依据。

两面针 Zanthoxyli radix 提取物中的生物碱类型繁多,其中苯骈[C]菲啶类、原阿片碱类、阿朴菲类以及喹啉类生物碱是其药效成分。Liang 等[45]应用 HPLC-ESI-MS/MS 鉴定了其中的 9 个生物碱。而 Cai 等[46]则详细研究了生物碱标品的电喷雾电离裂解行为,据此从提取物中鉴别了 10 个生物碱类成分,并指出特征离子的种类和丰度对于解析该类化合物的质谱数据具有重要作用。

百部 Stemonae radix 中的多环类生物碱,由于结构新颖且极具特点,被称为百部生物碱。Zhou 等[47]建立了专属性极强的 HPLC-MS 指纹图谱,以保证其在临床应用中的安全性。尽管各药材间的色谱峰存在差异,但所建立的 LC-MS 指纹图谱既可以为药材质量提供保证,又可为区分不同的百部属药材提供了重要依据。

4 单萜苷类化合物

单萜类化合物的基本骨架是由 2 个异戊二烯单元构成。其中环烯醚萜类化合物是 1 种较为特殊的单萜,具有环戊烷吡喃环系统,且分子内一般带有环烯醚键。在植物体内,环烯醚萜类化合物多以苷的形式存在。

Dong 等[48]采用 HPLC-ESI-MS/MS 法鉴定了芍药提取物中的 5 个单萜类化合物。Zhou 等[49]则应用 HPLC-ESI-MS/MS 对鸡矢藤 Paederia scandens 中的含硫环烯醚萜类化合物进行了研究,最后鉴定了 5 个该类化合物。Ren 等[50]则采用 HPLC-MS^n从栀子提取物中鉴定了 5 个环烯醚萜类化合物,研究尚待进一步深入。

5 二萜类化合物

二萜类化合物是以 20 个碳为骨架的碳氢化合物,通常可以用$(C_5H_8)_4$为通式加以表示。Ye 等[51]采用 ESI-MS 和 APCI-MS 两种方法研究了紫杉醇的裂解行为,其结果可用于从植物提取物中快速筛选紫杉醇类化合物。Yang 等[52]采用 HPLC-MS^n从丹参药材的甲醇-氯仿(7∶3)提取物中鉴定了 27 个丹参酮类化合物,阐明了其中的该类成分。

6 三萜类化合物

三萜类化合物由 30 个碳原子组成,在生源上是由鲨烯衍生化而来。许多五加科、豆科和桔梗科的植物以及某些动物和真菌中都富含三萜类化合物。Yang 等[53]采用 HPLC-ESI-MS^n鉴定灵芝了 32 个三萜类化合物,其中 6 个为新化合物。而关于川楝(川楝素)[54]、金线兰(齐墩果酸和熊果酸)[55]和印楝(印楝素)[56]等植物中三萜类化合物的 LC-MS 分析最近几年也有报道。

7 甾体皂苷元类化合物

最常见的甾体皂苷元是螺旋甾烷的衍生物,在其侧链上有一对特征的螺旋缩酮结构。植物甾醇、胆汁酸、类固醇激素、强心苷元、甾体皂苷元以及蟾毒等均属于此类化合物。Ye 等[57]应用 HPLC-DAD-APCI-MS 以及 IT 质量分析器对蟾酥中的蟾毒类成分进行了研究,最后鉴定了 35 个蟾蜍二烯内酯类成分,其中 4 个为新化合物。Huang 等[58]采用 SFE-LC-APCI-MS^n法研究了 β-谷甾醇、豆甾醇和麦角甾醇等化合物的裂解方式,并对花叶开唇兰中的植物甾醇类化合物进行了初步的研究。

8 其他化合物

存在于自然界的天然产物结构类型多种多样,除上述涉及的几种主要的化合物类型之外,还有很多类型的化合物。如牡丹皮中主富含苯乙酮、单萜以及没食子酚苷类成分。Xu 等[59]应用 HPLC-DAD-ESI-MS 和 Q-TOF 质量分析器研究了牡丹皮中的化学成分,并从中鉴定了 50 个化合物,包括 17 个单萜,14 个没食子酚苷,10 个苯乙酮,5 个酚酸,3 个黄酮以及 1 个三萜类化合物。

9 结语

LC-MS 及 LC-MS/MS 联用技术应用于天然产物成分研究,不需要对样品进行繁琐和复杂的前处理,具有高效快速、灵敏度高,尤其适于含量少、不

易分离得到或在分离过程中容易丢失的组分等诸多优点,因此自诞生以来便得到了突飞猛进的发展。由于中药的治疗机理是多种化学物质综合作用的结果,因此大力发展可对中药成分进行整体分析的 LC – MS 技术具有重大的意义。

但是,作为普遍应用时间不长的技术,其发展仍然不完善。如质谱数据解析必须要依靠大量的专业知识和技能,归纳出有用的信息,而且得到分析结果尚需得到进一步的确认。目前,液质联用技术在向着提高色谱分离度、降低对照品依赖性以及简化质谱和数据库信息提取过程的方向发展,主要表现在(1)超高压液相的发展[59]:应用 1.7 μm 粒径的填料,在更大程度上改善物质间的分离选择性,从而增强离子辨认度与归属性增强,并且最大可能地减小软电离技术中共流出物的离子抑制与和增强效应,从各个层次上使质谱图得以优化;(2)高分辨质谱检测器的应用[60]:TOF(飞行时间质谱仪)、Orbitrap(回旋共振质谱仪)等,通过获取精确分子量,得到未知峰的元素组成,使鉴定出的未知化合物的分子量误差小于 5 ppm,大大提高了化合物鉴定的准确率;(3)Q – TOF、IT – TOF 串联质谱技术的应用[61~62]:在获取准分子离子峰的基础上,进一步得到蕴含丰富结构信息的碎片离子峰,从而对结构进行快速、准确的鉴定;(4)各种鉴定策略的产生与发展:如基于“碎片 – 降解”相关性的产物鉴定法[63]、基于诊断离子的“DFIBES”快速鉴定法[64]以及未知组分的质谱从头鉴定策略[65],为中药复杂成分的快速检出与结构鉴定提供了新的技术策略。

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