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评 述

高效液相色谱整体柱在药物分离分析中的应用进展

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摘要 本文对高效液相整体柱在药物分离分析方面的应用进行了综述. 主要介绍了以烷氧基硅烷为主要原料, 采用溶胶-凝胶法制备的硅胶整体柱, 由于其具有微米级通孔结构和大的比表面积, 他们在高效、快速分离小分子物质方面得到广泛地应用. 对于聚合物整体柱, 主要介绍了包括分子印迹聚合物在内的有机聚合物整体柱在药物分离、生物样品的处理等方面的应用.

关键词
药物分析
分离
整体柱
高效液相色谱

1 引言

药物分析是运用化学、物理学、生物学以及微生物学方法和技术来研究化学结构已经明确的合成药物或天然药物及其制剂质量的一门学科. 它包括药物成品的化学检验、药物生产过程的质量控制、药物贮存过程的质量考察、临床药物分析、体内药物分析等.

高效液相色谱法是20世纪70年代迅速发展起来的一种高效、快速的新型分析分离技术. 高效液相色谱因其独特的优点已经广泛应用于药物的含量测定、组成分析、质量控制等方面. 在药典的所有分析方法中它是发展最快的一种分析方法. 高效液相色谱法在药物分析方面的应用主要包括定性分析、杂质的检查、含量测定、药物的稳定性研究、中草药成分及中成药成分分析、抗生素各组分的分离和测定及临床血药尿药浓度检测等方面.

整体柱是一种用有机或无机聚合方法在色谱柱内进行原位聚合的连续床固定相, 具有制备简单、重现性好、多孔性优越、能实现快速、高效分离等优点[1, 2]. 目前整体柱制备技术已日趋成熟, 其有机和无机聚合制备方法都得到很大发展[3~6]. 整体材料基质目前已被广泛地用于高效液相色谱分离分析, 是近年来得到迅速发展的新型色谱柱. 本文综述了高效液相色谱整体柱在药物分离分析方面的应用.

2 药物分离

整体柱具有极好的通透性, 在较大的流速下依然具有较高的柱效和较低的柱压降. 普通填充柱则无法实现这一点, 只能通过降低填料粒径来提高柱效, 但填料粒径的减少又不可避免地带来柱压降显著增加的弊端. 所以整体柱的优势在于分离速度快. 邹汉法等[7]制备了一种辛可宁印迹的手性整体柱, 可在 2 min 内实现非对映异构体辛可宁和辛可尼丁分离. 杨更亮等[8]以药物烟酰胺为模板分子制备了具有特定识别性能和分离能力的分子印迹聚合物, 模板分子与烟酸在 2 min 内快速分离. 张静等[9]以士的宁为模板分子, 合成了对士的宁具有特异识别能力的分子印迹整体柱, 并用于士的宁和马钱子碱的分离, 其分离因子为 3.5; 后来他们又合成了麻黄碱分子印迹聚合物整体柱, 在优化色谱条件下, 麻黄碱和伪麻

黄碱可以得到较好的分离[10]. 张倩倩等[11]合成的普萘洛尔分子印迹整体柱, 对模板分子具有很强的亲和力和特定的选择性, 且具有较大的结合容量, 可望将其应用于普萘洛尔的体内药物分析中. 何仲贵等[12]用C18 整体柱分离了利福平和它的四个相关物质. 朱岩等[13]用氯化十六烷基吡啶涂覆的ODS硅胶整体柱分离了药物中的抗癫痫药唑尼沙胺及其原料药和中间体. Tzanavaras等[14]用Chromolith® RP-18e整体柱在金思平片剂的溶出试验中对金思平进行了快速分离, 分离过程在 1 min内完成. Jira等[15]将片剂中的去炎松、脱氢皮质醇和地塞米松三种皮质激素固 相萃取后用C18 整体柱分离, 用时不超过 6 min. Aboul-Enein等[16]用Chromolith® RP-18e硅胶整体柱分析了片剂中的他达那非. 赵睿等[17]用制备的磺胺甲恶唑分子印迹整体柱直接对三种片剂中的磺胺甲恶唑进行了定量分析. Tzanavaras等[18]将整体柱与自动高效液相色谱系统联用对阿昔洛韦和它的主要杂质鸟嘌呤进行了定量分析; 用 5 cm的短整体柱在 4.0 mL min^{-1} 的流速下快速分离了尼美舒利, 用时在 1 min之内[19], 用高通量液相色谱方法分析了食物中的咖啡因[20]. Mrkvičková等[21]以整体柱作为固定相研究了新合成的两组有抗结核潜力的药物的亲脂性, 并在位置异构体的分离中明显的减少了分析时间, 优化了所有测试化合物的峰型. 孙汉文等[22]用茶碱分子印迹整体柱分离了黄嘌呤衍生物、咖啡因和茶碱等类似物, 咖啡因和茶碱在等度和梯度的情况下都能完全分离, 并用该整体柱对各种绿茶中的咖啡因和茶碱进行了定量分析. 尹艳凤等[23]以大黄素分子为模板, 合成了一系列分子印迹整体柱, 在优化的合成条件下制得的分子印迹整体柱能有效地分离大黄素及其类似物. 张亦弘等[24]建立了一种使用整体柱快速测定利福平注射液含量和有关物质的高效液相色谱方法, 在选定色谱条件下, 有关物质与主药分离良好.

3 体内药物分析

在药物研究中, 通过体内药物的检测可以了解给药后药物在体内的吸收、分布、代谢、排泄的情况, 为药物的药效、毒性的评价及其在体内作用机制的研究提供信息. 生物样品组成复杂, 样品预处理常很繁琐, 对检测结果有很大影响. 在血药分析中, 预处理是较为关键和困难的一步, 血浆样品一般采用液液萃取及固相萃取等常规样品预处理方法, 这些样品预处理方法通常具有样品耗用量大、较多使用有机溶剂对环境造成的污染等麻烦且需结合其他的样品净化处理步骤, 使得整个样品预处理过程繁琐. 由于整体柱制备成本低、使用寿命长且稳定性好, 并且能使分析物保留的同时除去生物大分子等物质, 所以被用于处理生物样品. 高效液相色谱整体柱具有的同时分离和分析的功能提高了体内药物分析和体内内源性物质分析测定的选择性和准确性.

Souverain[25]等通过用柱切换装置从体液中快速、直接的萃取药物及其代谢产物, 证明了整体柱材料的应用潜力. 该方法显示了极好的选择性及对每毫升血浆中纳克级的微量药物及其代谢产物有足够的灵敏度. Barnett等[26]采用高效液相色谱联用三(2, 2′二吡啶)钌化学发光检测方法分析了人的尿液和血液中抗精神病药喹硫平及其主要的活性代谢物和非活性代谢物, 能在 3 mL min^{-1} 的高流速下快速定量. Gundersen等[27]用整体柱和紫外检测器直接分析了血浆中血浆抗坏血酸和总抗坏血酸. Legido-Quigley[28]用聚苯乙烯烧结的微液相色谱柱直接分析了血浆中的一些药物, 沙美特罗昔萘酸酯的检测限是 12.5 ng. Šperlingová等[29]建立了高效液相色谱反相整体柱快速分析尿样中羧酸的方法, 该方法能同时测定苯乙烯和乙苯的芳烃代谢物扁桃酸和苯酰甲酸、甲苯的代谢物马尿酸、二甲苯的代谢物邻、间、对-甲基马尿酸等, 用反相整体柱测定的人尿液中的扁桃酸、苯酰甲酸及马尿酸的浓度与LiChrosorb反相色谱柱测定的结果没有明显的差别. Dear 等[30]通过开发压力极低的烷基键合硅胶整体柱, 使多柱联用成为可能, 从而明显提高了系统的分离能力. 整体柱在体液大体积进样方面也显示出非常好的能力, 峰型和分离能力都没有降低. van de Merbel 等[31]对液相色谱整体柱在生物定量分析领域的应用进行了研究, 通过提高流速, 运行时间比用传统反相色谱柱减少了三倍, 而分析物的分离度没有受到影响. Henion等[32]将整体柱与LC-MS联用测定了大鼠血浆中的哌甲酯及其去酯化代谢物利他林酸, 在3.5 mL min^{-1} 的流速下, 两种物质 15 s内就能分离. Zeng等[33]用整体柱分离后, 将血浆中的氟苯丙胺、替马西泮、奥沙西泮、它莫西芬用LC/MS/MS联用技术进行了定量分析. 在保证良好的色谱分离情况下, 运行时间大为缩短, 包括样品提取

和经过柱切换后整体柱分离在内, 整个过程 1.2 min 就能完成. Albu等[34]用LC/MS联用技术测定了血浆中的吲满酰胺及内标物, 该方法成功评价了两种缓释片剂的生物等效性. Papp等[35]用LC/MS/MS联用技术对绵羊血浆中的孟鲁司特进行了定量分析, 短的整体柱和快速梯度使整个过程在 1.5 min内就能完成. Stanley等[36]用液相色谱-串联质谱法直接进样分析沉降蛋白后的血浆中的酸性药物和马尿中的中性掺杂剂. 在两个分析样品中, 混合物中的被分析物在用反相整体柱分析前先用HLB固相萃取柱富集和净化, 当浓度为 10 ng mL^{-1} 时, 沉降蛋白后的血浆中的酸性药物和马尿中的中性掺杂剂的回收率分别是 91%和 80%. Vallano等[37]介绍了几种用高效液相色谱整体柱测定血浆中的环氧化酶-2 抑制剂罗非昔布和 3-异丙氧基-4-(4-甲磺酰基)-5,5′-二甲基-5(H)呋喃酮的方法. 分析物连同内标物是用 96 孔固相萃取技术从血浆中萃取出来的, 实验评价了用整体柱分析生物样品的重现性. Staub等[38]建立了一种定量测定全血中法医毒理学最常遇到的 8 种苯二氮卓类药物的方法, 该方法曾被用于两个真实案例并测定了约 30 种化合物; 然后他们用高效液相色谱-大气压电离质谱测定了 8 种苯二氮卓类药物, 使用硅胶整体柱分离加速了分析过程. Pistos等[39]建立了一种用高效液相色谱反相整体柱同时测定了固相萃取后的人血清中的对乙酰氨基酚、咖啡因、布他比妥的方法. Samanidou 等[40]用高效液相色谱整体柱同时测定了制剂和血液中的四种头孢菌素类抗生素. Aboul-Enein 等[41]通过测定血浆中的米安舍林及其三个代谢产物, 证明了用整体柱对血浆中米安舍林及其主要代谢产物快速定量分析的可行性. Liu等[42]首先用乙酸乙酯把托拉塞米和内标物利尿磺胺从血浆中提取出来, 然后用硅胶整体柱进行了分析. Indjova 等[43]用等度高效液相色谱同时测定了人血浆中的霉酚酸及其代谢物葡糖苷酸结合物. Zarghi 等[44~47]建立了用整体柱测定血浆中法莫替丁、塞来昔布、达美康和卡维地洛的方法. Wenk等[48]用反相硅胶整体柱测定了血浆中的新型三唑类抗真菌药伏立康唑及血浆和尿中的速尿, 经液液萃取后的血浆和尿样, 分析过程在 4 min之内完成, 该方法适用于药代动力学研究. Urbánek 等[49]用反相高效液相色谱整体柱和二极管阵列检测器同时测定了血清中的维生素A和维生素E, 运行时间不超过 2 min. Rouini等建立了用C18 高效液相色谱整体柱同时分离血浆中氯巴占及其主要代谢物[50]和曲马多[51]及其代谢物的方法. 该方法被用于氯巴占和曲马多及它们的主要代谢物的药代动力学研究. Karnes等[52]建立了测定健康志愿者和潜在急性心肌缺血患者血浆中的肌苷和次黄嘌呤的高效液相色谱方法. 该方法被用于血浆中内源性生物标志物的评价. Foroutan 等[53]用等度反相色谱同时测定了血浆中的阿莫西林和克拉维酸. Brunetto 等[54]用柱切换高效液相色谱方法对人尿中的氯沙坦、替米沙坦和缬沙坦进行了定量分析, 分析物先通过在线固相萃取过程萃取后再用C18 整体柱进行了分离.

4 天然药物分离

天然药物的来源有动物、植物和矿物之分, 其中以植物类为主. 由于天然药物的化学成分复杂, 其有效成分可能有一个, 也可以有多个, 这对于控制药品质量, 建立质量标准来说比较困难, 高效液相色谱整体柱可通过对天然药物的有效成分进行分离鉴定, 再测定有效成分的含量.

Heyden 等[55]通过检测甘草、鼠李、姜黄和朝鲜蓟 4 种中药, 提出了用 C18 整体柱为固定相建立指纹图谱的方法. 王芸等[56]用人血清白蛋白键合的硅胶柱和反相硅胶整体柱的全二维生物色谱, 分析了中药方剂龙胆泻肝汤中的生物活性成分. 邹汉法等[57]用氰基柱为第一维分离和反相硅胶整体柱为第二维分离的色谱模式, 建立了伞形科植物川芎和当归的甲醇提取物的分析方法. 结果证明, 与以前报道的结果相比, 检测到的色谱峰的数量增加了两倍, 而分析时间却减少了两倍. Schmidt 等用硅胶整体柱[58]代替传统硅胶柱[59]成功测定了魔鬼爪中的环烯醚萜苷成分哈帕酯苷, 更短的分析时间对魔鬼爪的提取物及其药物制剂的商业质量控制非常有价值. Körner 等[60]建立了一种用反相整体柱从秋水仙种子制成的浸膏制剂中将秋水仙碱和一些相关生物碱及降解产物分离的方法. 该方法对包括秋水仙碱在内的生物碱不用衍生化处理就能迅速分离, 并且有极好的线性、精密度和低定量限. Repollés 等[61]用反相高效液相色谱方法同时分离食品中的 11 种主要的黄酮苷元. 该方法被成功用于分析蜂胶和银杏等复杂中药样品中的这些化合物. Barnett[62]将高效液相色谱整体柱和酸性的高锰酸钾-三(2,2′二吡啶)钌(II)化学发光检测器

联用, 迅速、高灵敏的检测了从罂粟中提取的阿片类生物碱. 由于整体柱允许流动相高流速通过, 四种主要生物碱吗啡、可待因、东罂粟碱和蒂巴因在 2 min 之内就能测定. 谭天伟等[63]将正辛胺修饰的聚(甲基丙烯酸缩水甘油酯-乙二醇二甲基丙烯酸酯)整体柱用于快速分离纯化并测定了葛根中的葛根素.

5 手性化合物的分离

据统计, 大约有 40%的药物为手性化合物. 手性药物的两个对映体在药理活性及强度、毒副作用、体内代谢等各方面都可能体现出立体选择性. 手性药物的对映体拆分即手性分离是当今药物分析的前沿课题, 而高效液相色谱又是对映体分离分析中使用最多、应用最广的技术. 整体柱在手性化合物的分离方面应用最多的是将分子烙印聚合物用作液相色谱固定相进行手性拆分, 这也是分子烙印聚合物进展最大的领域, 到目前为止分子印迹技术在色谱手性分离中已取得了较大进展. 邹汉法等[64]综述了作为液相色谱和毛细管电泳固定相的整体柱的制备、特征及应用. 在 2002 年以前, 整体柱在手性分离方面的应用只有分子印迹整体柱, 且只有 4 篇文献. Lubda 等[65]用整体柱上化学键合环糊精制成的手性固定相分离手性化合物甲基苯巴比妥、去甲羟基安定、美沙酮和其他一些药物, 获得了好的分离效果; 通过键合手性阴离子交换剂叔丁基氨基甲酰喹啉制成的手性硅胶整体柱被成功用于分离一些 *N*-衍生化的氨基酸和舒洛芬等手性化合物[66]. 邹汉法等[67]制成的分子印迹整体柱用于快速分离对映异构体, *N*-苄氧羰酰-DL-色氨酸和芴甲氧羰基-DL-色氨酸在 3 min 之内成功分离, 结果证明分子印迹整体柱的柱长对对映异构体和非对映异构体的分离因子几乎没有影响; 用 l-延胡索乙素分子印迹整体柱直接测定延胡索中 dl-延胡索乙素[68], 在该整体柱上, dl-延胡索乙素能达到基线分离; 制备的(5S,11S)-特罗格尔碱(S-TB)的印迹整体柱, 实现了对特罗格尔碱消旋体的快速分离[69]. 杨更亮等[70]制备的分子印迹整体柱快速分离了那格列奈和它的 L-异构体. 图 1 是那格列奈及其 L-异构体的色谱分离图. 段宏泉等[71]以萘普生为模板分子制成的分子印迹整体柱, 能较好地将萘普生对映异构体分离, 分离度达到 1.55. Mallik 等[72]制备并评价了 α1-酸性糖蛋白键合的硅胶整体柱手性分离的选择性, 并与同一蛋白制成的常规硅胶手性柱进行了比较. 通过分离 *R*/*S*-华法林和 *R*/*S*-普萘洛尔, 硅胶整体柱显示出更强的保留、更好的分离能力和柱效.

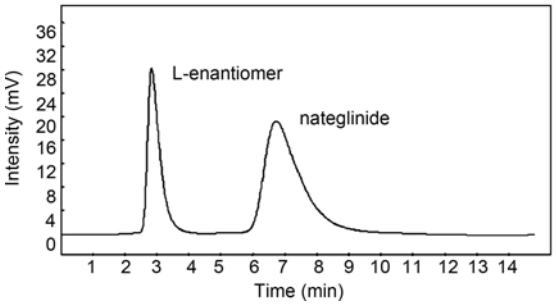

图 1 那格列奈及其 L-异构体的色谱分离图[70]. 分离柱尺寸, 150 mm×4.6 mm i.d.; 流动相, 已腈; 流速, 1.0 mL/min; 柱温, 25 ℃; 检测波长, 210 nm; 进样, 50 nmol.

6 固相萃取

在药物研究领域中, 对于复杂生物样品的预处理是十分必要的. 固相萃取技术因其高效及高选择性等特点备受分析工作者的青睐. 整体柱作为固相萃取吸附剂, 具有生物相容性好、可有效去除体系中的干扰物质、对药物具有较高的选择性等特点, 在去除干扰物质的同时能对复杂体系中的药物进行富集, 提高了检测灵敏度. 该类型的吸附材料可以反复使用几十次, 与传统的吸附剂相比, 极大地降低了操作成本, 为检测实际样品中的药物浓度提供了一种准确、快速、操作简单易行的新方法.

Xu 等[73]将人血浆中的蛋白酶抑制剂安普那韦和阿扎那韦用短的 C18 整体柱在线处理后用高效液相色谱-串联质谱法进行了测定. 杨更亮等[74~77]用弱离子交换整体柱在处理人血浆和尿样时, 能在除去体液中复杂物质的同时使药物很好的保留. 图 2 是人血浆中的抗高血压药多沙唑嗪经整体柱富集并洗脱后的色谱图. 冯钰锜等[78~82]将整体柱作为固相微萃取材料与高效液相色谱-质谱联用分析了尿样中的安非他明, 人血浆中的喜树碱和一些碱性药物及人血浆和尿样中的血管紧张素 II 受体拮抗剂和麻黄碱; 并将聚合物整体柱微萃取与高效液相色谱联用测定了鸡蛋中的磺胺嘧啶和磺胺二甲嘧啶残留及鱼肉中的四种四环素类抗生素[83, 84], 该方法不需要在萃取之

前先沉降蛋白和除去脂肪; 用聚合物整体柱微萃取和高效液相色谱-质谱联用法测定了牛奶、鸡蛋、鸡肉和鱼肉中的十三种磺胺类抗生素和七种痕量喹诺酮类抗生素; 用毛细管整体柱管内固相微萃取与高效液相色谱在线联用测定了血浆中 5 种氟喹诺酮类药物[85~87]; 并对环境水中的四种内分泌干扰物进行了在线萃取[88]. Wainer 等[89]建立了用整体柱自动固相萃取并用高效液相色谱-质谱测定人血浆环孢素 A 的方法. 为了克服固相萃取的限制, Namera 等[90]用装有键合十八烷基硅烷的硅胶整体柱的离心柱装置萃取了尿样中的安非他明和亚甲二氧苯丙胺. 陈朗星等[91]用分子印迹整体柱作为预柱对牛奶和蜂蜜中低浓度的六种四环素类抗生素固相萃取后进行了分析. 用整体柱作为萃取介质. Bones等[92]建立了一种分析天然水中一系列超痕量药物的简单富集方法. 固相萃取是用微反相硅胶整体柱在线进行的. 黄晓佳等[93]用高效液相色谱和包覆整体柱材料的搅拌棒测定了废水中的 6 种性激素.

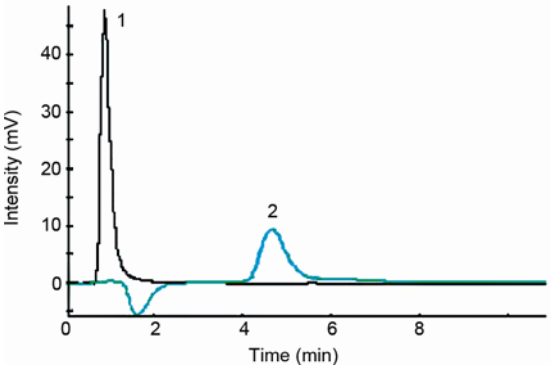

图 2　人血浆中的抗高血压药多沙唑嗪经整体柱富集并洗脱后的色谱图[74]. 富集流动相，去离子水；洗脱流动相，甲醇/水(70:30, *V*/*V*).

7　展望

近年来整体柱技术在药物分离分析方面得到了较快的发展. 由于硅胶整体柱具有高速、低压、高效及流速梯度洗脱等优异特性, 适合于小分子物质的高效快速分离, 但制备繁琐, 比表面积较小. 而聚合物整体柱虽然制备方法简单、机械强度适宜、能够实现对生物大分子的高效、快速、高通量分离分析等优点, 但还存在孔径及孔径分布难以控制、内部结构不均一、分离小分子物质柱效低等缺点. 因此, 发展新的制备手段和新的聚合体系, 探索分离机理, 对以上问题的研究并解决将使整体柱在新的应用领域得到发展.

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Application and development of monolithic stationary phases for HPLC in pharmaceutical analysis and separation

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Abstract: In recent years, monolithic supports as stationary phases in high-performance liquid chromatography (HPLC) have become a rapidly burgeoning field. This review summarizes the current achievements and their application in pharmaceutical analysis and separation. One kind is the silica monoliths prepared from tetraalkoxysilane by a sol-gel method with either micrometer-size through-pores or high specific surface areas and well suited for small molecules in HPLC modes. The other kind is the organic polymer-based monoliths including monolithic molecularly imprinted polymers in treatments of the samples before injected into the chromatograph in biological fluids. The analytical potential of these columns is demonstrated with separations involving various drugs in different chromatographic modes.

Keywords: pharmacertical analysis, separation, monolithic column, HPLC