在线二维液相色谱技术用于天然产物活性成分筛选中的研究进展

袁嘉明, 赖　亮, 汪锦才, 张婷婷, 江正瑾*

(暨南大学药学院, 药物分析研究中心, 广东 广州 511436)

摘要: 高效快速地分离和筛选复杂体系中的活性成分是从天然产物中发现先导化合物的关键。近年来, 在线二维液相色谱技术因具有分辨率高、自动化程度高以及集成方式灵活等优势在天然产物活性成分筛选研究中备受青睐。本文简要综述了在线二维液相色谱技术的特点及其在天然产物活性成分筛选领域中的应用研究进展, 主要从柱前、柱上以及柱后三大筛选模式的不同集成方式展开叙述, 并指出其不足, 以期为后续应用研究提供科学参考, 促进该技术在活性成分筛选领域的快速发展。

关键词: 二维液相色谱; 在线; 天然产物; 活性成分筛选

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Advances in online two-dimensional liquid chromatography for natural product screening

YUAN Jia-ming, LAI Liang, WANG Jin-cai, ZHANG Ting-ting, JIANG Zheng-jin*

(*Institute of Pharmaceutical Analysis, College of Pharmacy, Jinan University, Guangzhou 511436, China*)

Abstract: As a treasure resource of novel drug lead compounds, how to rapidly and high-efficiently screen and isolate active components from natural products is critical. Thanks to its high resolution, high automation and flexible integration, online two-dimensional liquid chromatography has great potential for screening active ingredients from complex matrices by integrating a highly specific bio-recognition process into a two-dimensional liquid chromatography system before, on or after the column separation. This review comprehensively summarized recent developments, applications and shortcomings of online two-dimensional liquid chromatography for natural product screening from different integration modes, including pre-column, on-column and post-column screening methods.

Key words: two-dimensional liquid chromatography; online; natural product; active compounds screening

天然产物泛指动物、植物和微生物体内的组成成分、内源性化学成分及其次级代谢产物等, 多年来, 天然产物因其固有的结构多样性一直作为药物先导化合物的主要来源而广受关注, 典型例子为抗疟药物青蒿素的发现[1]。然而, 因其结构多样性与复杂性, 从天然产物中快速筛选和鉴定具有生物活性的化学成分仍具备很大的技术挑战性[2]。随着现代分析技术的发展, 在线二维液相色谱技术已被开发用于从天然产物中快速分离和筛选潜在的生物活性成分, 并取得了阶段性的成果[3-5]。本文基于不同的在线集成方式综述了二维液相色谱技术在天然产物活性成分筛选方面的研究进展, 以期为天然产物活性成分的快速筛选提供技术参考。

1　二维液相色谱概述

天然产物成分极其复杂, 所含化合物的理化性质以及含量差异大, 传统一维液相色谱有限的峰容量以及分辨率往往不能满足复杂体系的分离要求[6]。相比

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*通讯作者 Tel: 86-20-85223604, E-mail: jzjjackson@hotmail.com

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于一维液相色谱, 基于两种独立分离机制的二维液相色谱技术可显著提高系统的峰容量、选择性以及对痕量组分的分析灵敏度, 并减少分析柱以及检测器的污染, 已广泛应用于天然产物的分析检测、制备分离和活性成分筛选等领域[4,7-9]。

根据第一维流分是否直接转移至第二维, 二维液相色谱可分为离线 (off-line) 模式与在线 (on-line) 模式两种类型, 其中, 在线模式因减少人为误差、缩短分析时间、提高分析通量与分离效率等优势而备受青睐。在线模式又可根据第一维流分是否全部转移至第二维, 分为全二维模式 (comprehensive two-dimensional liquid chromatography, LC×LC) 与中心切割二维模式 (heart-cutting two-dimensional liquid chromatography, LC-LC)[10]。全二维模式是指经第一维色谱分离的全部流分连续且直接地通过二位八通/十通阀等转入到第二维分离系统中; 而中心切割二维模式是将第一维收集的目标性流分直接通过六通阀转入到第二维色谱柱中进行分离[11]。图 1 为全二维模式典型装置示意图, 图 2 为中心切割二维模式的典型装置示意图。

2 在线二维液相色谱技术在天然产物活性成分筛选中的应用

从天然产物到单体先导化合物的发现, 成分分离是主要的限速因素, 如何快速锁定复杂体系中潜在活性成分群, 并从复杂体系中分离出来是成功与否的

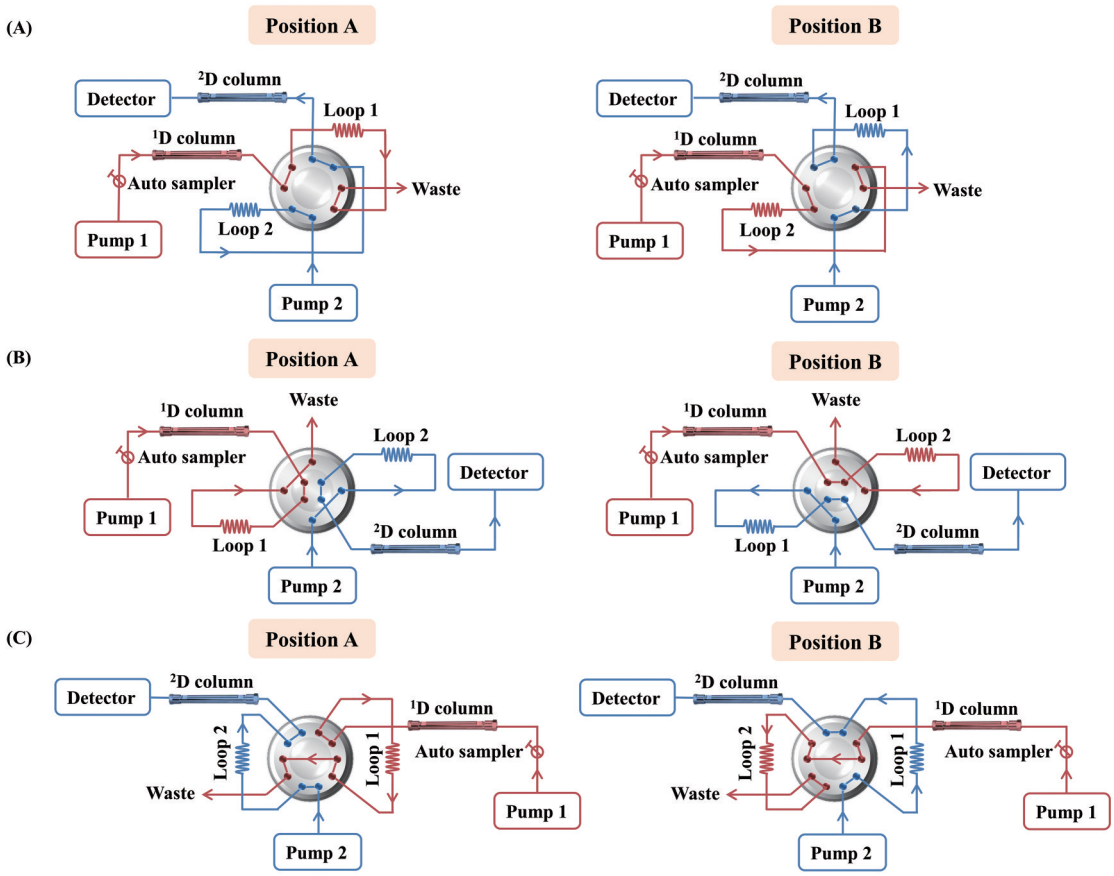

Figure 1 Schematic diagram of comprehensive two-dimensional liquid chromatography set-up based on two-position eight-port valve (A); dual two-position four-port valve (B); two-position ten-port valve (C)

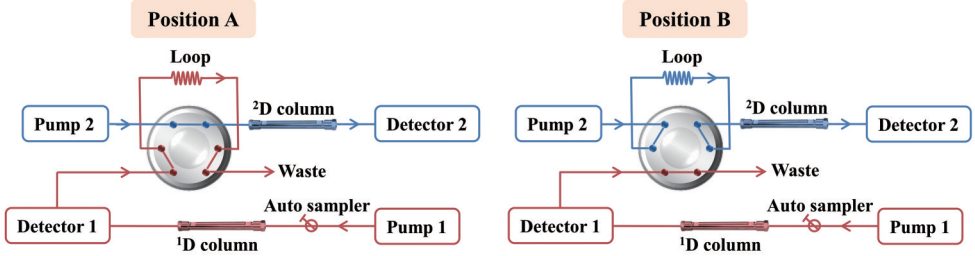

Figure 2 Schematic diagram of heart-cutting two-dimensional liquid chromatography set-up based on two-position six-port valve

关键。为了快速锁定、分离复杂体系中的活性物质，近年来涌现了一系列基于高效液相色谱技术 (high-performance liquid chromatography, HPLC) 与紫外、荧光、质谱等结构/活性检测仪器联用的筛选方法，如超滤法、磁珠垂钓法、细胞膜色谱法、固定化酶技术、在线生物测定及微流分活性评价等[12]。其中，在线二维液相色谱技术因其具有集成方式灵活、操作简便、自动化程度高等优点更适用于复杂多变的体系筛选，极大地提高了筛选效率，已在天然产物活性成分筛选方面取得了很大的进展。当前，在线二维液相色谱技术在天然产物活性成分筛选上大多以“小分子药物能与功能性蛋白、酶、生物膜等发生特异性生物识别相互作用”为理论依据，根据该生物识别过程在整个二维液相系统中所处的阶段，可分为柱前、柱上与柱后三种类型，本文将从柱前、柱上与柱后三大模式简述其技术特点及应用。

2.1 柱前模式

柱前模式是指将配体与受体预先混合孵育后注入二维液相系统，无亲和力或弱亲和力的小分子化合物呈现游离状态，而具有较高亲和力的潜在活性成分则被受体捕获形成复合物，利用洗脱液将配体洗脱，而后转移至液相系统中经进一步的分离分析与结构鉴定，从而筛选出复杂体系中具有潜在活性的单一化合物。在线二维液相色谱中较为典型的柱前筛选模式为尺寸排阻色谱/湍流色谱与HPLC联用，该模式主要基于不同分离机制的色谱柱组合后展现的强大分离能力以快速获得亲和配体，以下将对这两种技术进行简述。

2.1.1 尺寸排阻色谱与高效液相色谱联用 尺寸排阻色谱 (size-exclusion chromatography, SEC) 是一种根据分子的尺寸进行分离的液相色谱技术，是工业、食品、生物医学及蛋白质组学等研究领域中的常规技术[13]。在复杂体系活性成分筛选领域，游离小分子化合物与蛋白−配体复合物的尺寸差异使它们能在SEC柱中分离，蛋白−配体复合物因尺寸大先流出SEC柱，游离小分子化合物因尺寸小而被保留。收集蛋白−配体复合物进行在线解离，释放的配体被富集并转移至HPLC中进行分析[14]。Flarakos等[15]构建了在线^{2}D-SEC-LC-MS系统，并将其应用于两个不同类型的小型化合物库中筛选人血清白蛋白 (HSA) 的配体，以确定相对的蛋白−配体结合亲和力。该系统在第一维SEC柱中使用定制的Sephadex G-25作为尺寸排阻介质将蛋白−配体复合物与无亲和力的游离化合物分离，使用Waters Oasis HLB柱 (30 mm × 2 mm, 5 μm) 作为trap柱捕获未被SEC保留的蛋白−配体复合物与多余的蛋白质，利用pH变化使trap柱内的HSA蛋白变性以释放配体，同时去除HSA蛋白，最后，将trap柱捕获的配体转移至反相液相色谱中进行分离分析，重现性和重复性良好。尽管在线^{2}D-SEC-LC-MS系统能高效地获得亲和配体，但变性的蛋白质易污染反相色谱柱，影响色谱柱的寿命。

2.1.2 湍流色谱与高效液相色谱联用 湍流色谱法 (turbulent flow chromatography, TFC) 是指流动相以高流速 (5～10 mL·min^{-1}) 通过大粒径固定相时展现出湍流特性的色谱技术，该技术结合了“尺寸排阻”和传统的固定相色谱柱特性，能将生物大分子 (如蛋白质) 与小分子化合物快速分离，已被广泛应用于生物样本、食品、药品和环境等分析领域[16-18]。由于TFC柱具有选择性保留小分子化合物的特性，湍流色谱常作为一种在线前处理手段与液相分析系统联用，构成典型的中心切割二维模式，用于生物样本中小分子药物的纯化和富集，以及中药分析和活性成分筛选等。Song等[19,20]在湍流色谱−液质联用 (TFC-LC-MS) 技术的基础上引入在线加压提取模块 (PLE)，构建了全新的在线加压提取−湍流色谱−液质联用 (online PLE-TFC-LC-MS/MS) 系统，并成功应用于荒漠肉苁蓉 (*Cistanche deserticola* Ma) 以及远志 (*Polygala tenuifolia* Willd.)等中药材的有效成分分析，实现了中药样品提取、分离和分析一体化，对中药质量控制、化学成分的直接分析以及药效物质基础研究提供了新思路。

2009年，Zhou等[21]提出在线二维液相色谱与湍流色谱联用 (on-line ^{2}D-TFC-HPLC-MS) 技术，并成功用于贝母类生物碱中乙酰胆碱酯酶和丁酰胆碱酯酶抑制剂的快速筛选，其主要原理如图3所示。与常见的TFC-LC-MS技术不同的是，该技术在分析柱前使用了两个二位六通阀分别连接两根不同功能的TFC柱，首先，第一维TFC柱作为亲水亲脂平衡柱 (HLB) 用于分离复杂体系中游离的小分子与蛋白质−配体复合物，复合物不被TFC柱保留而储存于loop样品环内 (图3A)；之后，将第一个二位六通阀切换至B状态，洗脱液被注入样品环内进行复合物的在线解离，第二维TFC柱则作为复合阳离子交换/反相柱 (MCX) 用于捕获释放的配体，同时去除体系中的蛋白质与盐 (图3B)；最后，将第一个和第二个二位六通阀分别切换至A状态和B状态，潜在的活性小分子化合物被转移至HPLC分析柱中进行分离分析，实现活性成分的快速筛选 (图3C)。为了进一步扩展该方法对天然产物中不同成分的适用性和应用，Fu等[22]通过将第一维TFC柱与第二维TFC柱分别更换为复合阴离子交换/反相柱 (MAX) 与HLB柱，改变了该系统的保留机制，显著改善第一维TFC柱对天然产物中亲水性及弱酸性物质的保留，从而降

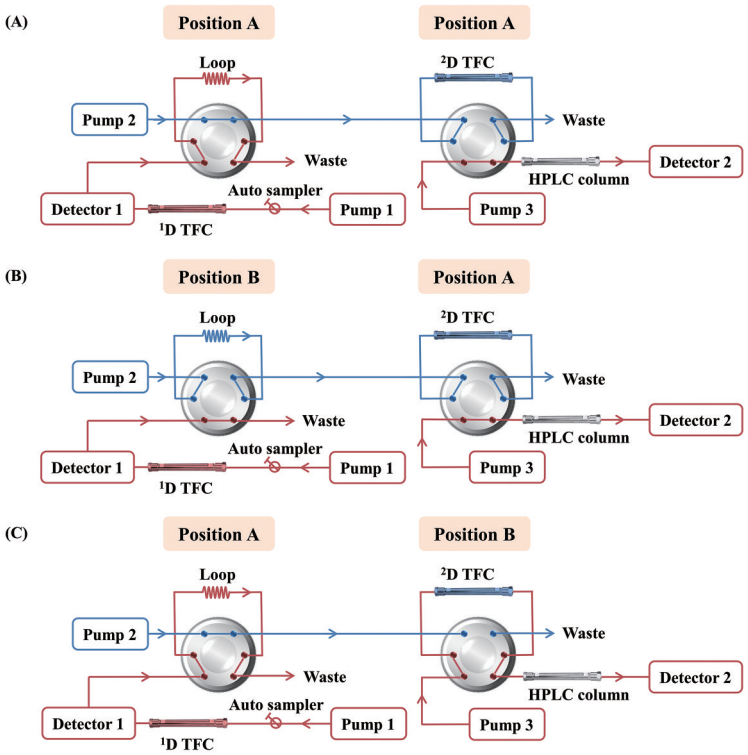

Figure 3 Schematic diagram of on-line ^{2}D-TFC-HPLC-MS set-up for screening active compounds. A: Reversed-phase TFC stage to separate the protein/ligand complex from the unbound free molecules; B: On-line dissociation process to release the bound ligands from the complexes and a second mixed-mode cation-exchange/reversed-phase TFC stage to trap the bound ligands; C: HPLC-MS analysis for identification and determination of the binding affinities

低假阳性率。该系统被用于在线筛选丹参中潜在的黄嘌呤氧化酶抑制剂, 并筛选出三种丹酚酸, 其中强活性成分丹酚酸C为黄嘌呤氧化酶抑制剂的首次报道。

^{2}D-TFC-HPLC-MS技术能实现配体与蛋白质的超快速分离, 通过不同的色谱柱组合能更广泛地适用于多种复杂体系潜在活性成分的快速筛选与分离鉴定, 相比于在线SEC-LC-MS联用技术而言, 该技术最大限度地减少分离材料和小分子之间的非特异性吸附。然而, 柱前筛选模式主要基于受体和配体之间的亲和作用, 并无法直接评估其生物活性, 之后仍需结合其他技术进行活性评价, 较为费时繁琐。在此基础上, 可采用失活蛋白进行同步对比, 以减少相应的非特异吸附。

2.2 柱上模式

柱上模式是指将生物活性大分子 (酶、功能性蛋白、生物膜等) 通过共价/非共价作用固定在不同材料表面, 并以此作为第一维或第二维液相色谱柱的固定相, 利用活性物质与固定化生物大分子间的亲和作用或基于固定化生物大分子的活性测试, 直接或间接地筛选出复杂体系中具有潜在活性的化合物。目前, 在线二维液相色谱中常见的柱上筛选模式包括固定化酶反应器/细胞膜色谱与高效液相色谱-质谱联用, 该模式集在线亲和垂钓与分离分析于一体以实现活性成分的快速筛选, 以下将对这两种技术进行简述。

2.2.1 固定化酶反应器与高效液相色谱联用

固定化酶反应器 (immobilized enzyme reactor, IMER) 可以定义为一个流通装置, 其中含有物理限制或化学键合的具催化活性的酶, 基于固定化酶反应器的二维或多维液相色谱已经成功应用于不同领域, 包括天然产物中抑制剂的筛选[23-25]。本文列举分析了IMER与高效液相色谱联用在天然产物活性成分筛选中的应用, 根据IMER与液相分析柱的集成顺序, 可分为IMER-HPLC (图4A) 与HPLC-IMER (图4B、C) 两种类型。

图4A展示了较为常见的IMER-HPLC二维液相活性成分筛选平台, 第一维IMER柱与第二维分析柱间通过二位六通阀连接, 该平台的基本过程为: 首先, 利用IMER在线捕获配体, 未结合成分则转移至废液口; 随后, 更换pump 1中的溶剂为洗脱液进行在线洗脱, 使固定化酶吸附的配体释放解离并储存在loop环或trap柱内; 最后, 通过自动阀切, pump 2中的流动相将富集的配体转移至HPLC分析柱内进行分离分析, 通过与UV或DAD检测器或质谱仪等联用, 进行成分指认与结构鉴定, 从而获得潜在的活性成分信息。Cornelio等[24]将牛组织蛋白酶D (CatD) 固定在熔融石英毛细管 (40 cm × 100 μm I.D.) 上制备了CatD-IMER,

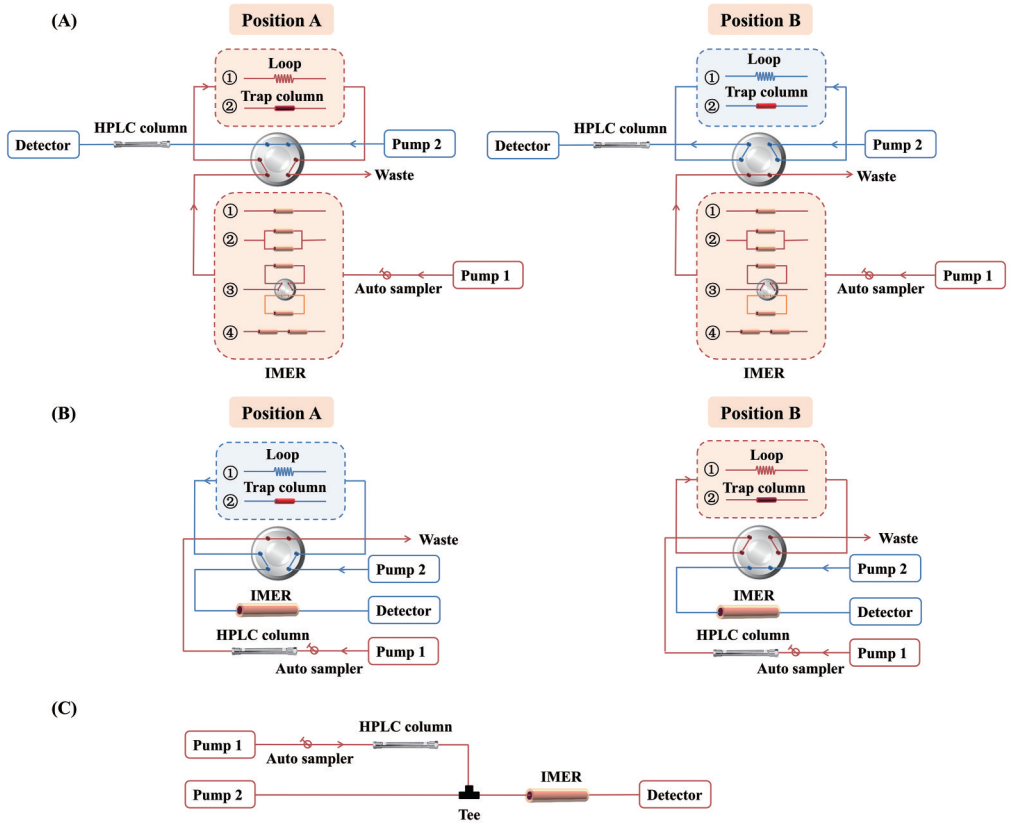

Figure 4 Schematic diagram of on-line IMER-HPLC (A) and HPLC-IMER (B, C) set-up for screening active compounds. IMER: Immobilized enzyme reactor

通过一个二位六通阀将其与C18柱相连, 构建在线二维液相色谱筛选平台, 并将其应用于12种天然产物粗提物中进行活性成分筛选, 从中筛选出26种潜在的活性化合物。相似地, Lima等[26]构建了固定化人核苷二磷酸激酶B反应器 (NME2-ICER)-HPLC筛选平台, 使用 (−)-表儿茶素没食子酸酯 (ECG) 验证了该方法在NME2配体筛选和NME2抑制剂鉴定中的潜在应用。与上述不同的是, Qiu等[27]在反应器材料上进行创新, 首次将α-葡萄糖苷酶固定在ZIF-90上并物理装载于注射器用过滤器中形成微反应器, 通过二位六通阀与C18柱相连, 构建在线二维液相色谱系统, 应用于从薯蓣果皮、金银花、信阳毛尖茶和熟地黄等天然产物中筛选α-葡萄糖苷酶抑制剂, 共筛选出11个活性化合物, 其中不乏首次报道或活性高于临床用药阿卡波糖的化合物, 展示了该系统在筛选天然产物中酶抑制剂方面的巨大潜力。为了增大酶固定量, Peng等[28]将黄嘌呤氧化酶固定在氨基官能化硅球表面并装填于不锈钢微柱中制成固定化黄嘌呤氧化酶微柱 (IXOM), 采用二位四通阀和二位六通阀作为连接元件, 将IXOM与HPLC-DAD-MS/MS相连, 构建了在线二维筛选平台, 应用于筛选和鉴定山银花粗提物及其人肝微粒体代谢物中的黄嘌呤氧化酶抑制剂, 获得9个生物活性化合物, 包括6个原型化合物和3个代谢物。为了更加快速高效的筛选, Seidl等[29]针对中/晚期阿尔茨海默病治疗的两个关键靶蛋白 [乙酰胆碱酯酶 (AChE) 和丁酰胆碱酯酶 (BChE)], 开发了一种较为新颖的基于AChE-BChE反应器与质谱联用的在线双酶平行筛选与鉴定平台, 该平台只需一次样品注射, 即可在不到6 min的时间内同时筛选两种不同的酶抑制剂, 但该系统仅使用阳性药加兰他敏作为概念验证, 并未扩大到其应用。然而, 上述二维液相筛选平台仅基于酶反应器与配体的亲和作用进行筛选, 必然存在不可忽视的非特异性吸附问题, 仍需要依靠其他活性检测手段进一步确证化合物的活性。为此, 笔者所在课题组[30]使用二位六通阀将乙酰胆碱酯酶反应器与失活的乙酰胆碱酯酶反应器并联, 用于延胡索提取物中活性成分的筛选, 被捕获的成分经洗脱后转移至样品环内, 随后进行二维的在线分离分析, 通过对比酶反应器与失活酶反应器所捕获配体的色谱峰/质谱数据的差异, 可以排除由于材料/酶引起的非特异性吸附, 最终确认8个活性化合物。

图4B与C均为HPLC-IMER集成模式, 即分析物首先经过第一维分析柱以其原始形式分离, 而后泵入

底物/缓冲液将目标性流分 (图4B) 或全部流分 (图4C) 通过二位六通阀/三通接头转移至IMER中进行反应, 流经IMER的少量分析物能在几分钟内转化为具有紫外吸收/荧光/电化学检测的产物, 常用于酶活性的测试, 也可以进行间接的酶抑制剂筛选。Yuan等[31]将这种HPLC-IMER-MS集成筛选平台应用于石蒜提取物中乙酰胆碱酯酶抑制剂的筛选, 并首次报道了一种新的乙酰胆碱酯酶抑制剂 (dihydro-latifaliumin C)。但是该模式除了底物消耗量大以外, 还容易受到有机流动相、pH等的限制, 常需加入稀释泵对第一维流动相进行稀释, 以免影响IMER的活性。

2.2.2 细胞膜色谱与高效液相色谱联用 细胞膜色谱 (cell membrane chromatography, CMC) 是指将具有生物活性的细胞膜固定在色谱填充材料表面作为色谱柱固定相的仿生亲和色谱技术, 首创自西安交通大学贺浪冲教授课题组, 已广泛应用于药物-受体相互作用的研究以及复杂体系中活性成分的筛选[32,33]。细胞膜色谱法因具有细胞膜活性与色谱分离的双重特性, 能实现对目标成分的特异性捕获以及分离, 具有巨大的应用潜力[4]。尽管CMC省时高效且特异性高, 但仍存在柱效低、柱寿命短及峰容量低等不足, 这限制了该技术的应用。为了提高柱效、峰容量与分离效率, 可在CMC后增加分析柱, 构建CMC与二维或多维液相联用的分析平台, 以下就二维细胞膜色谱与高效液相色谱联用常见的原理与集成方式进行简述, 如图5所示。

该平台为典型的全二维模式, 以一个两位十通阀将第一维的CMC色谱柱与第二维的分析柱相连, 十通阀上搭载的两个500 μL loop环用于不断地交替富集并储存自动阀切过程中的CMC流出成分。最初, loop 1环用于富集CMC流分, 一段时间后, 十通阀自动切换至B状态, 与此同时, loop 2环继续富集CMC流分, loop 1环内的流分则转移至第二维分析柱中进行分离, 如此循环交替, 直至获得CMC全部流分的色谱及质谱数据, 以进行全面的分析。Li等[34]以腹膜巨噬细胞膜 (PM) 作为固定相, 建立了一种新型的腹膜巨噬细胞/细胞膜色谱-在线-高效液相色谱/质谱法 (PM/CMC-online-HPLC/MS) 联用技术, 并成功应用于多穗金粟兰提取物中抗炎成分的快速检测、富集和鉴定。在此基础上, 为了减少假阳性结果, Chen等[35]提出了基于正常心脏CMC (NM/CMC) 与多柔比星 (DOX) 诱导的衰竭心肌CMC (FM/CMC) 模型比较的在线二维细胞膜色谱-高效液相色谱联用技术应用于从中药附子中筛选抗DOX诱导心力衰竭的活性成分, 该技术首次实现了正常CMC柱与病理性CMC柱之间潜在活性成分的可视化亲和力比较, 快速地筛选出4个潜在的活性成分。CMC与HPLC-MS在线耦合方法对天然产物中药物先导化合物的发现提供了一种自动化、高通量、实用性强的技术参考, 目前已广泛应用于天然产物中活性成分的筛选, 见表1[36-53]。

2.3 柱后模式

柱后模式是指样品经过二维液相分离后再进行活性测试, 即配体-受体相互作用过程发生在二维液相系统之后, 目前在天然产物活性成分筛选领域相关的报道主要为基于一维液相的在线生物测定法[2,54,55]以及柱后微流分活性评价法[56,57]。然而, 一维液相有限的峰容量以及分辨率并不能满足复杂体系中活性成分的有效分离, 为了减少活性检测干扰导致的假阳性或假阴性结果, 在线二维液相系统在复杂体系化合物的分离

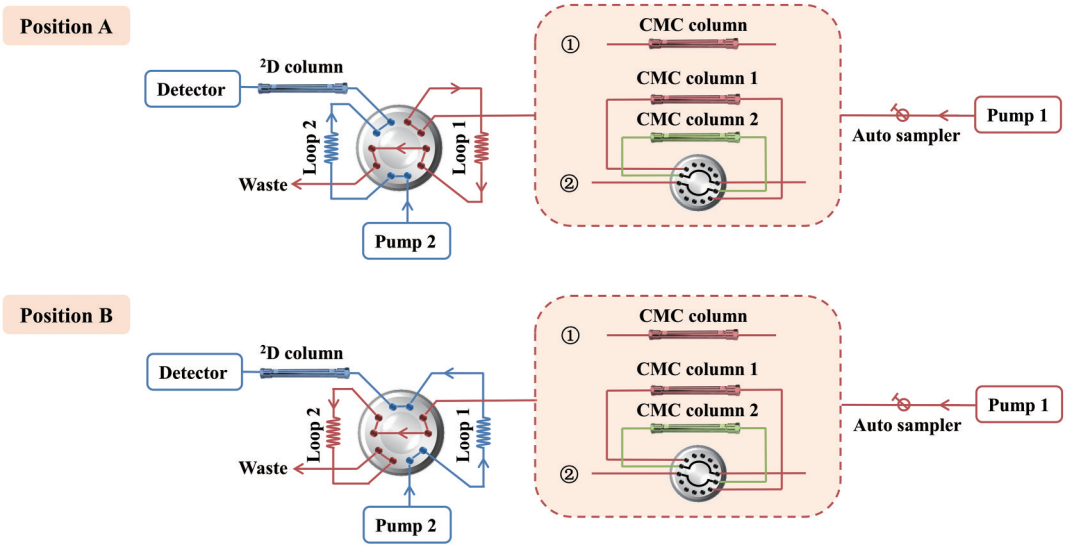

Figure 5 Schematic diagram of on-line CMC-HPLC set-up for screening active compounds

Table 1 Applications of online two-dimensional cell membrane chromatography in the screening of active compounds from natural products

Model method	^{1}D column	^{2}D column	Valve	Samples	Ref.
EGFR/CMC-online-HPLC/MS	EGFR/CMC column (10 mm × 2.0 mm, 5 μm)	VP-ODS column (150 mm × 2.0 mm, 5 μm)	Two-position ten-port with two enrich columns	*Scutellaria baicalensis* Georgi	[36]
VEGFR-CMC-online-LC/MS	VEGFR-CMC column (10 mm × 2.0 mm, 5 μm)	VP-ODS column (150 mm × 2.0 mm, 5 μm)	Two-position ten-port with one enrich column	*Aconitum carmichaeli* Debx.	[37]
A431/CMC-online-LC/MS	A431/CMC column (10 mm × 2.0 mm, 5 μm)	VP-ODS column (150 mm × 2.0 mm, 5 μm)	Two-position ten-port with two enrich columns	Radix Caulophylli	[38]
α1AAR-CMC-online-HPLC/MS	α1AAR-CMC column (10 mm × 2.0 mm, 5 μm)	VP-ODS column (150 mm × 2.0 mm, 5 μm)	Two-position eight-port with two loops	Radix Caulophylli	[39]
α1A/CMC-online-LC/MS	α1A/CMC column (10 mm × 2.0 mm, 5 μm)	VP-ODS column (150 mm × 2.0 mm, 5 μm)	Two-position ten-port with two enrich columns	Fructus Piperis	[40]
EGFR/CMC-online-HPLC-MS	EGFR/CMC column (10 mm × 2.0 mm, 5 μm)	VP-ODS column (150 mm × 2.0 mm, 5 μm)	Two-position ten-port with two enrich columns	*Curcuma longa*	[41]
FGFR4/CMC-online-LC/MS	FGFR4/CMC column (10 mm × 2.0 mm, 5 μm)	VP-ODS column (150 mm × 2.0 mm, 5 μm)	Two-position ten-port with one enrich column	*Brassica albla*	[42]
H1R-CMC-online-HPLC-MS	FGFR4/CMC column (10 mm × 2.0 mm, 5 μm)	VP-ODS column (150 mm × 2.0 mm, 5 μm)	Two-position ten-port with two enrich columns	Yujin injection	[43]
EGFR/CMC-online-HPLC-MS	EGFR/CMC column (10 mm × 2.0 mm, 5 μm)	VP-ODS column (150 mm × 2.0 mm, 5 μm)	Two-position ten-port with two enrich columns	Radix et Rhizoma Asari	[44]
RBL-2H3/CMC-online-HPLC/MS	RBL-2H3/CMC column (10 mm × 2.0 mm, 5 μm)	VP-ODS column (150 mm × 4.6 mm, 5 μm)	Two-position ten-port with two enrich columns	Shuanghuanglian injection	[45]
HPDLC/CMC-online-HPLC/MS	hPDLC/CMC column (10 mm × 2.0 mm, 5 μm)	VP-ODS column (150 mm × 2.0 mm, 5 μm)	Two-position ten-port with two enrich columns	*Scutellaria baicalensis* Georgi	[46]
Y367C-FGFR4-CMC-online-HPLC-MS	FGFR4/CMC column (10 mm × 2.0 mm, 5 μm)	VP-ODS column (150 mm × 2.0 mm, 5 μm)	Two-position ten-port with one enrich column	*Ligusticum wallichii*	[47]
HepG2/CMC/ECs-online-HPLC/TOF-MS	HepG2/CMC column (10 mm × 2.0 mm, 5 μm)	XBridge™ C18 column (100 mm × 3.0 mm, 3.5 μm)	Two-position ten-port with two enrich columns	Radix Scutellariae	[48]
MRGPRX2-HEK293/CMC-online-HPLC-ESI-IT-TOF-MS	FGFR4/CMC column (10 mm × 2.0 mm, 5 μm)	RP-18e column (100 mm × 4.6 mm, 5 μm)	Two-position ten-port with one enrich column	Kudiezi injection	[49]
MCF7/CMC-online-Capcell-C18/TOF-MS	MCF7/CMC column (10 mm × 2.0 mm, 5 μm)	Capcell-C18 column (100 mm × 3.0 mm, 5 μm)	Two-position eight-port with two loops	Rhizoma Corydalis, Radix Angelicae Dahurica	[50]
DU145/CMC-online-HPLC/TOF-MS	DU145/CMC column (10 mm × 2.0 mm, 5 μm)	C18 column (100 mm × 3.0 mm, 5 μm)	Two-position eight-port with two loops	Radix et Rhizoma Rhei	[51]
EGFR/CMC-online-HPLC-IT-TOF-MS	EGFR/CMC column (10 mm × 2.0 mm, 5 μm)	VP-ODS column (150 mm × 2.0 mm, 5 μm)	Two-position ten-port with two enrich columns	*Marsdenia tenacissima*	[52]
SiO_2-GQDs- EGFR/CMC-online-LC-IT-TOF-MS	EGFR/CMC column (10 mm × 2.0 mm, 5 μm)	VP-ODS column (250 mm × 4.6 mm, 5 μm)	Two-position six-port with one enrich column	*Peucedanum praeruptorum* Dunn	[53]

中展现出巨大的应用潜力[58]。Pandohee等[59]构建了中心切割二维液相系统与酸性高锰酸钾化学发光检测方法相结合的筛选平台以实现大麻样品中多种大麻素的完整分离与极微量成分的活性筛选。该平台将第一维Phenomenex Luna 5 u CN 100 Å柱 (4.60 mm × 150 mm, 5 μm) 与第二维Agilent Eclipse XDB-C18柱 (4.60 mm × 150 mm, 5 μm) 正交, 克服了气相色谱或一维液相色谱中大麻素发生热降解或分离度低等问题, 是首次对大麻进行全面的二维液相分析与筛选, 具有很大的研究潜力。为了实现全自动化高通量活性成分筛选, Coulerie等[60]将在线^{2}D-LC×LC-UV/ELSD分流系统与高通量筛选 (HTS) 平台结合, 用于红车轴草提取物中腺苷酸活化蛋白激酶 (AMPK) 激动剂与抑制剂的筛选。该技术流程如图6所示, 主要涉及三个步骤: ① 样品的制备与预纯化; ② ^{2}D-LC×LC的分离, 使用蒸发光散射器 (ELSD) 量化所有收集的流分并标准化浓度; ③ 流分转移至96孔板后进行活性检测。文中还使用该技术与^{1}D-LC分流系统进行对比, 结果表明^{2}D-LC×LC-UV/ELSD分流系统能筛选出更微量的活性成分, 避免了活性成分的漏检, 具有更高的筛选效率。但是, 基于柱后模式的筛选往往需要离线进行基于多孔板的活性测试, 繁琐的干燥、移液等步骤增加了筛选时间, 过程中的人为/系统误差给活性成分指认工作带来了很大的不确定性。

3 结论与展望

当前, 针对天然产物活性成分筛选的方法层出不穷, 在线二维液相色谱技术相对于其他方法而言, 具有操作简便、自动化程度高、省时高效、有效避免干扰等

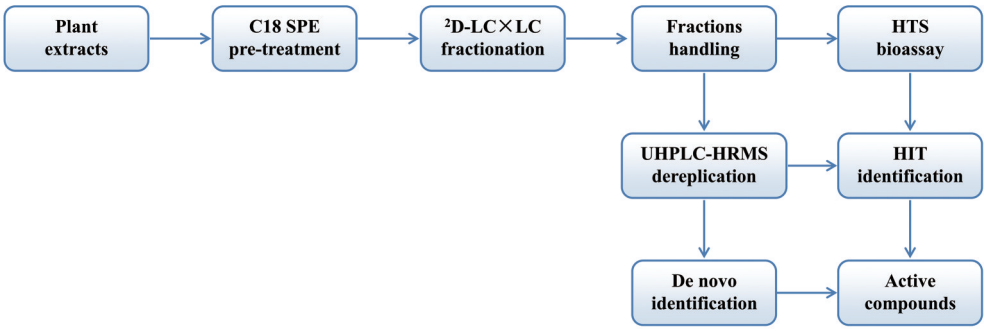

Figure 6 Technical flow chart of online ^{2}D-LC×LC-UV/ELSD platform for high-throughput screening of active compounds (Reproduced with permission of Ref. 60, Copyright of ©2016 American Chemical Society)

优点。然而, 不同在线集成方式的二维液相色谱技术存在自身局限性, 比如自身搭建的在线系统不成熟, 稳定性与重复性远差于商业化仪器; 其次, 柱前与柱上筛选模式常见的非特异吸附问题, 常需依靠对照实验进行对比, 从而排除假阳性结果, 增加了样品用量与实验成本; 再者, 柱上筛选模式对于靶蛋白的选择十分有限, 实验设计人员不仅需要考虑固定化方式可能引起的蛋白结构改变或破坏, 还需要考虑管路溶剂兼容性、阀切时间与流速等因素; 最后, 柱后筛选模式中液相色谱流动相与活性测试缓冲体系不兼容, 常需干燥、复溶后再进行活性检测, 大大增加了筛选时间, 不利于筛选稳定性差的样品, 此外, 同时具备高灵敏度与高分辨率一直是科研人员在该模式下追求的目标。

综上所述, 在线二维液相色谱技术在天然产物活性成分筛选领域展现了巨大的应用潜力, 针对不同的复杂样本以及活性测试体系, 可以根据以上提及的多种二维集成方式选择合适的筛选模式, 或者考虑其他的多维组合方式, 但这些技术的普及仍需在实践中不断改进与完善, 使之成为天然产物药物先导化合物筛选与药效物质基础研究的有力工具。展望未来, 快速、高通量、低成本、微型化、智能化的多维在线液相色谱技术必将是药物筛选领域的研究热点!

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