基于UPLC-MS/MS技术的内源性胆汁酸分析方法研究进展

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摘要: 胆汁酸 (bile acids, BAs) 是一类调节脂质、葡萄糖和能量代谢的内源性甾体物质。其作为宿主和肠道微生物代谢的关键信号分子, 在维持机体稳态和生理功能中发挥着重要的作用。生物体内BAs的准确定性定量在基础及临床研究中具有重要意义。过去几十年间, 酶法分析、酶联免疫分析法、核磁共振、色谱法等相关技术均相继应用于 BAs的检测。由于BAs结构多样、存在同分异构体, 且生物样本基质复杂, 给内源性BAs检测带来巨大挑战。超高效液相色谱−串联质谱 (UPLC-MS/MS) 是一种稳健性分析技术, 其结合了 UPLC快速的分离能力及MS/MS强大的结构鉴定功能, 使得生物样品中目标分析物的快速分离、准确定性及定量检测更加便利。UPLC-MS/MS因具有高选择性、高灵敏度、高准确性等优势, 近年来被广泛用于BAs的分析。本文主要对BAs的生物合成途径、样品前处理方法、常见分析检测技术进行总结, 并重点介绍了近五年来UPLC-MS/MS技术在内源性BAs分析中的应用现状, 旨在为内源性BAs准确检测及进一步研究和应用提供参考。

关键词: 胆汁酸; 生物基质; 样品前处理; 液质联用; 分析方法

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Advances in analytical methods for endogenous bile acids based on UPLC-MS/MS technology

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Abstract: Bile acids (BAs) are a group of endogenous steroid molecules that regulate lipid, glucose and energy metabolism. They play an important role in maintaining body homeostasis and physiological functions as key signaling molecules for host and gut microbial metabolism. The accurate characterization and quantification of BAs *in vivo* is of great importance in basic and clinical research. Over the past decades, enzymatic assay, enzyme-linked immunoassay, nuclear magnetic resonance (NMR), chromatography, and other related techniques have been developed and applied to the detection of BAs. The diverse structures of BAs, the existence of isomers and the complex matrix of biological samples pose great challenges for the detection of endogenous BAs. Ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) is a robust analytical technique that combines the rapid separation capacities of UPLC with the powerful structural identification capabilities of MS/MS, facilitating the more rapid separation, characterization and accurate quantitative of target analytes in biological samples. UPLC-MS/MS has been widely used in the quantitative analysis of BAs in recent years for its high selec-

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tivity, high sensitivity, and high accuracy. This paper summarized the biosynthetic pathways of BAs, sample pretreatment methods, common analytical detection techniques, and highlights the current status of the application of UPLC-MS/MS technology in the analysis of endogenous BAs over the past five years, to provide a reference for the accurate detection of endogenous BAs and further research development and application.

Key words: bile acid; biological matrix; sample preparation; UPLC-MS/MS; analytical method

胆汁酸 (bile acids, BAs) 是生物体内一类具有特殊理化性质的酸性类固醇的总称, 由肝脏中的胆固醇经羟基化和侧链氧化步骤合成后储存在胆囊中, 是胆固醇的最终代谢产物[1,2]。BAs作为信号分子在生物体内发挥着重要的调控功能[3,4], 在糖代谢、脂代谢、能量代谢及脂溶性维生素吸收等过程中发挥着关键作用[5-9]。同时研究表明, BAs是细胞表面受体G蛋白偶联受体、核激素受体的内源性配体[10], 是法尼醇X受体的天然激动剂[11,12]。生物体内BAs合成和代谢紊乱会导致多种疾病的发生和免疫功能的失调, 如肥胖、糖尿病、非酒精性脂肪肝等代谢性疾病[13,14], 上述疾病的发生和发展也会影响机体BAs的产生与循环[15,16]。

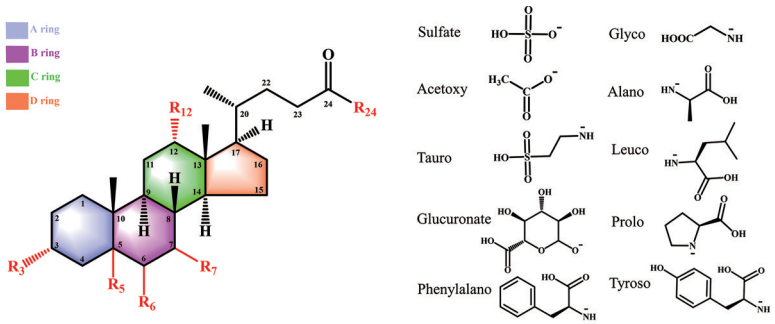
BAs作为关键的内源性分子, 在临床上逐渐被用于疾病的治疗与诊断。熊去氧胆酸胶囊 (商品名: 优思弗) 是美国食品药品监督管理局批准的第一个在临床上用于治疗原发性胆汁性胆管炎的药物[17], 也被用于非酒精性脂肪肝的治疗[18-21]; 此外, 药理研究表明, 鹅去氧胆酸 (chenodeoxycholic acid, CDCA) 在高剂量使用时具有溶解小型胆结石的作用[22]; 奥贝胆酸用于治疗原发性胆汁性胆管炎[23-25]; Zheng等[26]研究发现猪胆酸与糖尿病之间存在负相关, 提出用其代谢轮廓来评估人体发生代谢性异常风险的高低; Alamoudi等[27]发现, 原发性胆汁性肝硬化患者的尿液中总BAs含量较高, 而乙型肝炎病毒感染者的尿液中总BAs量仅略有增加, 尿液中胆酸 (cholic acid, CA) 和CDCA的增加在原发性硬化性胆管炎患者中明显高于乙型肝炎病毒患者。因此, 对BAs化合物进行全面表征及药理作用的深入研究, 对于新药的发现及临床中代谢性疾病的快速诊断具有重要意义。

BAs是一类天然甾体化合物, 是胆烷酸的羟基衍生物。BAs甾核四个环的稠合方式与植物甾醇相似, A/B环有顺式和反式两种稠合方式, B/C环和C/D环均为反式稠合。根据环上羟基及侧链羧基是否与基团发生结合, 可将BAs分为游离型BAs与结合型BAs, 游离型BAs如CA、熊去氧胆酸 (ursodeoxycholic acid, UDCA)、CDCA、去氧胆酸 (deoxycholic acid, DCA) 等。部分BAs在结构上只是个别基团的构型不同, 如UDCA和CDCA, 二者在结构上只是7位碳原子上连接的羟基构型 (*α*/*β*) 不同; 此外, 生物体内不同的游离型BAs在酶的催化作用下还可与氨基酸、葡糖醛酸、硫酸等发生一系列反应形成结合型BAs (图1)。

BAs的化学结构式相似, 存在多个同分异构体。BAs池组成的复杂性导致可用于快速和准确检测BAs分析技术的应用受到了一定的限制。BAs的检测方法可分为非色谱法和色谱法。非色谱法主要包括酶循环法、酶联免疫吸附测定 (enzyme-linked immunosorbent assay, ELISA)、核磁共振波谱 (nuclear magnetic resonance, NMR) 等; 色谱法如早期常用于BAs检测的薄层色谱 (thin-layer chromatography, TLC)、气相色谱 (gas chromatography)、气相色谱−质谱联用 (gas chromatography-mass spectrometry, GC-MS) 等, 近年来随着色谱技术的快速发展, 高效液相色谱−质谱联用 (high-performance liquid chromatography-mass spectrometry, HPLC-MS) 及超高效液相色谱−串联质谱 (ultra-performance liquid chromatography-tandem mass spectrometry, UPLC-MS/MS) 技术在BAs的分离检测中逐渐成为主流方法[28-30], 该类技术具有灵敏度高、特异性好、检出限低的特点, 被广泛用于生物样本中代谢物谱的全表征[31]。本文介绍了BAs的生物合成途径和常见分析技术, 主要对近五年来UPLC-MS/MS技术在生物样本中BAs检测方法进行了总结, 包括样本前处理方法、色谱分离方法及质谱检测方法, 并对BAs检测未来的发展趋势做了展望, 以期为今后BAs检测方法的开发提供思路。

1 BAs生物合成途径简介

BAs是胆固醇的最终代谢产物, 在肝脏中主要通过经典途径和替代途径这两种生物合成途径产生[32,33], 包含多种酶的参与 (图2), 由此产生的胆汁酸被称为初级胆汁酸。经典途径是通过胆固醇-7*α*-羟化酶 (cholesterol 7*α*-hydroxylase, CYP7A1) 介导进行初级BAs的合成, 该途径产生约75%的初级BAs, 由于经典途径中的中间代谢产物为中性甾醇类化合物, 故经典途径又被称为“中性途径”, 该类中性甾醇只存在于肝脏中, 后进一步被合成生物体内主要的胆汁酸: CA和CDCA。生物体内的初级BAs产生在较小程度上依赖于替代途径中甾醇27-羟化酶 (sterol 27-hydroxylase,



$R_{3,5,6,7,12}$	R_{24}
α/β-H	OH
α/β-OH	-$C_2H_4NO_2$(Glyco)
C=O	-$C_3H_6NO_2$(Alano)
SO_4H(Sulfate)	-$C_2H_6NO_3S$(Tauro)
C_2H_3O(Acetoxy)	-$C_6H_{12}NO_2$(Leuco)
-$C_6H_9O_7$(Glucuronate)	-$C_6H_9O_7$(Glucuronate)
	-$C_5H_8NO_2$(Prolo)
	-$C_9H_{10}NO_2$(Phenylalano)
	-$C_9H_{10}NO_3$(Tyroso)

Figure 1　Structure profile of bile acids (BAs)

CYP27A1) 的催化作用, 该途径又被称为酸性途径, 主要产生CDCA。在啮齿类动物中, 大多数CDCA在酶的作用下被很快转化为鼠胆酸, 当机体中的代谢处于非平衡状态时该途径在初级BAs的合成中变得尤为重要[34]。在肝脏中形成的大部分初级BAs可在酶的作用下与甘氨酸、牛磺酸等常见的氨基酸发生结合生成亲水作用较强的结合型BAs。由这两种途径产生的初级BAs被分泌到胆囊中, 当生物体进食后初级BAs被释放到肠道中后在肠道微生物的作用下发生水解、脱羟基化及差向异构化等一系列的反应而生成次级BAs, 如DCA、石胆酸 (lithocholic acid, LCA)、UDCA等。当BAs到达小肠远端时, 大部分BAs会被小肠上皮细胞重新吸收通过肠肝循环返回至肝脏, 而少量BAs会进入大肠通过粪便排出体外。

2　生物样本前处理方法

BAs的生物合成途径表明, BAs主要存在于肝脏、胆囊、肠道、肠道内容物、粪便中。血清、血浆、肝脏、肠内容物、粪便、尿液、胆囊等为常见的待测样本[35]。样品前处理的主要目的是去除对目标分析物产生干扰的成分如脂质、蛋白质、无机盐等[36], 减少基质效应对目标化合物检测时的影响。此外, 为降低基质效应对待测化合物检测的影响, 内标法被广泛用于样品的定量中, Krautbauer等[37]将稳定同位素标记物用于人类血清中BAs的含量测定, 采用5种 d_5-牛磺酸结合型胆汁酸作为内标, 结果精密度、准确度方面有了较高的提升。常见的样品前处理方法如蛋白沉淀、固液萃取、液液萃取、固相萃取、衍生化等[35]; 近年来研究者逐渐将一些新方法用于样本前处理中, 如固相萃取中采用新型填料对目标化合物进行富集。在不同的生物样本之间, 存在一定的异质性且BAs浓度较低, 故对样品前处理的操作及仪器检测的灵敏度要求较高, UPLC-MS/MS具有较高的灵敏度和较低的检测限可以满足大多数生物样本的分析需求, 且与GC-MS技术相比, UPLC-MS/MS样本前处理中无需复杂的衍生化操作; 相较于酶循环法中BAs的含量测定高度依赖于酶的纯度, UPLC-MS/MS样本前处理中无需酶的参与[38], 故本部分将重点讨论这些常见生物样本采用UPLC-MS/MS分析时的前处理操作。

2.1　常见样本前处理方法

2.1.1　血清/血浆、尿液　血液样本的采集具有微创性, 且含有丰富的生物学信息, 故血液样本是代谢组学研究中的首选生物体液[39]。尿液是一种淡黄色液体, 除包含水外, 还有无机盐、尿素、尿酸等成分, 是临床疾病检验中常见的待测样本[40], BAs作为一类两亲性的化合物, 在尿液中也占有一定比例。有研究表明, 血清和血浆之间BAs的浓度没有显著差异[41,42], 但由于血浆中含有的抗凝剂可能会对BAs的检测有干扰, 因此较常使用血清作为待测样本。

在使用UPLC-MS/MS技术对血浆/血清、尿液样本进行检测分析时, 前处理方式采用较多的是蛋白沉淀法[43-45]。一般步骤是在血清/血浆或尿液中加入3、4倍样品体积的含/不含有内标化合物的沉淀试剂, 涡旋加

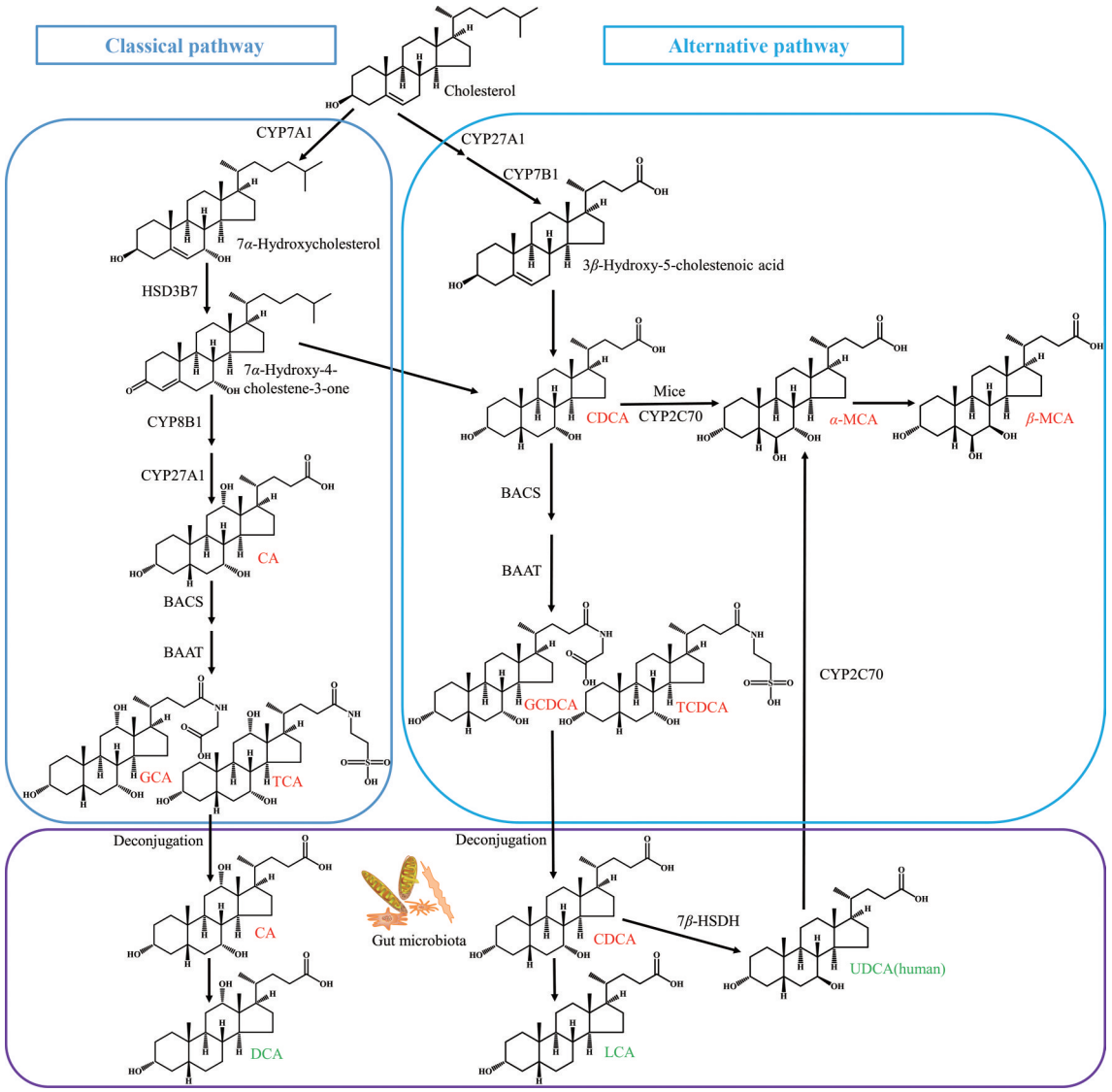


Figure 2 Biosynthesis and metabolism of bile acids. Red: Primary BAs; Green: Secondary BAs; BAAT: Amino acid *N*-acyltransferase; BACS: BA-coenzyme A synthase; CA: Cholic acid; CDCA: Chenodeoxycholic acid; CYP27A1: Sterol-27α-hydroxylase; CYP7A1: Cholesterol-7α-hydroxylase; CYP7B1: Oxysterol-7α-hydroxylase; CYP8B1: Sterol-12α-hydroxylase; CYP2C70: Sterol-6β-hydroxylase; DCA: Deoxycholic acid; GCA: Glycocholic acid; GCDCA: Glycochenodeoxycholic acid; HSD3B7: 3β-Hydroxy Δ5-C27 steroid dehydrogenase; LCA: Lithocholic acid; TCA: Taurocholic acid; TCDCA: Taurochenodeoxycholic acid; UDCA: Ursodeoxycholic acid; α-MCA: α-Muricholic acid; β-MCA: β-Muricholic acid; 7β-HSDH: 7β-Hydroxysteroid dehydrogenase

速蛋白沉淀后, 在4 ℃离心机中高速离心, 取出上层清液后, 采用N_2吹仪或真空浓缩仪干燥上清液, 最后用一定体积的特定试剂 (流动相) 复溶后上样分析。尿液中包含的基质成分种类较少, 目前已有报道直接以尿液或对尿液进行简单稀释后作为待测样本采用LC-MS技术获取尿液中代谢物的指纹图谱[46,47]。Zhu等[28]以人类尿液为待测样本, 采用酶水解法和含0.1%甲酸的乙腈溶液分别对50 μL尿液进行处理, 以测定尿液中结合型BAs和游离型BAs的含量。

2.1.2 胆汁 胆汁是一种深绿色到黄褐色的液体, 胆汁中BAs的含量高低与机体的生理状态有着密切的关联[1]。胆汁中主要以初级BAs为主, 包含的BAs种类较少但含量很高, 故在UPLC-MS/MS测定前需要进行一定比例的稀释[48], 一般先将胆汁样本用纯水进行稀释或直接以高倍量的有机试剂沉淀基质后再进行下一步操作。Mi等[49]将胆汁样品用纯水稀释后, 与内标液和缓冲液混合, 装入经甲醇活化后的C18固相萃取柱上, 依次用水和甲醇冲洗, 收集的甲醇洗脱液作为待测样本采用UPLC-MS/MS对猪胆汁中的19种BAs进行分析, 回收率为89.1%～100.2%。

2.1.3 肝脏 许多肝脏疾病如肝内胆汁淤积、非酒精性脂肪肝、慢性肝炎、肝癌等疾病的发生发展与BAs的

种类和水平有着较大的关联, 但由于肝脏样本的不易获得性, 故关于肝脏中BAs的分析数据多是在实验动物中获得。在实验中肝脏样本的前处理中较多采用的是液液萃取技术[50], Wang等[51]在肝脏的样本前处理中将一定量的肝脏中加入适量去离子水后进行匀浆, 在组织匀浆液中加入适量冰乙腈对目标化合物两次萃取后, 萃取液低温高速离心取上清真空干燥后, 用适当比例的流动相复溶后进行分析。

2.1.4 肠道内容物和粪便 肠道内容物及粪便中含有丰富的BAs, 且BAs的数目和含量可以反映宿主肠道的稳态情况, 故以肠道内容物和粪便为待测样本进行BAs测定的研究逐渐成为热点。与液体样本相比, 粪便及肠道内容物样本基质更为复杂, 一般步骤包括冻干、均质、萃取富集、浓缩等[52]。该类样本在以BAs为分析对象的前处理过程中采用较多的是单向萃取技术[53], 一般先将待测样本用有机溶剂进行稀释后, 在4 ℃离心机中高速离心后取上清弃去下层蛋白和其他沉淀物[54]。Yu等[55]采用双相萃取技术对粪便中代谢物轮廓进行了全面表征, 在样本前处理中对样本进行冻干除去液体后, 依次加入一定比例的甲基叔丁基醚、甲醇和水后超声提取20 min, 高速离心后加入一定量的超纯水进行诱导相分离, 对粪便中的极性与非极性成分进行表征。

2.2 新技术在前处理中的应用

在MS检测中, 基质效应主要通过影响电喷雾离子源对待测化合物的信号响应强度从而导致目标成分的检测不准确[56]。较多研究主要是通过对待测样本进行复杂的纯化步骤、空白基质配制标准曲线、减少进样体积、同位素标记内标法等措施来降低基质效应对待测化合物定性定量的影响[57]。因基质效应无法完全避免, 目前大多数研究者常采用内标降低基质效应对待测化合物的影响[37,58,59], 从而通过UPLC-MS/MS实现待测生物样品中化合物的准确定量。在样本的前处理中, 有效减少基质效应的同时还应注意待测化合物的富集, Lyu等[60]在尿液样本的前处理中采用了一种含有共价有机框架填料的固相萃取小柱, 通过该方法对样本中的BAs进行富集处理后, 测定的8种BAs的灵敏度相对于传统方法提高了9.37～54.30倍, 明显降低了基质效应的影响。Silveira等[61]建立了一种用于快速测定血清BAs的在线分子印迹固相萃取结合UPLC-MS/MS方法, 将合成的一种包裹牛血清白蛋白的分子印迹聚合物作为萃取相, 成功从人类血清中分离出9种BAs。

3 不同检测技术在BAs分析中的应用

在过去几十年中, 已报道了采用不同平台的多种方法对生物体内BAs化合物进行检测分析 (表1)。如简单且较为方便的技术酶循环法[62,63], 目前被广泛用于临床中血清总BAs含量的测定, 但该方法并不能显示出不同BAs的含量差异, 且样本中含有其他干扰物质时对BAs浓度的测定影响较大[64]; ELISA在实验及临床中使用也较为普遍, 作为一种特殊的试剂分析法, 通过抗原-抗体反应可特异性定量某类BAs, 但ELISA总耗时长、对操作人员熟悉程度要求高、试剂盒昂贵且一次能够定量的BAs的种类过少; NMR是有机物结构测定的一种强有力的手段, 作为一种无损检测技术在某种特定BAs的定性检测中扮演着重要的作用[65,66], 但由于其复杂的操作而受到一定的使用限制。临床试验中所采用的非色谱法对BAs进行定性及含量测定, 专属性及灵敏度虽比较高, 但只能实现待测样本中总BAs或个别BAs的含量测定, 无法实现同时对多种BAs的表征和含量测定。

色谱法作为一种分离和分析方法, 在复杂体系中化合物的分离定量中有着广泛的应用。色谱分离技术最早在1946年用于BAs化合物的分离, Silberman等[67]采用衍生化的方法将胆酸和去氧胆酸转变成有色酯类化合物后, 通过以$MgCO_3$为填料的色谱柱, 采用苯∶石

Table 1 Characteristics of different bile acids detection technologies[24]. ELISA: Enzyme-linked immunosorbent assay; GC-MS: Gas chromatography-mass spectrometry; LC-MS: Liquid chromatography-mass spectrometry; NMR: Nuclear magnetic resonance; SFC-MS: Supercritical fluid chromatography-mass spectrometry; TLC: Thin-layer chromatography

Method	Advantage	Disadvantage
Enzyme cycle method	Simple operation, low cost, routine clinical detection method of TBAs	Only applicable to C_{24}-steroids containing C_3-OH
ELISA	Easy operation, single instrumentation, short analysis time	Proneness of antibodies to cross reactions
NMR	Simple sample pretreatment, small sample size, non-destructive testing	Lower sensitivity than MS
TLC	Easy operation, low cost	More often used for qualitative analysis, results are easily influenced by environmental factors
GC-MS	Suitable for the analysis of volatile/semi-volatile compounds	Complex sample pretreatment
SFC-MS	Short analysis time, simple sample treatment	High cost
LC-MS	High stability, low detection limit, high sensitivity, high separation capacity, suitable for high throughput sample detection	High cost, complicated instrumental operation

油 = 1∶1为洗脱剂进行洗脱从而实现两种胆汁酸的分离。TLC[68,69]在BAs的定性分析中扮演着重要的角色, 因操作简便且成本较低在实验中使用较为频繁, 但TLC在检测过程中易受薄层板、展开剂及展开环境的湿度温度等因素的影响, 且BAs化合物同分异构体多理化性质相似, 容易出现多个BAs点重合在一起从而导致定性错误。GC多用于分离测定沸点低易挥发的化合物, 在BAs的检测中需进行衍生化改变化合物的理化性质后进行检测[70-72], 样品前处理操作繁琐费时, 在早期的研究中GC-MS的检测能力强于LC-MS, 故该技术在BAs的前期检测中是常用的检测技术[70,73], VandenHeuvel等[74]于1960年首次将GC-MS用于BAs的分离检测中, 共检测到4种甲基衍生化后的BAs。超临界流体色谱 (supercritical fluid chromatography, SFC) 是一种以超临界流体为流动相的色谱分离技术[75,76], 与GC和LC不同的是SFC可充分利用超临界流体扩散速度快且黏度小的优点实现化合物的快速分离, 在手性药物和中药成分的分离方面应用较多[77,78], Scalia等[79]采用甲醇改性后的二氧化碳为流动相, 实现了人十二指肠中共8种甘氨酸和牛磺酸结合型BAs的分离定量; Taguchi等[80]采用SFC-MS技术用于大鼠血浆中24种BAs的分离和定量, 与GC-MS相比, SFC-MS具有检测时间短、分离能力强和灵敏度高的优点。

HPLC作为目前广泛使用的BAs定性定量的技术之一[81], 对混合物具有强的分离能力且灵敏度、特异性较高, 但当样品中含有较低浓度的BAs或以游离型BAs为主对光谱吸收较差时, HPLC所配置的检测器类型便成了限制BAs检测的主要因素; MS具有特异性强、灵敏度高、样品用量少及分析速度快等多个优点, 被广泛用于各类化合物的鉴定, 但对生物样品及中药等复杂体系中化合物进行鉴定分析时, MS常与分离技术结合使用。在色谱-质谱联用技术中, 对BAs化合物的表征前期多采用GC-MS[73,82]、SFC-MS[80]等技术; 近年来, 采用UPLC-MS/MS对待测生物样本中BAs的全面表征受到越来越多学者的关注[29,83]。

4 UPLC-MS/MS技术在BAs化合物分析中的应用现状

近年来, 随着液相色谱与质谱技术的快速发展, 越来越多的学者将液相色谱与质谱联用技术用于BAs的鉴定与分析 (表2[28,29,51,84-107])。UPLC-MS/MS较其他联用技术相比, 分析时间较短且样品前处理过程简单快速。本部分将重点介绍近五年来基于UPLC-MS/MS技术的BAs分析方法研究进展。

自2004年沃特世公司推出了第一台UPLC仪器以来[108], UPLC-MS/MS已成为生物样品中BAs检测的主流方法。Lin等[29]在自建BAs数据库的基础上, 采用UPLC-MS/MS技术对生物样本进行检测, 对检测出的化合物通过保留时间、离子峰强度、一级质谱及二级质谱进行四维数据挖掘[109], 通过包含491种BAs的内部数据库获得了292种BAs的分子式; 此外, 还对含有羟基、邻羟基、羰基及结合型BAs的保留时间及裂解规律进行归纳总结, 为后续采用LC-MS对新型BAs的鉴定提供了依据; 最后将建立的方法用于胆汁淤积性大鼠尿液和粪便中BAs的检测, 发现胆汁淤积性大鼠粪便和尿液中磺酸化和葡糖醛酸化的BAs显著增加, 为胆汁淤积症的临床诊断提供了新思路。Hu等[104]基于UPLC-MS/MS技术建立了对大鼠血清中游离型和甘氨酸结合型BAs全面表征的平行衍生化分析策略, 使用两种结构类似物分别对大鼠血清中的BAs进行衍生化处理后进行检测分析, 该衍生化反应在室温下20 min内完成且产物得率 > 99%, 通过衍生化处理后的BAs检测灵敏度较未衍生化前提高了10～400倍, 最终在大鼠血清中共检测并鉴定出221种BAs, 并通过MRM数据采集模式对27种BAs进行准确定量, 该策略可使每个化合物都有相应的内标, 从而降低基质效应保证化合物定量的准确性, 在其他代谢物的定性定量中也常使用[110,111]。

在样品前处理与数据处理方面, Zhu等[105]将化学衍生化与质谱技术相结合后对生物样本中的BAs进行了深入挖掘, 将衍生化试剂与BAs侧链上的羧基反应后以提高其在MS中的电离效率, 并基于UPLC-Q-TOF技术建立了衍生化后BAs辅助交替双碰撞能量扫描质谱策略, 根据不同类型BAs衍生化后在高碰撞能和低碰撞能下产生的碎片离子特征, 对小鼠肝脏、肠道及肠道内容物进行BAs分析, 最终在小鼠肝脏、肠道、肠道内容物及粪便中鉴定出341个BAs, 其中, 首次在小鼠肠道、肠道内容物及粪便中检测并表征出丙氨酸和脯氨酸结合型BAs。Ma等[106]开发了用于对未知BAs进行鉴别BAsFinder软件 (https://BAsfinder.github.io/.), 基于对84种已商品化BAs标准品在MS/MS中的碎片离子片段对BAs碎裂规律进行推测, 经过峰提取、对齐的谱图在MS/MS库中搜索获得代表性的质谱图, 并通过产生的特征碎片离子和中性丢失基团最终实现对未知BAs的注释, 分别在人类血浆和尿液中注释了112和244种BAs。

5 总结与展望

综上所述, 多种技术被用于BAs的分析检测中。ELISA是临床实验中常用的检测特定BAs的方法之一, 但由于抗原抗体与不同结构的BAs之间存在交叉反应, 导致该方法在对单个BA进行定量时受到一定

Table 2 UPLC-MS/MS method for the detection of bile acids in biological samples. LLE: Liquid-liquid extraction; LOD: Limit of detection; LOQ: Limit of quantitation; PPT: Protein precipitation; QQQ: Triple quadrupole; Q-TOF: Quadrupole time of flight; SPE: Solid phase extraction; UPLC-MS/MS: Ultra-performance liquid chromatography-tandem mass spectrometry. *nmol·L^{-1}; #nmol·g^{-1}

Sample type	Sample pretreatment	BAs (number)	Mass analyzer	LOD, LOQ /ng·mL^{-1}	Ref.
Human serum, urine	Enzymatic hydrolysis, PPT	32	QQQ	LOQ 4.1*	[28]
Human serum, urine	Enzymatic hydrolysis, PPT	70	Q-TOF		[28]
Rat/human serum, urine; rat bile, feces, liver, spleen, kidney, small intestine, large intestine	PPT, LLE	292	Q-TOF QQQ		[29]
Mice serum, liver	PPT, LLE	39	Q-TOF	LOD 2−100 LOQ 3−500	[51]
Rat serum, urine, bile, liver	PPT	42	QQQ	LOQ 1.02−12.8#	[84,85]
Human serum, cerebrospinal fluid	PPT, online-SPE	17	QTrap	LOQ 0.2−500	[86]
Human plasma	PPT	26	Quadrupole-orbitrap	LOQ 1−1 000	[87]
Human plasma, serum	PPT	18	Qtrap		[88]
Human plasma	PPT	21	QQQ	LOQ 50−2 500*	[89]
Ratatouille bile	Ultrasonic extraction	30	Qtrap		[90]
Human urine	SPE	27	Q-TOF	LOQ 3.0−20 000*	[91]
Mice serum	PPT	21	Q-TOF	LOQ 5−10 000	[92]
Human serum, urine	SPE	49	QQQ	LOD 0.5−15* LOQ 2−50*	[93]
Mice serum, plasma, liver	PPT, solid-liquid extraction	36	QQQ	LOD 0.01−1.18 LOQ 0.02−3.58	[94]
Human serum	PPT	15	QQQ	LOQ 5−5 000	[95]
Rat serum	PPT	25	QTrap	LOD 0.01−0.1 LOQ 0.02−0.2	[96]
Human urine	Enzymatic hydrolysis	40	QQQ		[97]
Mice serum, feces	PPT, LLE	60	QQQ	LOD 0.5−13.0* LOQ 1.6−43.2*	[98]
Macaque plasma, bile, liver; cells, cell culture medium	PPT, SPE	19	QQQ	LOD 0.05−10 LOQ 0.1−10	[99]
Carp bile, plasma	PPT	30	QQQ	LOQ 1−50	[100]
Human follicular fluid	High-speed centrifugal	24	Q-TOF	LOD 0.01−0.43 LOQ 0.03−1.29	[101]
Human feces	LLE	12	QQQ	LOD 0.07−0.47# LOQ 0.23−1.56#	[102]
Bile and feces of mice	LLE	10	QQQ	LOD 0.5; LOQ 1.0	[103]
Rat serum	SPE	27	QQQ	LOD 0.004−0.4	[104]
Rat serum	SPE	221	QQQ		[104]
Mouse liver, small intestine, large intestine, large intestine contents, feces, serum	PPT, solid-liquid extraction, Derivatization	341	Q-TOF		[105]
Human plasma	PPT	112	Quadrupole-orbitrap		[106]
Human urine	SPE	244	Quadrupole-orbitrap		[106]
Human feces	LLE	21	Q-TOF	LOD 5−10 LOQ 15−30	[107]

的限制。在BAs的初期检测中, GC及GC-MS是检测BAs的主要手段。近年来, 随着LC-MS分离度与检测灵敏度的快速提升, 该技术逐渐被用在BAs的定性定量分析中, 可通过MRM数据采集策略, 对单个BA更加准确的定量。

然而, 在采用LC-MS对BAs进行分析时, 由于同分异构体的存在, 不同BAs的色谱保留行为及碎片离子高度相似。另外基质效应对BAs检测信号强度的响应有明显影响, 不同BAs在同一样本中的浓度相差多个数量级。因此, 为获得最理想的检测结果, 分析过程中流动相的考察与各项参数的优化极为重要; 在样本前处理过程中应尽可能对BAs进行富集及去除基质。此外, 可采用二维液相色谱、离子淌度质谱等对同分异构体实现分离后, 根据保留时间差异及碰撞横截面积的不同对化合物进行定性定量分析; 同分异构体在二级质谱中显示出相似的碎片离子, 但产生的不同碎片离子强度比具有一定区别, 在数据处理过程中应注意进行归纳总结, 以便于对未知BAs的结构进行推测。

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References

[1] Monte MJ, Marin JJG, Antelo A, et al. Bile acids: chemistry, physiology, and pathophysiology [J]. World J Gastroenterol, 2009, 15: 804-816.

[2] Hofmann AF, Hagey LR. Bile acids: chemistry, pathochemistry, biology, pathobiology, and therapeutics [J]. Cell Mol Life Sci, 2008, 65: 2461-2483.

[3] Schaap FG, Trauner M, Jansen PLM. Bile acid receptors as targets for drug development [J]. Nat Rev Gastroenterol Hepatol, 2014, 11: 55-67.

[4] Baes M, Gulick T, Chol HS, et al. A new orphan member of the nuclear hormone receptor superfamily that interacts with a subset of retinoic acid response elements [J]. Mol Cell Biol, 1994, 14: 1544-1552.

[5] Rajani C, Jia W. Bile acids and their effects on diabetes [J]. Front Med, 2018, 12: 608-623.

[6] Wei M, Shao Y, Liu QR, et al. Bile acid profiles within the enterohepatic circulation in a diabetic rat model after bariatric surgeries [J]. Am J Physiol Gastrointest Liver Physiol, 2018, 314: G537-G546.

[7] Ahmad TR, Haeusler RA. Bile acids in glucose metabolism and insulin signalling-mechanisms and research needs [J]. Nat Rev Endocrinol, 2019, 15: 701-712.

[8] Vallim TQA, Tarling EJ, Edwards PA. Pleiotropic roles of bile acids in metabolism [J]. Cell Metab, 2013, 17: 657-669.

[9] Yu HC, Hou SC, Cui B, et al. Research progress on the role of bile acids in regulating glycolipid metabolism [J]. Acta Pharm Sin (药学学报), 2020, 55: 1419-1430.

[10] Forman BM, Goode E, Chen J, et al. Identification of a nuclear receptor that is activated by farnesol metabolites [J]. Cell, 1995, 81: 687-693.

[11] Xu G, Pan LX, Erickson SK, et al. Removal of the bile acid pool upregulates cholesterol 7α-hydroxylase by deactivating FXR in rabbits [J]. J Lipid Res, 2002, 43: 45-50.

[12] Watanabe M, Houten SM, Wang L, et al. Bile acids lower triglyceride levels *via* a pathway involving FXR, SHP, and SREBP-1c [J]. J Clin Invest, 2004, 113: 1408-1418.

[13] Huang FJ, Zheng XJ, Ma XH, et al. Theabrownin from Pu-erh tea attenuates hypercholesterolemia *via* modulation of gut microbiota and bile acid metabolism [J]. Nat Commun, 2019, 10: 4971.

[14] Jiao TY, Ma YD, Guo XZ, et al. Bile acid and receptors: biology and drug discovery for nonalcoholic fatty liver disease [J]. Acta Pharmacol Sin, 2022, 43: 1103-1119.

[15] Li T, Chiang JYL. Bile acid signaling in metabolic disease and drug therapy [J]. Pharmacol Rev, 2014, 66: 948-983.

[16] Inagaki T, Choi M, Moschetta A, et al. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis [J]. Cell Metab, 2005, 2: 217-225.

[17] Laurin J, Lindor KD, Crippin JS, et al. Ursodeoxycholic acid or clofibrate in the treatment of non-alcohol-induced steatohepatitis: a pilot study [J]. Hepatology, 1996, 23: 1464-1467.

[18] Li X, Liao M, Pan Q, et al. Combination therapy of obeticholic acid and ursodeoxycholic acid in patients with primary biliary cholangitis who respond incompletely to ursodeoxycholic acid: a systematic review [J]. Eur J Gastroenterol Hepatol, 2020, 32: 1116-1122.

[19] Al-Alaiyan S, Elsaidawi W, Alanazi AM, et al. Ursodeoxycholic acid and SMOFlipid for treating parenteral nutrition associated cholestasis in infants [J]. Cureus, 2022, 14: e22060.

[20] Tang R, Wei Y, Li Y, et al. Gut microbial profile is altered in primary biliary cholangitis and partially restored after UDCA therapy [J]. Gut, 2018, 67: 534-541.

[21] Mueller M, Thorell A, Claudel T, et al. Ursodeoxycholic acid exerts farnesoid X receptor-antagonistic effects on bile acid and lipid metabolism in morbid obesity [J]. J Hepatol, 2015, 62: 1398-1404.

[22] Roda E, Bazzoli F, Labate AM, et al. Ursodeoxycholic acid *vs*. chenodeoxycholic acid as cholesterol gallstone-dissolving agents: a comparative randomized study [J]. Hepatology, 1982, 2: 804-810.

[23] Hirschfield GM, Mason A, Luketic V, et al. Efficacy of obeticholic acid in patients with primary biliary cirrhosis and inadequate response to ursodeoxycholic acid [J]. Gastroenterology, 2015, 148: 751-761.

[24] Younossi ZM, Ratziu V, Loomba R, et al. Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial [J]. Lancet, 2019, 394: 2184-2196.

[25] Wang W, Luo P, Miao XL, et al. Regulation of obeticholic acid on serum lipids and bile acids and gut microbiota of non-alcoholic steatohepatitis mice induced by methionine and choline deficiency diet [J]. Acta Pharm Sin (药学学报), 2022, 57: 3203-3213.

[26] Zheng X, Chen T, Zhao A, et al. Hyocholic acid species as novel biomarkers for metabolic disorders [J]. Nat Commun, 2021, 12: 1487.

[27] Alamoudi JA, Li W, Gautam N, et al. Bile acid indices as biomarkers for liver diseases I: diagnostic markers [J]. World J Hepatol, 2021, 13: 433-455.

[28] Zhu P, Zhang J, Chen Y, et al. Analysis of human C24 bile acids metabolome in serum and urine based on enzyme digestion of conjugated bile acids and LC-MS determination of unconjugated bile acids [J]. Anal Bioanal Chem, 2018, 410: 5287-5300.

[29] Lin M, Chen X, Wang Z, et al. Global profiling and identification of bile acids by multi-dimensional data mining to reveal a way of eliminating abnormal bile acids [J]. Anal Chim Acta, 2020, 1132: 74-82.

[30] Sarafian MH, Lewis MR, Pechlivanis A, et al. Bile acid profiling and quantification in biofluids using ultra-performance liquid

chromatography tandem mass spectrometry [J]. Anal Chem, 2015, 87: 9662-9670.

[31] Cheng K, Brunius C, Fristedt R, et al. An LC-QTOF MS based method for untargeted metabolomics of human fecal samples [J]. Metabolomics, 2020, 16: 46.

[32] Russell DW. The enzymes, regulation, and genetics of bile acid synthesis [J]. Annu Rev Biochem, 2003, 72: 137-174.

[33] Chiang JY. Regulation of bile acid synthesis: pathways, nuclear receptors, and mechanisms [J]. J Hepatol, 2004, 40: 539-551.

[34] Jia W, Wei M, Rajani C, et al. Targeting the alternative bile acid synthetic pathway for metabolic diseases [J]. Protein Cell, 2021, 12: 411-425.

[35] Zhao X, Liu Z, Sun F, et al. Bile acid detection techniques and bile acid-related diseases [J]. Front Physiol, 2022, 13: 826740.

[36] Dettmer K, Aronov PA, Hammock BD. Mass spectrometry-based metabolomics [J]. Mass Spectrom Rev, 2006, 26: 51-78.

[37] Krautbauer S, Buchler C, Liebisch G. Relevance in the use of appropriate internal standards for accurate quantification using LC-MS/MS: tauro-conjugated bile acids as an example [J]. Anal Chem, 2016, 88: 10957-10961.

[38] Griffiths WJ, Sjövall J. Bile acids: analysis in biological fluids and tissues [J]. J Lipid Res, 2010, 51: 23-41.

[39] Vignoli A, Tenori L, Morsiani C, et al. Serum or plasma (and which plasma), that is the question [J]. J Proteome Res, 2022, 21: 1061-1072.

[40] Kopp BT, Joseloff E, Goetz D, et al. Urinary metabolomics reveals unique metabolic signatures in infants with cystic fibrosis [J]. J Cyst Fibros, 2019, 18: 507-515.

[41] Peng Z, Zhang Q, Mao Z, et al. A rapid quantitative analysis of bile acids, lysophosphatidylcholines and polyunsaturated fatty acids in biofluids based on ultraperformance liquid chromatography coupled with triple quadrupole tandem massspectrometry [J]. J Chromatogr B Analyt Technol Biomed Life Sci, 2017, 1068-1069: 343-351.

[42] Jäntti SE, Kivilompolo M, Ohrnberg L, et al. Quantitative profiling of bile acids in blood, adipose tissue, intestine, and gall bladder samples using ultra high performance liquid chromatography-tandem mass spectrometry [J]. Anal Bioanal Chem, 2014, 406: 7799-7815.

[43] Shen Y, Liu K, Luo X, et al. A simple and reliable bile acid assay in human serum by LC-MS/MS [J]. J Clin Lab Anal, 2022, 36: e24279.

[44] Zhou K, Wang J, Xie G, et al. Distinct plasma bile acid profiles of biliary atresia and neonatal hepatitis syndrome [J]. J Proteome Res, 2015, 14: 4844-4850.

[45] Sangaraju D, Shi Y, Parys MV, et al. Robust and comprehensive targeted metabolomics method for quantification of 50 different primary, secondary, and sulfated bile acids in multiple biological species (human, monkey, rabbit, dog, and rat) and matrices (plasma and urine) using liquid chromatography high resolution mass spectrometry (LC-HRMS) analysis [J]. J Am Soc Mass Spectrom, 2021, 32: 2033-2049.

[46] Williams RE, Major HJ, Lock EA, et al. *D*-Serine-induced nephrotoxicity: a HPLC-TOF/MS-based metabonomics approach [J]. Toxicology, 2005, 207: 179-190.

[47] Lenz EM, Bright J, Knight R, et al. Cyclosporin A-induced changes in endogenous metabolites in rat urine: a metabonomic investigation using high field ^{1}H NMR spectroscopy, HPLC-TOF/MS and chemometrics [J]. J Pharm Biomed Anal, 2004, 35: 599-608.

[48] Humbert L, Maubert MA, Wolf C, et al. Bile acid profiling in human biological samples: comparison of extraction procedures and application to normal and cholestatic patients [J]. J Chromatogr B Analyt Technol Biomed Life Sci, 2012, 899: 135-145.

[49] Mi S, Lim DW, Turner JM, et al. Determination of bile acids in piglet bile by solid phase extraction and liquid chromatography-electrospray tandem mass spectrometry [J]. Lipids, 2016, 51: 359-372.

[50] Suzuki Y, Kaneko R, Nomura M, et al. Simple and rapid quantitation of 21 bile acids in rat serum and liver by UPLC-MS-MS: effect of high fat diet on glycine conjugates of rat bile acids [J]. Nagoya J Med Sci, 2013, 75: 57-71.

[51] Wang X, Wang F, Lu Z, et al. Semi-quantitative profiling of bile acids in serum and liver reveals the dosage-related effects of dexamethasone on bile acid metabolism in mice [J]. J Chromatogr B Analyt Technol Biomed Life Sci, 2018, 1095: 65-74.

[52] Kakiyama G, Muto A, Takei H, et al. A simple and accurate HPLC method for fecal bile acid profile in healthy and cirrhotic subjects: validation by GC-MS and LC-MS [J]. J Lipid Res, 2014, 55: 978-990.

[53] Franco P, Porru E, Fiori J, et al. Identification and quantification of oxo-bile acids in human faeces with liquid chromatography-mass spectrometry: a potent tool for human gut acidic sterolbiome studies [J]. J Chromatogr A, 2019, 1585: 70-81.

[54] Cao H, Huang H, Xu W, et al. Fecal metabolome profiling of liver cirrhosis and hepatocellular carcinoma patients by ultra performance liquid chromatography-mass spectrometry [J]. Anal Chim Acta, 2011, 691: 68-75.

[55] Yu S, Fan J, Zhang L, et al. Assessment of biphasic extraction methods of mouse fecal metabolites for liquid chromatography-mass spectrometry-based metabolomic studies [J]. J Proteome Res, 2021, 20: 4487-4494.

[56] Pascoe R, Foley JP, Gusev AI. Reduction in matrix-related signal suppression effects in electrospray ionization mass spectrometry using on-line two-dimensional liquid chromatography [J]. Anal Chem, 2001, 73: 6014-6023.

[57] Matuszewski BK, Constanzer ML, Chavez-Eng CM. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS [J]. Anal Chem, 2003, 75: 3019-3030.

[58] Vogeser M, Seger C. Pitfalls associated with the use of liquid chromatography-tandem mass spectrometry in the clinical laboratory [J]. Clin Chem, 2010, 56: 1234-1244.

[59] Berg T, Strand DH. ^{13}C labelled internal standards-a solution to minimize ion suppression effects in liquid chromatography-tandem mass spectrometry analyses of drugs in biological samples? [J]. J Chromatogr A, 2011, 1218: 9366-9374.

[60] Lyu J, Li H, Yin D, et al. Analysis of eight bile acids in urine of gastric cancer patients based on covalent organic framework enrichment coupled with liquid chromatography-tandem mass spectrometry [J]. J Chromatogr A, 2021, 1653: 462422.

[61] Silveira AT, Barbosa AMC, Faria HD, et al. Online restricted access molecularly imprinted solid-phase extraction coupled with liquid chromatography-mass spectrometry for the selective determination of serum bile acids [J]. Analyst, 2022, 147: 2779-2792.

[62] Tan RF, Wo YB, Li FP. Method comparison of 2 biochemical analyzers for total bile acids [J]. Int J Lab Med (国际检验医学杂志), 2017, 38: 254-256.

[63] Murphy GM. Billing BH, Baron DN. A fluorimetric and enzymatic method for the estimation of serum total bile acids [J]. J Clin Pathol, 1970, 23: 594-598.

[64] Wang L. Study of the effect of cross-contamination between reagents of biochemical analysers on the determination of total bile acids [J]. Int J Lab Med (国际检验医学杂志), 2014, 35: 1069-1070.

[65] Ijare OB, Bezabeh T, Albiin N, et al. Simultaneous quantification of glycine- and taurine-conjugated bile acids, total bile acids, and choline-containing phospholipids in human bile using ^{1}H NMR spectroscopy [J]. J Pharm Biomed Anal, 2010, 53: 667-673.

[66] Gowda GAN. NMR spectroscopy for discovery and quantitation of biomarkers of disease in human bile [J]. Bioanalysis, 2011, 3: 1877-1890.

[67] Silberman H, Silberman-Martyncewa S. The chromatographic separation of bile acids the separation of cholic and desoxycholic acid [J]. J Biol Chem, 1946, 165: 359-363.

[68] Fujihira E, Takahashi N, Minato A, et al. A new solvent system for thin-layer chromatographical separation of taurine conjugated trihydroxy bile acids in rat bile [J]. Chem Pharm Bull, 1972, 20: 2719-2721.

[69] Touchstone JC, Levitt RE, Levin SS, et al. Separation of conjugated bile acids by reverse phase thin layer chromatography [J]. Lipids, 1980, 5: 386-387.

[70] Eguchi T, Miyazaki H, Nakayama F. Simultaneous determination of keto and non-keto bile acids in human serum by gas chromatography with selected ion monitoring [J]. J Chromatogr, 1990, 525: 25-42.

[71] Ma XK, Ying J, Wang GG. Determination of goose deoxycholic acid by capillary gas chromatography with pre-column derivatization [J]. Chin J Biochem Pharm (中国生化药物杂志), 1997, 18: 194-196.

[72] Zhang C, Chen AK, Peng HM. et al. Determination of ursodeoxycholic acid by capillary gas chromatography [J]. Chin J Pharm (中国医药工业杂志), 2000, 31: 504-506.

[73] Elliott WH. Identification of sterols and bile acids by computerized gas chromatography-mass spectrometry [J]. Lipids, 1980, 15: 764-769.

[74] VandenHeuvel WJA, Sweeley CC, Horning EC. Microanalytical separations by gas chromatography in the sex hormone and bile acid series [J]. Biochem Biophys Res Commun, 1960, 3: 33-36.

[75] Gere D. Supercritical fluid chromatography [J]. Science, 1983, 222: 253-259.

[76] Liu N. Advances in the application of supercritical fluid chromatography [J]. Mil Med Sci (军事医学), 2021, 45: 558-561.

[77] Kozlov O, Kalíková K, Gondová T, et al. Fast enantioseparation of indole phytoalexins in additive free supercritical fluid chromatography [J]. J Chromatogr A, 2019, 1596: 209-216.

[78] Huang Y, Zhang T, Zhao Y, et al. Simultaneous analysis of nucleobases, nucleosides and ginsenosides in ginseng extracts using supercritical fluid chromatography coupled with single quadrupole mass spectrometry [J]. J Pharm Biomed Anal, 2017, 144: 213-219.

[79] Scalia S, Games DE. Analysis of conjugated bile acids by packed-column supercritical fluid chromatography [J]. J Chromatogr, 1992, 574: 197-203.

[80] Taguchi K, Fukusaki E, Bamba T. Simultaneous and rapid analysis of bile acids including conjugates by supercritical fluid chromatography coupled to tandem mass spectrometry [J]. J Chromatogr A, 2013, 1299: 103-109.

[81] Sardella R, Gioiello A, Ianni F, et al. HPLC/ELSD analysis of amidated bile acids: an effective and rapid way to assist continuous flow chemistry processes [J]. Talanta, 2012, 100: 364-371.

[82] Nittono H, Takei H, Unno A, et al. Diagnostic determination system for high-risk screening for inborn errors of bile acid metabolism based on an analysis of urinary bile acids using gas chromatography-mass spectrometry: results for 10 years in Japan [J]. Pediatr Int, 2009, 51: 535-543.

[83] Prinville V, Ohlund L, Sleno L. Targeted analysis of 46 bile acids to study the effect of acetaminophen in rat by LC-MS/MS [J]. Metabolites, 2020, 10: 26.

[84] Brock WJ, Beaudoin JJ, Slizgi JR, et al. Bile acids as potential biomarkers to assess liver impairment in polycystic kidney disease [J]. Int J Toxicol, 2018, 37: 144-154.

[85] Xie G, Wang Y, Wang X, et al. Profiling of serum bile acids in a healthy Chinese population using UPLC-MS/MS [J]. J Proteome Res, 2015, 14: 850-859.

[86] Reinicke M, Schröter J, Müller-Klieser D, et al. Free oxysterols and bile acids including conjugates-simultaneous quantification in human plasma and cerebrospinal fluid by liquid chromatogra-

phy-tandem mass spectrometry [J]. Anal Chim Acta, 2018, 1037: 245-255.

[87] Shi Y, Gao Y, Parys MV, et al. Definitive profiling of plasma bile acids as potential biomarkers for human liver diseases using UPLC-HRMS [J]. Bioanalysis, 2018, 10: 917-932.

[88] Krautbauer S, Liebisch G. LC-MS/MS analysis of bile acids [J]. Methods Mol Biol, 2018, 1730: 103-110.

[89] Fu X, Xiao Y, Golden J, et al. Serum bile acids profiling by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and its application on pediatric liver and intestinal diseases [J]. Clin Chem Lab Med, 2019, 58: 787-797.

[90] Cao Y, Song QQ, Li J, et al. Chemical profiling for bile acid derivatives in yak bile [J]. China J Chin Mater Med (中国中药杂志), 2019, 44: 2538-2543.

[91] Chen X, Zhang X, Xu B, et al. The urinary bile acid profiling analysis of asymptomatic hypercholanemia of pregnancy: a pseudo-targeted metabolomics study [J]. Clin Chim Acta, 2019, 497: 67-75.

[92] Ghaffarzadegan T, Essen S, Verbrugghe P, et al. Determination of free and conjugated bile acids in serum of Apoe (−/−) mice fed different lingonberry fractions by UHPLC-MS [J]. Sci Rep, 2019, 9: 3800.

[93] Ulaszewska MM, Mancini A, Garcia-Aloy M, et al. Isotopic dilution method for bile acid profiling reveals new sulfate glycine-conjugated dihydroxy bile acids and glucuronide bile acids in serum [J]. J Pharm Biomed Anal, 2019, 173: 1-17.

[94] Gómez C, Stücheli S, Kratschmar DV, et al. Development and validation of a highly sensitive LC-MS/MS method for the analysis of bile acids in serum, plasma, and liver tissue samples [J]. Metabolites, 2020, 10: 282.

[95] Danese E, Negrini D, Pucci M, et al. Bile acids quantification by liquid chromatography-tandem mass spectrometry: method validation, reference range, and interference study [J]. Diagnostics (Basel), 2020, 10: 462.

[96] Hu T, An Z, Shi C, et al. A sensitive and efficient method for simultaneous profiling of bile acids and fatty acids by UPLC-MS/MS [J]. J Pharm Biomed Anal, 2020, 178: 112815.

[97] Wang WX, Chen L, Wang GY, et al. Urinary bile acid profile of newborns born by cesarean section is characterized by oxidative metabolism of primary bile acids: limited roles of fetal-specific CYP3A7 in cholate oxidations [J]. Drug Metab Dispos, 2020, 48: 662-672.

[98] Choucair I, Nemet I, Li L, et al. Quantification of bile acids: a mass spectrometry platform for studying gut microbe connection to metabolic diseases [J]. J Lipid Res, 2020, 61: 159-177.

[99] Shiffka SJ, Jones JW, Li L, et al. Quantification of common and planar bile acids in tissues and cultured cells [J]. J Lipid Res, 2020, 61: 1524-1535.

[100] Wei X, Yao T, Fall FN, et al. An integrated bile acids profile determination by UHPLC-MS/MS to identify the effect of bile acids supplement in high plant protein diet on common carp (*Cyprinus carpio*) [J]. Foods, 2021, 10: 2465.

[101] Yang X, Wu R, Qi D, et al. Profile of bile acid metabolomics in the follicular fluid of PCOS patients [J]. Metabolites, 2021, 11: 845.

[102] Shafaei A, Rees J, Christophersen CT, et al. Extraction and quantitative determination of bile acids in feces [J]. Anal Chim Acta, 2021, 1150: 338224.

[103] Zheng J, Ye C, Hu B, et al. Bile acid profiles in bile and feces of obese mice by a high-performance liquid chromatography-tandem mass spectrometry [J]. Biotechnol Appl Biochem, 2021, 68: 1332-1341.

[104] Hu T, Li H, Xu B, et al. Parallel derivatization strategy for comprehensive profiling of unconjugated and glycine-conjugated bile acids using ultra-high performance liquid chromatography-tandem mass spectrometry [J]. J Steroid Biochem Mol Biol, 2021, 214: 105986.

[105] Zhu QF, Wang YZ, An N, et al. Alternating dual-collision energy scanning mass spectrometry approach: discovery of novel microbial bile-acid conjugates [J]. Anal Chem, 2022, 94: 2655-2664.

[106] Ma Y, Cao Y, Song X, et al. BAFinder: a software for unknown bile acid identification using accurate mass LC-MS/MS in positive and negative modes [J]. Anal Chem, 2022, 94: 6242-6250.

[107] Porru E, Scicchitano D, Interino N, et al. Analysis of fecal bile acids and metabolites by high resolution mass spectrometry in farm animals and correlation with microbiota [J]. Sci Rep, 2022, 12: 2866.

[108] Plumb R, Castro-Perez J, Granger J, et al. Ultra-performance liquid chromatography coupled to quadrupole-orthogonal time-of-flight mass spectrometry [J]. Rapid Commun Mass Spectrom, 2004, 18: 2331-2337.

[109] Sumner LW, Amberg A, Barrett D, et al. Proposed minimum reporting standards for chemical analysis Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI) [J]. Metabolomics, 2007, 3: 211-221.

[110] Girard MFC, Ruskic D, Böhm G, et al. Automated parallel derivatization of metabolites with SWATH-MS data acquisition for qualitative and quantitative analysis [J]. Anal Chim Acta, 2020, 1127: 198-206.

[111] Hu T, Sun Y, Li H, et al. Dual derivatization strategy for the comprehensive quantification and double bond location characterization of fatty acids by ultra-high performance liquid chromatography-tandem mass spectrometry [J]. J Chromatogr A, 2021, 1639: 461939.