液相色谱-串联质谱技术在临床检验中的应用研究进展

易可可[1,2],谢 洁[2],江 游[2],黄泽建[2],龚晓云[2],翟 睿[2],乔晓婷[2],刘梅英[2],戴新华[2],方 向[2],时国庆[1]

(1.北京科技大学化学与生物工程学院,北京 100083;
2.中国计量科学研究院前沿计量科学中心,质谱仪器工程技术研究中心,北京 100029)

摘要:液相色谱-串联质谱(LC-MS/MS)技术具有高灵敏度、高特异性、高分辨率和高效率的优点。近年来随着仪器灵敏度的提高,LC-MS/MS在常规临床检验中显示出极大的潜力,并在疾病早期预防和诊断中发挥着不可替代的作用。本文对LC-MS/MS在新生儿疾病筛查、维生素D检测、内分泌激素检测、肽类和蛋白质定量分析等临床检验方面的研究进展进行综述,并讨论了未来面临的挑战。

关键词:液相色谱-串联质谱(LC-MS/MS);临床检验;应用研究
中图分类号:O657.63 **文献标志码**:A **文章编号**:1004-2997(2022)06-0804-13
doi:10.7538/zpxb.2021.0146

Progress in the Application of Liquid Chromatography-Tandem Mass Spectrometry in Clinical Diagnosis

YI Ke-ke[1,2], XIE Jie[2], JIANG You[2], HUANG Ze-jian[2], GONG Xiao-yun[2], ZHAI Rui[2], QIAO Xiao-ting[2], LIU Mei-ying[2], DAI Xin-hua[2], FANG Xiang[2], SHI Guo-qing[1]

(1. *School of Chemical and Biological Engineering, University of Science and Technology, Beijing* 100083, *China*; 2. *Mass Spectrometry Engineering Technology Research Center, Center for Advanced Measurement Science, National Institute of Metrology, Beijing* 100029, *China*)

Abstract: The blood, body fluid and other samples of patients collected by clinical laboratory can be detected to find disease markers through different methods, so as to provide clinicians with an important basis for disease prevention, diagnosis, curative effect and prognosis. LC-MS/MS is an analytical instrument with high sensitivity, high specificity, high resolution and high efficiency. In recent years, with the improvement of instrument sensitivity, LC-MS/MS showed great superiority, practicability and development potential in analytical and clinical practices, thus many diseases can be diagnosed accurately, quickly and sensitively in the early stage. For example, LC-MS/MS was used for neonatal screening, and it could screen more than 30 disease markers including various amino acids and carnitine simultaneously. Comparing with immunoassay, LC-MS/MS applied to endocrine hormones can simultaneously determine a variety of

国家重点研发计划项目(2021YFC2401100);中国计量科学研究院基本业务费项目(AKY1934)
本文共同第一作者易可可,谢洁;通信作者谢洁,时国庆

analytes and provide structural information of analytes. It has the advantages of high throughput and high specificity. Due to the expensive instruments and the cost of training, only a few clinical laboratories have the ability to develop and verify LC-MS/MS method. Based on the inherent limitations of the instrument, such as matrix effect and ion suppression effect, the efficiency of the method was decreased, resulting in challenges in the process of method development. In addition, the technical levels of different clinical laboratories are uneven, so it is necessary to establish corresponding reference measurement methods and reference ranges for clinical analysis. The standardization of quantitative results is significant for laboratory to build the traceability system. The research progress of the application of LC-MS/MS in clinical practices was introduced, and the advantages and disadvantages of the application of LC-MS/MS in neonatal screening, vitamin D detection, endocrine hormone detection, therapeutic drug concentration monitoring and protein quantitative analysis were discussed in the review. Finally the possible challenges and the recent advancement that clinical laboratories may confront in the application of LC-MS/MS were discussed.

Key words: liquid chromatography-tandem mass spectrometry (LC-MS/MS); clinical diagnosis; applied research

对临床实验室收集的患者血液、体液等样品进行各项指标检测,可以提供对疾病预防、诊断、治疗方案和预后判断的重要依据。传统的检测方法(如基于抗原抗体结合的免疫测定(IA))是常规临床检测方法之一[1],但临床实践中发现,IA 法会出现选择性差或假阳性结果[2]。液相色谱-串联质谱(LC-MS/MS)技术凭借高灵敏度和高选择性的优点,可提供更准确的分析结果,在临床检验中发挥着重要作用。

质谱的电离模式通常可分为硬电离[3]和软电离[4-5],其中硬电离模式目前仅限于分析具有挥发性和热稳定的化合物[6],不适用于分析临床样本;软电离允许样品分子在电离过程中保持完整形态[7],有利于检测临床小分子分析物,电喷雾电离是临床质谱最常见的电离模式。完整的样品分子进入串联质谱后,在一级质谱中选择特定化合物的母离子,在碰撞池中将母离子碰撞裂解,随后采用二级质谱的 MRM 或 SRM 模式获得对应的离子对信息[8-9]。相比于其他方法如紫外分光光度法、荧光光谱法和免疫测定法等,串联质谱具有更高的选择性、准确性、分辨率、灵敏度等优势[10-11],可通过 MRM/SRM 模式实现对多种分析物的同时准确定量[12]。

基于液相色谱分离的 LC-MS/MS 是目前临床实验室中最常用的检测方法[13-15]。由于临床检验的样本通常是基质复杂的生物液体或固体,不适于直接通过质谱分析,须对样品进行预处理和色谱分离。其中,预处理过程中样品制备不仅会影响色谱的分离效果,还会影响分析物信号强度。有时需要根据所采用 LC-MS/MS系统的检出限浓缩或稀释样品,并通过优化液相色谱条件将目标物与干扰成分分离[11, 16-17]。

本文基于 LC-MS/MS 在临床检验中的应用研究进展进行综述,拟讨论 LC-MS/MS 在新生儿筛查[18]、维生素 D 检测[19]、内分泌激素检测[20]、血药浓度监测[21]、蛋白定量分析[22]等应用中存在的独特优势和局限性,并讨论目前临床实验室在 LC-MS/MS 应用中可能面临的挑战,并对其发展趋势和应用前景进行展望。

1　LC-MS/MS 在临床检验的应用研究进展

目前,LC-MS/MS 在临床中可检测项目多达 500 余项,主要的检测项目列于表 1。该技术使多种疾病在早期得以被准确、快速、高灵敏地诊断。

表 1 临床质谱检测项目

Table 1 Detectable items of MS in clinical application

序号 No.	检测项目 Test item	项目数量 Item quantity	疾病标志物 Disease marker
1	新生儿遗传代谢病筛查[23-29]	200 余项	新生儿筛查,可一次性筛查 30～40 种遗传代谢病,包括氨基酸、肉碱、有机酸、胆汁酸、脂肪酸及各种代谢物等的检测
2	维生素[30-35]	10 余项	25-羟基维生素 D_2/D_3、1,25-双羟基维生素 D_2/D_3、维生素 A/C/E/K、维生素 B 族等
3	治疗药物实时监测[36-39]	100 余项	抗免疫排斥类药物、抗抑郁和抗精神病类药物、抗 HIV 药物、抗肿瘤药物等、治疗浓度小需要需要精确定量的药物
4	疼痛药物管理[36]	120 余项	镇静剂类、兴奋剂类、致幻剂类等
5	药物毒物滥用[40-42]	10 余项	吗啡、芬太尼、可待因、氧可酮、生物碱等
6	内分泌/类固醇激素类[43-48]	70 余项	睾酮、孕酮、雌酮、雌二醇、17-羟孕酮、皮质醇等超过 15 种类固醇,激素类等免疫方法测量结果不准确的激素
7	神经递质类化合物[49]	10 余项	儿茶酚胺:多巴胺、去甲肾上腺素、肾上腺素;儿茶酚胺代谢产物:甲氧基肾上腺素、甲氧基去甲肾上腺素
8	高血压/糖尿病[50]	50 余项	同型半胱氨酸、1,5-脱水葡萄糖醇、肾素活性-血管紧张素、醛固酮、皮质醇、氨基酸、有机酸等
9	蛋白/多肽类[51-56]	10 余项	胰岛素、IGF-1、甲状腺球蛋白、血红蛋白等

1.1 新生儿遗传代谢疾病筛查

先天性代谢缺陷(IEM)是一类罕见的遗传疾病,其成因复杂,且多属于进行性疾病,病情恶化后可导致新生儿严重的损伤甚至残疾,因此早期筛查、诊断和治疗具有重要意义。LC-MS/MS 技术能够快速、准确地对新生儿筛查中的多种疾病标志物进行定性、定量分析[57-58]。该技术可通过收集干血斑(DBS)制备样本,对婴儿伤害小且简单[59]。通常直接用含有一定量同位素内标的提取液从干血斑中提取目标物,然后直接进行 LC-MS/MS 分析,预处理简便、省时,2 min 内即可完成 1 个样本的检测,具备极高的检测效率和通量。随着方法的不断开发,单个样本中可同时检测的分析物种类不断增加。目前,市售的试剂盒可以同时分析单个样品中的 30 多种标志物,如山东英盛的新生儿遗传代谢病筛查试剂盒,可检测 13 种氨基酸、31 种肉碱和琥珀酰丙酮,列于表 2。此外,将高效液相色谱替换为超高效液相色谱,与串联质谱联用,不仅扩大了疾病诊断的范围,还能进一步提高仪器的灵敏度,一些标志物的检出限可达1 ng/mL[25,29],为临床实验室提供了更强大的分离各种疾病标志物的能力[60]。

LC-MS/MS 技术自 20 世纪被用于新生儿筛查,至今已经发展成熟,其缺点在于设备昂贵、对实验室条件和技术人员的要求较高,且需要实验室具有一定水平的质量控制能力。此外,由于新生儿筛查临床样本中各分析物的浓度受疾病和新生儿母亲营养水平影响较大,因此各分析物具有较宽的参考范围。IEM 患儿出生后,短时间内一些代谢物可能保持在正常水平,但随年龄增长后出现变化,有时仅依靠 LC-MS/MS 的定量结果不足以构成诊断的充分条件。目前,第二代测序技术作为 LC-MS/MS的二级诊断方式或二者组合的形式被广泛使用,这种组合策略可降低 LC-MS/MS 的误诊风险[61]。

1.2 维生素 D

维生素 D(VD)的缺乏会导致少儿佝偻病和成年人的软骨病,是一个世界性的健康问题,对 VD 的浓度水平检测具有诊断意义[62]。VD 在肝脏中转化为 25-羟基维生素D(25(OH)D),

表 2　氨基酸、肉碱和琥珀酰丙酮的 LC-MS/MS 测定试剂盒

Table 2　LC-MS/MS kit for determination of amino acids, carnitine and succinyl acetone

分析物 Analyte	缩写 Abbreviation	灵敏度 Sensitivity/(μmol/L)	线性范围 Linear range/(μmol/L)	参考范围 Reference range/(μmol/L)
甘氨酸	Gly	50	400～4556	98～1200
缬氨酸	Val	0.8	35～2240	63～330
亮氨酸/异亮氨酸/羟基脯氨酸	Leu/Ile/Pro-OH	1.0	35～2240	70～330
苯丙氨酸	Phe	0.5	20～1280	21～120
酪氨酸	Tyr	1.0	20～1280	25.8～320
丙氨酸	Ala	2.0	175～2000	62.9～650
蛋氨酸	Met	0.8	10～640	8～55
瓜氨酸	Cit	1.0	15～960	4～45
鸟氨酸	Orn	0.6	23～1900	42～400
精氨酸	Arg	0.5	10～640	1.64～66.5
脯氨酸	Pro	0.8	65～740	61～500
琥珀酰丙酮	SA	0.8	1.2～100	0～3.0
游离肉碱	C0	0.2	8.5～544	8～60
乙酰肉碱	C2	0.1	5～320	3.4～57
丙酰肉碱	C3	0.04	1～64	0.2～5
丙二酰肉碱/3-羟基-丁酰肉碱	C3DC/C4OH	0.04	1～64	0.02～0.3
丁酰肉碱	C4	0.04	0.2～51.2	0.05～0.53
甲基丙二酰肉碱/3-羟基-异戊酰肉碱	C4DC/C5OH	0.04	0.2～51.2	0.07～0.61
异戊酰肉碱	C5	0.03	0.2～51.2	0.04～0.45
异戊烯酰肉碱	C5：1	0.03	0.2～51.2	0～0.12
戊二酰肉碱/3-羟基-己酰肉碱	C5DC/C6OH	0.03	0.2～51.2	0.01～0.3
己酰肉碱	C6	0.02	0.05～12.8	0.01～0.15
己二酰肉碱	C6DC	0.02	0.05～12.8	0.01～0.25
辛酰肉碱	C8	0.02	0.05～12.8	0.02～0.2
辛烯酰肉碱	C8：1	0.02	0.05～12.8	0.02～0.38
癸酰肉碱	C10	0.01	0.05～12.8	0.01～0.3
癸烯酰肉碱	C10：1	0.01	0.05～12.8	0.01～0.27
癸二烯酰肉碱	C10：2	0.01	0.05～12.8	0～0.12
十二碳酰肉碱	C12	0.02	0.1～25.6	0.02～0.34
十二碳烯酰肉碱	C12：1	0.02	0.1～25.6	0.01～0.3
十四碳酰肉碱	C14	0.02	0.2～51.2	0.02～0.5
十四碳烯酰肉碱	C14：1	0.02	0.2～51.2	0.01～0.3
十四碳二烯酰肉碱	C14：2	0.02	0.2～51.2	0～0.15
3-羟基-十四碳酰肉碱	C14OH	0.02	0.2～51.2	0～0.04
十六碳酰肉碱	C16	0.08	2.5～160	0.15～6.31
十六碳烯酰肉碱	C16：1	0.08	2.5～160	0.01～0.46
3-羟基-十六碳酰肉碱	C16OH	0.08	2.5～160	0～0.06
3-羟基-十六碳烯酰肉碱	C16：1OH	0.08	2.5～160	0～0.1
十八碳酰肉碱	C18	0.04	0.65～41.6	0.1～1.85
十八碳烯酰肉碱	C18：1	0.04	0.65～41.6	0.17～3
十八碳二烯酰肉碱	C18：2	0.04	0.65～41.6	0.06～0.87
3-羟基-十八碳酰肉碱	C18OH	0.04	0.65～41.6	0～0.05
3-羟基-十八碳烯酰肉碱	C18：1OH	0.04	0.65～41.6	0～0.06

其中 25-羟基维生素 D_3(25(OH)D_3)是主要的存在形式,同时也存在少量 25-羟基维生素 D_2(25(OH)D_2)。由于 25(OH)D 总含量高于其他任何 VD 代谢物且相对稳定,临床通过检测 25(OH)D 作为评价人体 VD 营养水平的可靠指标,将 25(OH)D 低于 20 ng/mL (50 nmol/L)定义为 VD 缺乏[69]。目前,血清样品中25(OH)D的检测主要采用基于抗原与抗体特异性结合的 IA 法或 LC-MS/MS 法,IA 法成本低廉,预处理过程易于自动化和高通量检测,但其主要缺点是抗体与25(OH)D代谢物之间存在交叉反应,并且检测容易受到温度、基质、抗体状态等条件影响[70]。此外,该方法仅能得到 25(OH)D 总含量,无法分别定量 25(OH)D_2 和 25(OH)D_3。LC-MS/MS 技术不仅能区分微量复杂样本中 25(OH)D_2 和 25(OH)D_3,同时具有很高的灵敏度,这是由于通过色谱分离或同位素标记法来筛选候选的生物标记物,解决了同分异构体干扰的问题[63],因此,LC-MS/MS 被国际公认为测定 25(OH)D 的"金标准"[64]。

然而,在临床实践中发现,VD 存在少量差向异构体的类似物,差向异构体在一些人群如婴儿体内的含量足以影响诊断结果,需要进行长时间的色谱分离才能得到满意的分离效果。此外,由于 25(OH)D 为甾体类化合物,除 C、H、O 外只含有较少的其他原子,因此电离效率较低,且电离后表现出较差的、非特征性的碎裂特性[65],限制了 VD 临床检测的灵敏度。一些研究[66-67]使用样品衍生化或在流动相中添加盐来提高灵敏度,但衍生化步骤繁琐、耗时且不便于大规模分析应用。Tai 等[68]开发了一种基于 LC-MS/MS 的测定人血清中 25(OH)D 的参考测量测序程序,采用 LLE 预处理样品,不经过衍生化,分析时长为 45 min,25(OH)D 的检出限为 0.375 nmol/L。Liebisch 等[69]改进了该方法后,使分析时间缩短至 4 min。Kassim 等[70]开发了一种基于衍生化的检测血清中 12 种 VD 代谢物的 LC-MS/MS 方法,分析时间长达 34 min,但检出限可达 fmol 水平。Wan 等[71]基于衍生化开发了检测 VD 代谢物的新方法,同时采用液液萃取-固相萃取(LLE-SPE)联用的预处理方法,与单独的 SPE 相比,降低了 2～4 倍的离子抑制,分析时间为13 min,定量限为 0.005～0.02 nmol/L。Mena-Bravo 等[72]基于二维液相色谱(2DLC)提出了 SPE-2DLC-MS/MS 方法,用于人血清中 VD 代谢物的绝对定量分析,2DLC 可以分离 25(OH)D_3 和差向异构体,检出限范围在 0.023～0.225 nmol/L,RSD 小于 11.6%。最近,Kassim 等[34]报道了在不使用衍生化的情况下,25(OH)D_2 和25(OH)D_3的检出限分别达到 0.012 和 0.037 nmol/L,由于采用了在线 SPE 方法,进一步纯化提取物需要较长时间,总分析时长为 35 min。因此,开发更短时间和少量样本即可同时分析 VD 及其代谢物的新方法,将有助于 VD 缺乏相关疾病的临床诊断。通过 LC-MS/MS 同时检测维生素 D 和其代谢物的相关信息列于表 3。

1.3 内分泌激素

人体内某种激素浓度发生变化可能会对其他激素产生影响,因此,同时测量多种内源性激素在临床和生理学研究中具有重要意义[73]。但许多激素具有相似的分子结构,且激素含量较低,样品受性别、年龄等因素影响较大[74-76],准确定量分析内分泌激素是目前临床最具挑战性的问题之一。以临床中常见的肾上腺皮质癌为例,其病理特征是在男性和绝经后女性体内过量分泌雌激素,而且患者的 17-羟基孕酮、雄烯二酮和硫酸脱氢表雄酮等多种激素也在生理周期内异常上升[77],须在 1 次检测中同时筛选多种激素才能分析诊断,常规检测涉及激素种类有限,难以满足要求。采用 LC-MS/MS 法能同时检测这些激素含量,通过色谱分离,使具有相似化学结构的类固醇得到优异的纯化效果,同时缩短了分析时间,能快速得到高重现性的结果,这对揭示内分泌失调等疾病的发病机理,疾病诊断或预防的标志物以及相关治疗方案具有巨大潜力[78]。

目前,LC-MS/MS 已用于测量几乎所有类别的内分泌激素[79],Enver 等[80]采用 LC-MS/MS 建立了婴儿肾上腺激素含量的参考范围,可检测 14 种内分泌激素。Handelsman 等[81]通过 LC-MS/MS 测量10 904份血清样本,认为男性雄激素水平受到年龄、身高和体重等因素的影响,男性的睾酮及其 2 种生物活性代谢物双氢睾酮和雌二醇等内分泌激素水平呈现从 35 岁逐渐下降,80岁后下降更显著的年龄特征。然

表 3　维生素 D 的 LC-MS/MS 分析

Table 3　LC-MS/MS analysis of vitamin D

分析物 Analyte	预处理方法 Pretreatment method	检测技术 Analytical method	分析时间 Analysis duration/min	检出限 LOD/(nmol/L)	线性范围 Reference range/(nmol/L)	参考文献 Reference
VD_2,VD_3,25(OH)D_2,25(OH)D_3	PP、衍生化	LC-MS/MS	34	0.20～0.86*	50～1250	[70]
维生素 D	PP、SPE	LC-MS/MS	35	0.012～0.182	0.5～100	[34]
25(OH)D_2,25(OH)D_3	LLE-SPE、衍生化	LC-MS/MS	13	0.005～0.02**	0.2～1000	[71]
25(OH)D_2,25(OH)D_3	LLE	LC-MS/MS	45	0.375	600～1000	[68]
VD_2,VD_3,25(OH)D_2,25(OH)D_3,3-epi-25(OH)D_3	SPE	LC-MS/MS	28	0.023～0.225	0.75～625	[72]
25(OH)D_2,25(OH)D_3,3-epi-25(OH)D_2,3-epi-25(OH)D_3	LLE	LC-MS/MS	4	2.1～7.3**	2.1～460	[69]

注:* 表示单位为 fmol;** 表示结果为 LOQ

而,LC-MS/MS 在检测内分泌激素方面存在一些缺点。首先,检测样本时会受到基质效应和离子抑制等现象的影响,如来自不同人群的血液样本基质成分不同,显示出不同的离子抑制效果,同时,由于样本基质复杂,可能会发生同量异位素干扰导致定量误差,造成误诊。其次,雌激素离子化效率低,虽然通过衍生化引入带电部分能提高离子化效率,但许多雌激素衍生物会产生非特异性产物离子[20]。近年来,有报道[82]采用 LC-MS/MS 法测定人血清中包含雌激素在内的类固醇,通过在流动相中添加氟化铵盐来替代衍生化,但该方法仅限于检测孕妇体内几种高表达的内源性雌激素,尚不适用于检测低含量的类固醇化合物。此外,LC-MS/MS 在内分泌激素检测的应用时间相对较短,实验室内部方法的开发具有一定难度,实验室建立的各分析物参考范围仍需要经过实践检验,实现标准化以尽量减少实验室内和实验室间的差异问题[83]。

1.4　血药浓度监测

血药浓度监测包括治疗药物检测(TDM)、疼痛药物管理[84-85]和毒理学检测[86]等,其中 TDM 是血药浓度监测在临床中最常见的应用。在临床实践中,由于免疫抑制药物的治疗窗口狭窄且患者个体差异较大,须准确测量血液中的免疫抑制药物浓度来确定给药窗口,以制定个性化给药方案,从而避免药物副作用。LC-MS/MS 方法可在单个血样常规分析中对任意免疫抑制剂的组合方案进行准确定量,是目前实现 TDM 的最佳选择[87]。

TDM 中常用的免疫抑制剂包括他克莫司(TAC)、西罗莫司(SIR)、依维莫司(EvE)和环孢素 A(CsA)等,药物中约 75%～95%的成分会与红细胞结合,所以目前 TDM 的临床实践主要基于全血样本,其他非常规临床材料(如组织、细胞、尿液和其他体液的基质)也可用于 TDM,但应用较少。此外,由于全血样本储存时间短,将全血制备成 DBS 为临床提供了一种简单的样本基质替代方案,Sadilkova 等[88]研究表明,通过 LC-MS/MS 在 DBS 和全血中进行免疫抑制剂测量的结果具有良好的相关性。

在样本前处理阶段,由于全血样本中血细胞和蛋白质含量较高,大分子蛋白质和细胞会堵塞色谱柱,无法直接注入色谱柱进行分离。此外,全血样本中药物浓度通常较低,且存在大量干扰物质,导致基质干扰严重,因此,为提高方法的灵敏度,有必要去除干扰物质来净化样品,并通过浓缩分析物来增强信号。蛋白质沉淀(PP)、液液萃取(LLE)和固相萃取(SPE)是常用于 TDM 的样品预处理方法,PP 使用有机溶剂如甲醇、乙腈、丙酮或这些溶剂的混合物沉淀全血中的蛋白质,将硫酸锌添加到全血样本中进行溶血可以辅助沉淀蛋白质。LLE 通常使用甲醇、乙腈和叔丁基甲醚或它们的混合物萃取样品,但蒸发和复溶步骤可能会导致分析物的损失。SPE 无需蒸发和复溶步骤,减少了分析时间,但成本较高。临床实验室需要根据具体分析目标优化合适的预处理方案或组合方案,具体报道列于表 4。

表 4 免疫抑制剂的 LC-MS/MS 定量分析

Table 4 Quantification of immunosuppressantare by LC-MS/MS

分析物 Analyte	预处理方法 Pretreatment method	定量限 LOQ/(ng/mL)	线性范围 Reference range/(ng/mL)	分析时间 Analysis duration/min	参考文献 Reference
TAC、SIR、EvE、CsA	PP/乙腈	0.1～1	0.1～500	8	[90]
TAC、SIR、EvE、CsA	PP/甲醇,硫酸锌	0.5～5	0.5～2000	3.5	[91]
TAC、SIR、EvE、CsA	PP/甲醇-硫酸锌(60∶30,*V*/*V*)	2～9	2～1000	2.5	[92]
TAC、CsA	PP/乙腈,硫酸锌	14.6～17.5	10～1974	7	[93]
TAC、SIR、EvE、CsA	PP/硫酸锌	1～27.7	1.0～1483	—	[94]

Juliana 等[89]采用 LC-MS/MS 同时分析了全血样本中 4 种免疫抑制药物的 235 种组合方案,以探究器官移植后患者的最佳免疫抑制剂给药量,体现了 LC-MS/MS 在临床中为患者提供准确的个性化诊疗方案的巨大优势。然而,LC-MS/MS 存在设备较昂贵、只能在具备条件的实验室使用等缺点。未来,随着质谱仪更加自动化、小型化,使 TDM 从实验室分析走向现场快速分析成为可能。

1.5 肽类和蛋白质定量分析

蛋白质是生命过程的关键角色,定量检测蛋白质对于疾病的预防、诊断和治疗至关重要。目前,质谱已成为蛋白质定量分析必不可少的工具,其应用包括肿瘤标志物检测[95]、蛋白质或肽类药物定量[96]和靶向蛋白质组学分析[97-98],Sabbagh 等[99]对蛋白质定量分析适用方法进行了概述。

基于质谱的蛋白质定量分析要求检测结果具有高灵敏度、高稳定性、高通量以及良好的重复性,挑战性较高。首先,由于蛋白质在体外条件下易被降解,对样本基质的稳定性具有更严格的要求,预处理方法时长须小于 6 h[100]。其次,不同蛋白质在血清或血浆样本中丰度具有较大差异,须通过液相分离、使用 SPE 富集、适配体亲和富集或使用包括修饰的磁性纳米材料等技术富集含量较低的蛋白质。此外,不同于临床其他分析物,蛋白质属于大分子化合物,实验室常用的三重四极杆质谱无法直接定量分析其天然存在形式,需使用胰蛋白酶进行消化处理生成特征肽段,然后将这些特征肽段作为目标分析物,并严格优化包括孵育时间、反应温度以及蛋白酶用量在内的消化条件,以确保特征肽段与目标蛋白质的物质的量比例接近。可通过不同类型的质谱仪实现肽类和蛋白质的定量分析,包括基质辅助激光解吸-飞行时间质谱(MALDI-TOF MS)、离子阱质谱、单四极杆质谱、四极杆-飞行时间串联质谱、三重四极杆质谱以及高分辨质谱。大多数情况下使用三重四极杆的 SRM/MRM 模式即可实现对多个目标物的高度并行分析[101]。

目前,基于质谱的蛋白质定量分析方法日趋成熟,然而也存在不足:长时间的色谱分离和有限的通量限制了该方法进一步发展;样品制备中胰蛋白酶消化和低丰度蛋白质的富集,大大延长了分析时间;大多数易于定量的蛋白质尚未建立各自的参考范围[102],由于蛋白质丰度的变化可能受多种非疾病因素影响,因此定量结果还需要其他技术印证。

2 结论与展望

基于 LC-MS/MS 的定量分析方法在临床检验中变得越来越重要,目前,已建立了 LC-MS/MS 对氨基酸、肉碱、VD、一些内分泌激素和蛋白质等分析物定量检测的参考测量程序,并提供了可溯源至国际单位制的标准物质,随着标准化规模的扩大,LC-MS/MS 在临床分析中将得到更多认可。目前面临的主要挑战有:1) 受限于现有仪器的灵敏度,一些分析物的定量分析需要提前进行衍生化处理,增加了分析时间和使用成本,不利于临床大规模分析应用;2) 由于临床样本基质的复杂性,大多数分析物尚不明确其定量参考范围,或缺少相应的 LC-MS/MS 参考测量测序程序,不同个体间的定量差异须受到重视;3) LC-MS/MS设备昂贵,开发新方法的成本较高,因此分析物的定量分析通常仅限于实验室内,限制了临床应用的进一步发展。未来,随着

质谱仪器小型化,仪器成本将得到控制,现场LC-MS/MS检测将成为可能。

参考文献:

[1] WU A H B. A selected history and future of immunoassay development and applications in clinical chemistry[J]. Clinica Chimica Acta, 2006, 369(2): 119-124.

[2] KETHA H, KAUR S, GREBE S K, SINGHET R J. Clinical applications of LC-MS sex steroid assays[J]. Current Opinion in Endocrinology & Diabetes and Obesity, 2014, 21(3): 217-226.

[3] MARSHALL A G, HENDRICKSON C L, JACKSON G S. Fourier transform ion cyclotron resonance mass spectrometry: A primer[J]. Mass Spectrometry Reviews, 1998, 17(1): 1-35.

[4] HOPWOOD J. Review of inductively coupled plasmas for plasma processing[J]. Plasma Sources Science & Technology, 1992, 1(2): 109-116.

[5] HILLENKAMP F, KARAS M, BEAVIS R C, CHAIT B T. Matrix-assisted laser desorption/ionization mass spectrometry of biopolymers[J]. Analytical Chemistry, 1991, 63(24): 1193A-1203A.

[6] GROSS J H. Mass spectrometry[M]. Berlin: Springer, 2017: 29-84.

[7] ADAWAY J E, KEEVIL B G, OWEN L J. Liquid chromatography tandem mass spectrometry in the clinical laboratory[J]. Annals of Clinical Biochemistry, 2015, 52(1): 18-38.

[8] GROSS J H. Mass spectrometry[M]. Berlin: Springer, 2017: 539-612.

[9] RIFAI N, HORVATH A R, WITTWER C T. Principles and applications of clinical mass spectrometry[M]. Berlin: Elsevier, 2018: 33-65.

[10] van BREEMEN R B, MARTINEZ E M. Best practice in mass spectrometry for LC-MS[M]. Hoboken, USA: John Wiley & Sons Inc, 2013: 205-216.

[11] LEUNG K S, FONG B M. LC-MS/MS in the routine clinical laboratory: has its time come?[J]. Analytical and Bioanalytical Chemistry, 2014, 406(9/10): 2 289-2 301.

[12] CARVALHO V M. The coming of age of liquid chromatography coupled to tandem mass spectrometry in the endocrinology laboratory[J]. Journal of Chromatography B, 2012, (883/884): 50-58.

[13] HIMMELSBACH M. 10 years of MS instrumental developments-impact on LC-MS/MS in clinical chemistry[J]. Journal of Chromatography B, 2012, (883/884): 3-17.

[14] UNGER S, WENG N. Handbook of LC-MS bioanalysis: best practices, experimental protocols, and regulations[M]. Hoboken, USA: John Wiley & Sons, 2013: 185-204.

[15] HAGE D S. Principles and applications of clinical mass spectrometry[M]. Berlin: Elsevier, 2018: 1-32.

[16] STONE J. Mass spectrometry for the clinical laboratory[M]. Berlin: Elsevier, 2017: 37-62.

[17] WELLS D A. Principles and applications of clinical mass spectrometry[M]. Berlin: Elsevier, 2018: 67-91.

[18] ADAMKIN D H. Metabolic screening and postnatal glucose homeostasis in the newborn[J]. Pediatric Clinics of North America, 2015, 62(2): 385-409.

[19] JENSEN B P, SARAF R, MA J, BERRY S, GRANT C C, CAMARGO JR C A, SIES C W. Quantitation of 25-hydroxyvitamin D in dried blood spots by 2D LC-MS/MS without derivatization and correlation with serum in adult and pediatric studies[J]. Clinica Chimica Acta, 2018, 481: 61-68.

[20] KUSHNIR M M, ROCKWOOD A L, BERGQUIST J. Liquid chromatography-tandem mass spectrometry applications in endocrinology[J]. Mass Spectrometry Reviews, 2010, 29(3): 480-502.

[21] SYED M, SRINIVAS N R. A comprehensive review of the published assays for the quantitation of the immunosuppressant drug mycophenolic acid and its glucuronidated metabolites in biological fluids[J]. Biomedical Chromatography, 2016, 30(5): 721-748.

[22] VIDOVA V, SPACIL Z. A review on mass spectrometry-based quantitative proteomics: Targeted and data independent acquisition[J]. Analytica Chimica Acta, 2017, 964: 7-23.

[23] BLEYLE L, HUIDEKOPER H H, VAZ F M, SINGH R, STEINER R D, DEBARBER A E. Update on newborn dried bloodspot testing for cerebrotendinous xanthomatosis: An available high-throughput liquid-chromatography tandem

mass spectrometry method[J]. Molecular Genetics and Metabolism Reports, 2016, 7: 11-15.

[24] KUBASKI F, MASON R W, NAKATOMI A, SHINTAKU H, XIE L, van VLIES N N, CHURCH H, GIUGLIANI R, KOBAYASHI H, YAMAGUCHI S, SUZUKI Y, ORII T, FUKAO T, MONTANO A M, TOMATSU S. Newborn screening for mucopolysaccharidoses: a pilot study of measurement of glycosaminoglycans by tandem mass spectrometry[J]. Journal of Inherited Metabolic Disease, 2017, 40(1): 151-158.

[25] BURLINA A B, POLO G, SALVIATI L, DURO G, ZIZZO C, DARDIS A, BEMBI B, CAZZORLA C, RUBERT L, ZORDAN R, DESNICK R J, BURLINA A P. Newborn screening for lysosomal storage disorders by tandem mass spectrometry in North East Italy[J]. Journal of Inherited Metabolic Disease, 2018, 41(2): 209-219.

[26] CHO S E, KWAK J R, LEE H, SEO D H, SONG J. Triplex tandem mass spectrometry assays for the screening of 3 lysosomal storage disorders in a Korean population[J]. Clinica Chimica Acta, 2016, 454: 20-27.

[27] GHORABA D A, MOHAMED M M, ZAKI O K. Screening of diseases associated with abnormal metabolites for evaluation of HPLC in organic aciduria profiling[J]. Egyptian Journal of Medical Human Genetics, 2014, 15(1): 69-78.

[28] MALACA S, MARCHEI E, BARCELÓ MARTÍN B, MINUTILLO A, PICHINI S. Novel fast ultra-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) and extraction of ethylglucuronide in meconium samples[J]. Drug Testing and Analysis, 2019, 11(9): 1 471-1 475.

[29] GOUDA A S, NAZIM W S. Development of a simple method for the analysis of phenylalanine in dried blood spot using tandem mass spectrometry [J]. Egyptian Journal of Medical Human Genetics, 2020, 21(1): 1-6.

[30] CHIN S, OSMAN J, JAMAL R. Simultaneous determination of 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 in human serum by ultra performance liquid chromatography: An economical and validated method with bovine serum albumin [J]. Clinica Chimica Acta, 2018, 485: 60-66.

[31] ZHANG H, QUAN L, PEI P, LIN Y, FENG C, GUAN H, WANG F, ZHANG T, WU J, HUO J. Simultaneous determination of Vitamin A, 25-hydroxyl vitamin D3 α-tocopherol in small biological fluids by liquid chromatography-tandem mass spectrometry[J]. Journal of Chromatography B, 2018, 1 079: 1-8.

[32] MÜLLER M J, STOKES C S, VOLMER D A. Quantification of the 3α and 3β epimers of 25-hydroxyvitamin D 3 in dried blood spots by LC-MS/MS using artificial whole blood calibration and chemical derivatization[J]. Talanta, 2017, 165: 398-404.

[33] ZHANG H Q L. Simultaneous determination of Vitamin A, 25-hydroxyl vitamin D3 α-tocopherol in small biological fluids by liquid chromatography-tandem mass spectrometry[J]. J Chromatogr B Analyt Technol Biomed Life Sci, 2018, 2(111): 1-8.

[34] ABU KASSIM N S, SHAW P N, HEWAVITHARANA A K. Simultaneous determination of 12 vitamin D compounds in human serum using online sample preparation and liquid chromatography-tandem mass spectrometry[J]. Journal of Chromatography A, 2018, 1 533: 57-65.

[35] WANG D, YU S, ZOU Y, ZHANG Y, QIU L, CHEN L. Distribution of free 25OHD in elderly population based on LC-MS/MS[J]. The Journal of Steroid Biochemistry and Molecular Biology, 2020, 200: 105 672.

[36] BODOR G S. Quantitative, multidrug pain medication testing by liquid chromatography: tandem mass spectrometry (LC-MS/MS)[M]. New York: Humana Press, 2016: 223-240.

[37] BROZMANOVÁ H, KACÍŘOVÁ Ĭ, UŘINOVSKÁ R, ŠIŠTÍK P, GRUNDMANN M. New liquid chromatography-tandem mass spectrometry method for routine TDM of vancomycin in patients with both normal and impaired renal functions and comparison with results of polarization fluoroimmunoassay in light of varying creatinine concentrations[J]. Clinica Chimica Acta, 2017, 469: 136-143.

[38] LIU T, KOTHA R R, JONES J W, POLLI J E, KANE M A. Fast liquid chromatography-tandem mass spectrometry method for simultaneous determination of eight antiepileptic drugs and an

active metabolite in human plasma using polarity switching and timed selected reaction monitoring [J]. Journal of Pharmaceutical and Biomedical Analysis, 2019, 176: 112 816.

[39] WILLEMAN T, JOURDIL J F, GAUTIER-VEYRET E, BONAZ B, STANKE-LABESQUE F. A multiplex liquid chromatography tandem mass spectrometry method for the quantification of seven therapeutic monoclonal antibodies: Application for adalimumab therapeutic drug monitoring in patients with Crohn's disease[J]. Analytica Chimica Acta, 2019, 1 067: 63-70.

[40] SCHEIDWEILER K B, BARNES A J. Quantification of eight cannabinoids including cannabidiol in human urine via liquid chromatography tandem mass spectrometry[M]. LC-MS in Drug Analysis, New York: Humana Press, 2019, 1 872: 11-12.

[41] CAPPELLE D, DE DONCKER M, GYS C, KRYSIAK K, DE KEUKELEIRE S, MAHO W, CRUNELLE C L, DOM G, COVACI A, VAN NUIJS A L, NEELS H. A straightforward, validated liquid chromatography coupled to tandem mass spectrometry method for the simultaneous detection of nine drugs of abuse and their metabolites in hair and nails[J]. Analytica Chimica Acta, 2017, 960: 101-109.

[42] CHEN R, NING Z, ZHENG C, YANG Y, ZHANG C, OU X, CHEN K, YU H, WEI X, ZHAO Q, HE J. Simultaneous determination of 16 alkaloids in blood by ultrahigh-performance liquid chromatography-tandem mass spectrometry coupled with supported liquid extraction[J]. Journal of Chromatography B, 2019, 1 128: 121 789.

[43] BEN-DOR S H A. Finding a needle in a haystack: the advantages of liquid chromatography-tandem mass spectrometry (LC-MS/MS) in determination of sex hormones in children[J]. Pediatric Endocrinology Reviews, 2016, 4(13): 714-719.

[44] BERNSTONE L, JAYANTI A, KEEVIL B. A simplified, rapid LC-MS/MS assay for serum and salivary creatinine[J]. Clinical Mass Spectrometry, 2019, 11: 21-26.

[45] FAQEHI A M M, COBICE D F, NAREDO G, MAK T C S, UPRETI R, GIBB F W, BECKETT G J, WALKER B R, HOMER N Z M, ANDREW R. Derivatization of estrogens enhances specificity and sensitivity of analysis of human plasma and serum by liquid chromatography tandem mass spectrometry[J]. Talanta, 2016, 151: 148-156.

[46] KARVALY G, KOVÁCS K, MÉSZÁROS K, KOCSIS I, PATÓCS A, VÁSÁRHELYI B. The comprehensive characterization of adrenocortical steroidogenesis using two-dimensional ultra-performance liquid chromatography-electrospray ionization tandem mass spectrometry[J]. J Pharm Biomed Anal, 2018, 153: 274-283.

[47] ZHOU Y, CAI Z. Determination of hormones in human urine by ultra-high-performance liquid chromatography/triple-quadrupole mass spectrometry[J]. Rapid Communications in Mass Spectrometry, 2020, 34(s1): e8583.

[48] SHAFAEI A, CROFT K, HODGSON J, BOYCE M C. Simultaneous quantitative analysis of polyphenolic compounds in human plasma by liquid chromatography tandem mass spectrometry[J]. Journal of Separation Science, 2019, 42(18): 2 909-2 921.

[49] WOO H I, YANG J S, OH H J, CHO Y Y, KIM J H, PARK H D, LEE S Y. A simple and rapid analytical method based on solid-phase extraction and liquid chromatography-tandem mass spectrometry for the simultaneous determination of free catecholamines and metanephrines in urine and its application to routine clinical analysis[J]. Clinical Biochemistry, 2016, 49(7/8): 573-579.

[50] LUO A, EL GIERARI E T M, NALLY L M, STURMER L R, DODD D, SHI R Z. Clinical utility of an ultrasensitive urinary free cortisol assay by tandem mass spectrometry[J]. Steroids, 2019, 146: 65-69.

[51] GARCIA-AC A, DUY S V, SAUVÉ S, MOLDOVAN F, ROULLIN V G, BANQUY X. Quantification of peptides in human synovial fluid using liquid chromatography-tandem mass spectrometry[J]. Talanta, 2018, 186: 124-132.

[52] IPPOUSHI K, WAKAGI M, HASHIMOTO N, TAKANO-ISHIKAWA Y. Absolute quantification of the α, α′, and β subunits of β-conglycinin from soybeans by liquid chromatography/tandem mass spectrometry using stable isotope-labelled peptides[J]. Food Research International, 2019, 116: 1 223-1 228.

[53] WANG Z, CHEN Q, WU Q, LI Q, CHEN D, CHU X. Evaluation of mutual interference

between bovine α-lactalbumin peptide and its isotope-labeled peptide in whey protein analysis using liquid chromatography-tandem mass spectrometry[J]. Journal of Chromatography A, 2018, 1 533: 94-101.

[54] LIU W, CAO Y, REN Y, XU X, HE L, XIA R, TU P, WANG Y, SONG Y, LI J. Simultaneously quantitative analysis of peptides and chemical components in Cervus and Cucumis polypeptide injection (Songmeile®) using reversed phase liquid chromatography-hydrophilic interaction liquid chromatography-tandem mass spectrometry[J]. Journal of Chromatography A, 2019, 1 617: 460 827.

[55] YANG T, CHEN F, XU F, WANG F, XU Q, CHEN Y. A liquid chromatography-tandem mass spectrometry-based targeted proteomics assay for monitoring P-glycoprotein levels in human breast tissue[J]. Clin Chim Acta, 2014, 436: 283-289.

[56] YU C, HUANG S, WANG M, ZHANG J, LIU H, YUAN Z, WANG X, HE X, WANG J, ZOU L. A novel tandem mass spectrometry method for first-line screening of mainly beta-thalassemia from dried blood spots[J]. J Proteomics, 2017, 154: 78-84.

[57] ADAWAY J E, KEEVIL B G, OWEN L J. Liquid chromatography tandem mass spectrometry in the clinical laboratory[J]. Annals of Clinical Biochemistry, 2015, 52(1): 18-38.

[58] THIBOONBOON K, LEELAHAVARONG P, WATTANASIRICHAIGOON D, VATANAVICHARN N, WASANT P, SHOTELERSUK V, PANGKANON S, KUPTANON C, CHAISOMCHIT S, TEERAWATTANANON Y. An Economic Evaluation of Neonatal Screening for Inborn Errors of Metabolism Using Tandem Mass Spectrometry in Thailand[J]. PLOS One, 2015, 10(8): e134782.

[59] CHACE D H, MILLINGTON D S, TERADA N, KAHLER S G, ROE C R, HOFMAN L F. Rapid diagnosis of phenylketonuria by quantitative analysis for phenylalanine and tyrosine in neonatal blood spots by tandem mass spectrometry [J]. Clinical Chemistry, 1993, 39(1): 66-71.

[60] MOAT S J, GEORGE R S, CARLING R S. Use of dried blood spot specimens to monitor patients with inherited metabolic disorders[J]. International Journal of Neonatal Screening, 2020, 6(2): 26.

[61] LUO X, WANG R, FAN Y, GU X, YU Y. Next-generation sequencing as a second-tier diagnostic test for newborn screening[J]. Journal of Pediatric Endocrinology & Metabolism, 2018, 31(8): 927.

[62] HOSSEIN-NEZHAD A, HOLICK M F. Vitamin D for health: a global perspective[J]. Mayo Clinic Proceedings, 2013, 88(7): 720-755.

[63] STEPMAN H C, VANDERROOST A, VAN UYTFANGHE K, THIENPONT L M. Candidate reference measurement procedures for serum 25-Hydroxyvitamin D3 and 25-Hydroxyvitamin D2 by using isotope-dilution liquid chromatography-tandem mass spectrometry[J]. Clinical Chemistry, 2011, 57(3): 441-448.

[64] MÜLLER M J, VOLMER D A. Mass spectrometric profiling of vitamin D metabolites beyond 25-Hydroxyvitamin D[J]. Clinical Chemistry, 2015, 61(8): 1 033-1 048.

[65] HEWAVITHARANA A K, ABU KASSIM N S, SHAW P N. Standard addition with internal standardisation as an alternative to using stable isotope labelled internal standards to correct for matrix effects-comparison and validation using liquid chromatography-tandem mass spectrometric assay of vitamin D[J]. Journal of Chromatography A, 2018, 1 553: 101-107.

[66] HURST E A, HOMER N Z, DENHAM S G, MACFARLANE E, CAMPBELL S, BOSWINKEL M, MELLANBY R J. Development and application of a LC-MS/MS assay for simultaneous analysis of 25-hydroxyvitamin-D and 3-epi-25-hydroxyvitamin-D metabolites in canine serum[J]. The Journal of Steroid Biochemistry and Molecular Biology, 2020, 199: 105 598.

[67] SCURRIA A, LINO C, PITONZO R, PAGLIARO M, CIRIMINNA R. Vitamin D3 in fish oil extracted with limonene from anchovy leftovers [J]. Chemical Data Collections, 2020, 25: 100 311.

[68] TAI S S C, BEDNER M, PHINNEY K W. Development of a candidate reference measurement procedure for the determination of 25-Hydroxyvitamin D3 and 25-Hydroxyvitamin D2 in human serum using isotope-dilution liquid chromatography-tandem mass spectrometry[J]. Analytical

Chemistry, 2010, 82(5): 1 942-1 948.

[69] LIEBISCH G, MATYSIK S. Accurate and reliable quantification of 25-hydroxy-vitamin D species by liquid chromatography high-resolution tandem mass spectrometry[J]. Journal of Lipid Research, 2015, 56(6): 1 234-1 239.

[70] ABU KASSIM N S, GOMES F P, SHAW P N, HEWAVITHARANA A K. Simultaneous quantitative analysis of nine vitamin D compounds in human blood using LC-MS/MS[J]. Bioanalysis, 2016, 8(5): 397.

[71] WAN D, YANG J, BARNYCH B, HWANG S H, LEE K S S, CUI Y, NIU J, WATSKY M A, HAMMOCK B D. A new sensitive LC/MS/MS analysis of vitamin D metabolites using a click derivatization reagent, 2-nitrosopyridine [J]. Journal of Lipid Research, 2017, 58(4): 798-808.

[72] MENA-BRAVO A, PRIEGO-CAPOTE F, LUQUE DE CASTRO M D. Two-dimensional liquid chromatography coupled to tandem mass spectrometry for vitamin D metabolite profiling including the C3-epimer-25-monohydroxyvitamin D3 [J]. Journal of Chromatography A, 2016, 1 451: 50-57.

[73] KOAL T, SCHMIEDERER D, PHAM-TUAN H, RÖHRING C, RAUH M. Standardized LC-MS/MS based steroid hormone profile-analysis [J]. The Journal of Steroid Biochemistry and Molecular Biology, 2012, 129(3/5): 129-138.

[74] FOLKERD E J, LØNNING P E, DOWSETT M. Interpreting plasma estrogen levels in breast cancer: Caution needed[J]. Journal of Clinical Oncology, 2014, 32(14): 1 396-1 400.

[75] KRATZ A L K B. Case records of the massachusetts general hospital[J]. New England Journal of Medicine, 1998, 15(339): 1 063-1 072.

[76] KUSHNIR M M, ROCKWOOD A L, BERGQUIST J, VARSHAVSKY M, ROBERTS W L, YUE B, BUNKER A M, MEIKLE A W. High-sensitivity tandem mass spectrometry assay for serum estrone and estradiol[J]. American Journal of Clinical Pathology, 2008, 129(4): 530-539.

[77] ROSSI C, CICALINI I, VERROCCHIO S, DI DALMAZI G, FEDERICI L, BUCCI I. The potential of steroid profiling by mass spectrometry in the management of adrenocortical carcinoma [J]. Biomedicines, 2020, 8(9): 314.

[78] CARVALHO V M. The coming of age of liquid chromatography coupled to tandem mass spectrometry in the endocrinology laboratory[J]. Journal of Chromatography B, 2012, (883/884): 50-58.

[79] WUDY S A, SCHULER G, SáNCHEZ-GUIJO A, HARTMANN M F. The art of measuring steroids[J]. The Journal of Steroid Biochemistry and Molecular Biology, 2018, 179: 88-103.

[80] ENVER E O, VATANSEVER P, GURAN O, BILGIN L, BORAN P, TURAN S, HAKLAR G, BEREKET A, GURAN T. Adrenal steroids reference ranges in infancy determined by LC-MS/MS[J]. Pediatric Research, 2022, 92(1): 265-274.

[81] HANDELSMAN D J, YEAP B, FLICKER L, MARTIN S, WITTERT G A, LY L P. Age-specific population centiles for androgen status in men[J]. European Journal of Endocrinology, 2015, 173(6): 809-817.

[82] PREINDL K, BRAUN D, AICHINGER G, SIERI S, FANG M, MARKO D, WARTH B. A generic liquid chromatography-tandem mass spectrometry exposome method for the determination of xenoestrogens in biological matrices[J]. Analytical Chemistry, 2019, 91(17): 11 334-11 342.

[83] KOAL T, SCHMIEDERER D, PHAM-TUAN H, RÖHRING C, RAUH M. Standardized LC-MS/MS based steroid hormone profile-analysis [J]. The Journal of Steroid Biochemistry and Molecular Biology, 2012, 129(3/5): 129-138.

[84] GUDALA K, BANSAL D, VATTE R, GHAI B, SCHIFANO F, BOYA C. High prevalence of neuropathic pain component in patients with low back pain: evidence from meta-analysis[J]. Pain Physician, 2017, 20(5): 343-352.

[85] STEPHANSON N, SANDQVIST S, LAMBERT M S, BECK O. Method validation and application of a liquid chromatography-tandem mass spectrometry method for drugs of abuse testing in exhaled breath[J]. J Chromatogr B, 2015, 985: 189-196.

[86] SEMPIO C, SCHEIDWEILER K B, BARNES A J, HUESTIS M A. Optimization of recombinant β-glucuronidase hydrolysis and quantification of eight urinary cannabinoids and metabolites by liquid chromatography tandem mass spectrometry [J]. Drug Test Anal, 2018, 10(3): 518-529.

[87] KUHLIN J, STURKENBOOM M G G, GHIMIRE S, MARGINEANU I, van den ELSEN S H J, SIMBAR N, AKKERMAN O W, JONGEDIJK E M, KOSTER R A, BRUCHFELD J, TOUW D J, ALFFENAAR J C. Mass spectrometry for therapeutic drug monitoring of anti-tuberculosis drugs[J]. Clinical Mass Spectrometry, 2019, 14: 34-45.

[88] SADILKOVA K, BUSBY B, DICKERSON J A, RUTLEDGE J C, JACK R M. Clinical validation and implementation of a multiplexed immunosuppressant assay in dried blood spots by LC-MS/MS [J]. Clinica Chimica Acta, 2013, 421: 152-156.

[89] DINÉIA P J, SANCHES A D, APARECIDA R F, FEBBA A C, ROSSO C F, TEDESCO-SILVA J H, MEDINA DE A P J O. Simultaneous determination of everolimus, sirolimus, tacrolimus, and cyclosporine-a by mass spectrometry[J]. Transplantation Proceedings, 2020, 52(5): 1 402-1 408.

[90] PANIAGUA-GONZÁLEZ L, LENDOIRO E, OTERO-ANTÓN E, MOLINA-PÉREZ E, VARO-PÉREZ E, LÓPEZ-RIVADULLA M, CRUZ A, DE-CASTRO-RÍOS A. A multidrug LC-MS/MS method for the determination of five immunosuppressants in oral fluid[J]. Bioanalysis, 2019, 11(16): 1 509-1 521.

[91] KRNÁČ D, REIFFOVÁ K, ROLINSKI B. A new HPLC-MS/MS method for simultaneous determination of Cyclosporine A, Tacrolimus, Sirolimus and Everolimus for routine therapeutic drug monitoring[J]. Journal of Chromatography B, 2019, 1 128: 121 772.

[92] DINÉIA P J, SANCHES A D, APARECIDA R F, FEBBA A C, ROSSO C F, TEDESCO-SILVA J H, MEDINA DE A P J O, CASARINI D E. Simultaneous determination of everolimus, sirolimus, tacrolimus, and cyclosporine-a by mass spectrometry[J]. Transplantation Proceedings, 2020, 52(5): 1 402-1 408.

[93] SHEIKHOLESLAMI M N, VOSOUGH M, ESFAHANI H M. On the performance of multivariate curve resolution to resolve highly complex liquid chromatography-full scan mass spectrometry data for quantification of selected immunosuppressants in blood and water samples[J]. Microchemical Journal, 2020, 152: 104 298.

[94] BRESSÁN I G, GIMÉNEZ M I, LLESUY S F. Validation of a simple liquid chromatography coupled to tandem mass spectrometry method for the simultaneous determination of tacrolimus, sirolimus, everolimus and cyclosporin A in dried matrix on paper discs[J]. Journal of Mass Spectrometry and Advances in the Clinical Lab, 2021, 19: 7-19.

[95] MACKLIN A, KHAN S, KISLINGER T. Recent advances in mass spectrometry based clinical proteomics: applications to cancer research[J]. Clinical Proteomics, 2020, 17: 17.

[96] van BERGEN W, HECK A J R, BAGGELAAR M P. Recent advancements in mass spectrometry-based tools to investigate newly synthesized proteins[J]. Current Opinion in Chemical Biology, 2021, 66: 102 074.

[97] LI X, WANG W, CHEN J. Recent progress in mass spectrometry proteomics for biomedical research[J]. Science China Life Sciences, 2017, 60(10): 1 093-1 113.

[98] VIDOVA V, SPACIL Z. A review on mass spectrometry-based quantitative proteomics: Targeted and data independent acquisition[J]. Analytica Chimica Acta, 2017, 964: 7-23.

[99] SABBAGH B, MINDT S, NEUMAIER M, FINDEISEN P. Clinical applications of MS-based protein quantification[J]. Proteomics-Clinical Applications, 2016, 10(4): 323-345.

[100] YI J, KIM C, GELFAND C A. Inhibition of intrinsic proteolytic activities moderates preanalytical variability and instability of human plasma[J]. Journal of Proteome Research, 2007, 6(5): 1 768-1 781.

[101] PAN S, AEBERSOLD R, CHEN R, RUSH J, GOODLETT D R, MCINTOSH M W, ZHANG J, BRENTNALL T A. Mass spectrometry based targeted protein quantification: methods and applications[J]. Journal of Proteome Research, 2009, 8(2): 787-797.

[102] LIU Y, BUIL A, COLLINS B C, GILLET L C, BLUM L C, CHENG L Y, VITEK O, MOURITSEN J, LACHANCE G, SPECTOR T D, DERMITZAKIS E T, AEBERSOLD R. Quantitative variability of 342 plasma proteins in a human twin population[J]. Molecular Systems Biology, 2015, 11(2): 786.

(收稿日期:2021-08-30;修回日期:2022-10-08)