2021 年 7 月 色 谱 Vol.39 No.7
July 2021 Chinese Journal of Chromatography 730~743

技术与应用 DOI: 10.3724/SP.J.1123.2020.10016

超高效液相色谱-四极杆-飞行时间质谱法快速辨识芪玉三龙汤化学成分

黄梦文[1], 吴 欢[1,2,3*], 于 伟[1], 王 影[1], 汪逢灿[1],
张纯纯[1], 周龙生[1], 李泽庚[1,4]

(1. 安徽中医药大学科研实验中心, 安徽 合肥 230038; 2. 中药研究与开发安徽省重点实验室, 安徽 合肥 230012; 3. 中药复方安徽省重点实验室, 安徽 合肥 230012; 4. 安徽省教育厅中医药防治肺系重大疾病重点实验室, 安徽 合肥 230038)

摘要:利用超高效液相色谱-四极杆-飞行时间质谱(UPLC-QTOF-MS)的数据非依赖性采集(DIA)技术,结合靶向筛查方法,快速辨识芪玉三龙汤(Qi-Yu-San-Long decoction, QYSLD)化学成分。以 Waters ACQUITY UPLC BEH C_{18}柱(100 mm×2.1 mm, 1.7 μm)为色谱柱,流速为 0.2 mL/min,柱温为 35 ℃,进样量为 2 μL,以 0.1%(v/v)甲酸水溶液-乙腈为流动相进行梯度洗脱。采用电喷雾电离(ESI)源,以全信息串联质谱(MS^E)技术在正、负离子模式下分别采集 QYSLD 复杂组分的质谱数据。通过检索文献和在线数据库,建立 QYSLD 中各单味药材化学成分库。将采集到的样品原始质谱数据与 QYSLD 化学成分库导入天然产物后处理筛查(UNIFI)平台;在 UNIFI 平台中设置参数(保留时间偏差为±0.1 min,精确质量数偏差阈值为$\pm5\times10^{-6}$,正离子模式下,选择$[M+H]^+$和$[M+Na]^+$为准分子离子或加合离子,负离子模式下,选择$[M-H]^-$和$[M+HCOO]^-$)。经 UNIFI 平台对 MS^E 模式下采集的质谱数据与自建数据库中成分作靶向筛查,结合化合物准分子离子、质谱裂解途径及部分对照品进行结构确认。从 QYSLD 中共识别出 166 种化学成分,其中皂苷类 22 种,生物碱类 13 种,黄酮类 27 种,萜类 32 种,氨基酸类 20 种,苯丙素类 16 种,有机酸类 9 种,甾醇类 6 种,蒽醌类 6 种,其他类 15 种。其中 16 种成分使用对照品作验证。研究建立的方法能够快速、可靠的表征 QYSLD 中的化学成分,为该复方的药效物质及质量控制研究奠定了基础。

关键词:超高效液相色谱-四极杆-飞行时间质谱;数据非依赖性采集;靶向筛查;芪玉三龙汤

中图分类号:O658 **文献标识码**:A **文章编号**:1000-8713(2021)07-0730-14

Rapid identification of chemical components in Qi-Yu-San-Long decoction by ultra high performance liquid chromatography-quadrupole time-of-flight mass spectrometry

HUANG Mengwen[1], WU Huan[1,2,3*], YU Wei[1], WANG Ying[1], WANG Fengcan[1], ZHANG Chunchun[1], ZHOU Longsheng[1], LI Zegeng[1,4]

(*1. Scientific Research & Experiment Center, Anhui University of Chinese Medicine, Hefei 230038, China; 2. Anhui Province Key Laboratory of Research and Development of Chinese Medicine, Hefei 230012, China; 3. Anhui Province Key Laboratory of Chinese Medicinal Formula, Hefei 230012, China; 4. Key Laboratory of Prevention and Treatment of Major Pulmonary Diseases with Traditional Chinese Medicine, Department of Education of Anhui Province, Hefei 230038, China*)

收稿日期:2020-11-06

* **通讯联系人**.Tel:(0551)65165073,E-mail:wuhuancpu@163.com.

基金项目:国家自然科学基金(81903765);国家级大学生创新训练项目(医学自然)(202006180216);安徽省高校自然科学基金重点项目(KJ2019A0478);安徽省教育厅中医药防治肺系重大疾病重点实验室开放基金项目(JYTKF2020-5).

Foundation item: National Natural Science Foundation of China (No. 81903765); National College Student Innovation Training Project (Medical Nature) (No. 202006180216); Key Project of Natural Science Foundation for the Higher Education Institutions of Anhui Province (No. KJ2019A0478); Open Fund Project of Key Laboratory of Traditional Chinese Medicine for Prevention and Treatment of Major Pulmonary Diseases of Anhui Provincial Department of Education (No. JYTKF2020-5).

Abstract: Qi-Yu-San-Long decoction (QYSLD) is a classic traditional Chinese medicine prescription consisting of ten types of herbal medicines, including Astragali Radix, Polygonati Odorati Rhizoma, Scolopendra, Pheretima, *Solanum nigrum* L., *Hedyotis diffusa* Willd., Coicis Semen, *Euphorbia helioscopia* L., Curcumae Rhizoma, and Fritillariae Cirrhosae Bulbus, combined in a ratio of 15∶5∶3∶3∶10∶10∶10∶3∶5∶3 by weight. QYSLD has been used to treat non-small cell lung cancer (NSCLC) for over 20 years in clinical practice, and its curative effect is considered credible. However, the chemical constituents of QYSLD have not been revealed because of their complexity, which has significantly hindered the systematic clarification of the efficacy of the materials and quality evaluation. In this study, a reliable strategy based on the data-independent acquisition (DIA) technology of ultra high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) combined with a targeted screening method was established to investigate the chemical components of QYSLD. A 2-μL aliquot from each vial was injected into a Waters ACQUITY UPLC BEH C_{18} column (100 mm×2.1 mm, 1.7 μm) to separate complex components. The temperature of the column was 35 ℃, and the flow rate was set at 0.2 mL/min. The mobile phase consisted of 0.1% formic acid aqueous solution and acetonitrile. Detection was conducted using an Xevo G2-XS QTOF-MS with a LockSpray capable-electrospray interface. The data for complex components in QYSLD were collected by full-information tandem mass spectrometry (MS^E) in the positive and negative ion modes. In the MS^E mode, data acquisition was performed using a mass spectrometer by rapidly switching from a low-collision-energy (CE) scan to a high-CE scan during a single LC run. Thus, accurate precursor and fragment ions were collected in a single run, which was helpful for the structural elucidation of multiple components in QYSLD. In addition, systematic information on isolated chemical compounds was collected and distinguished from the ten individual herbs in QYSLD using databases such as China Academic Journals Full-text database (CNKI), PubMed, Web of Science, Medline, and ChemSpider. Accordingly, a self-building library of QYSLD, including the component name, molecular formula, and structure of the components from the herbs, was established. Subsequently, the raw MS^E data of the collected samples and the self-building chemical composition library were imported into a natural product post-processing screening (UNIFI) platform for targeted screening of the chemical components in QYSLD. The parameters for UNIFI platform were as follows: the retention time deviation was ±0.1 min; an error margin of no more than 5×10^{-6} for the identified compounds was allowed; positive adducts, including $[M+H]^+$ and $[M+Na]^+$, were selected; and negative adducts, including $[M-H]^-$ and $[M+HCOO]^-$, were selected. The results showed that a total of 166 compounds were initially identified, including 22 saponins, 13 alkaloids, 27 flavonoids, 32 terpenes, 20 amino acids, 16 phenylpropanoids, 9 organic acids, 6 sterols, 6 anthraquinones, and 15 other components. Among them, sixteen components were confirmed unambiguously with the reference substances. To better understand the chemical contribution of individual herbs to the entire decoction, the attributes of each component were summarized. This study provides a foundation for exploring the pharmacodynamic substances of QYSLD.

引用本文:黄梦文,吴欢,于伟,王影,汪逢灿,张纯纯,周龙生,李泽庚. 超高效液相色谱-四极杆-飞行时间质谱法快速辨识芪玉三龙汤化学成分. 色谱,2021,39(7):730-743.

HUANG Mengwen, WU Huan, YU Wei, WANG Ying, WANG Fengcan, ZHANG Chunchun, ZHOU Longsheng, LI Zegeng. Rapid identification of chemical components in Qi-Yu-San-Long decoction by ultra high performance liquid chromatography-quadrupole time-of-flight mass spectrometry. Chinese Journal of Chromatography, 2021, 39(7): 730-743.

Key words: ultra high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS); data independent acquisition (DIA); targeted screening strategy; Qi-Yu-San-Long decoction (QYSLD)

芪玉三龙汤(Qi-Yu-San-Long decoction, QYSLD)是由黄芪、玉竹、天龙、地龙、龙葵、白花蛇舌草、薏苡仁、泽漆、莪术、川贝母十味中药组方而成,是临床用于治疗非小细胞肺癌(NSCLC)的名老中医验方[1]。现代药理学研究[2-4]表明,QYSLD 抑制 NSCLC 的作用可能与其调控 PI3K/Akt/mTOR 和 Wnt/β-catenin 信号转导通路分子表达有关。虽然 QYSLD 能有效治疗 NSCLC,但其发挥抑制 NSCLC 作用的物质基础尚未明确。中药(复方)中的复杂化学组分具有多靶点和协同效应的治疗作用特点,对这些复杂化学组分进行解析是揭示中药(复方)药效物质基础并对其进行质量控制的关键步骤。因此,有必要建立一种快速、可靠的分析方法全面表征 QYSLD 中的复杂化学成分。

近年来,具有高灵敏度、高分辨率的超高效液相色谱-四极杆-飞行时间质谱(UPLC-QTOF-MS)已广泛用于中药(复方)化学成分的分离和结构表征中[5,6]。质谱数据采集主要分为数据依赖性采集和数据非依赖性采集(DIA),其中 DIA 技术无需对样品中的化合物作预选择,而是直接采集经色谱柱分离的所有化合物的质谱信息[7,8]。作为典型的 DIA 技术之一,全信息串联质谱(MS^E)是一种能够实现"低碰撞能"和"高碰撞能"交替扫描以获得高精确的母离子及碎片离子信息,并能够依据母离子和碎片离子的色谱行为进行关联归属的数据采集方法[9]。由于中药(复方)众多成分之间含量差异大、结构类型和理化性质多样,传统人工解谱费时耗力;在存在基质背景信号干扰的情况下,质谱响应信号低或含量很少的成分在 MS^E 模式中的信号贡献较小,这些成分在分析鉴定过程中,常易产生漏判。因此,依托于天然产物后处理筛查(UNIFI)平台的靶向筛查方法便成了很好的补充。UNIFI 平台可极大减轻以往质谱数据分析的工作量,能对 MS^E 低能量下采集的数据自动进行离子流提取和分子式确定,并与自建库中成分作靶向筛查,然后根据 MS^E 高能量下的碎片信息推导化合物裂解途径[10,11],最后在预设的过滤条件下输出识别成分的结构信息,对中药及复方中的化学成分进行快速识别。

本研究在前人研究的基础上构建了 QYSLD 化学成分库,并将其导入 UNIFI 平台;然后利用UPLC-QTOF-MS 的 DIA 技术采集样品信息,再结合靶向筛查方法快速辨识 QYSLD 的化学物质基础,共辨识出 166 种化合物,并通过对照品对其中 16 种主要化学成分予以确认。该研究将为下一步研究 QYSLD 中发挥抑制 NSCLC 的药效成分奠定基础,同时也为其他中药(复方)成分分析提供方法参考。

1 实验部分

1.1 仪器、试剂与材料

Acquity I Class 型 UPLC-Xevo G2-XS 型 QTOF-MS,配有 UNIFI 平台(美国 Waters 公司),KQ-500DB 型数控超声波清洗器(昆山市超声仪器有限公司),RE-3000A 型旋转蒸发仪(上海亚荣生化仪器厂),Milli-Q 超纯水净化系统(美国 Millipore 公司)。

QYSLD(安徽中医药大学第一附属医院院内制剂)。对照品:黄芪甲苷(批号:MUST-19091308,纯度 99.8%)、黄芪皂苷 I(批号:MUST-20042906,纯度 99.1%)、黄芪皂苷 II(批号:MUST-20051008,纯度 99.8%)、毛蕊异黄酮(批号:MUST-19120901,纯度 99.8%)、毛蕊异黄酮-7-*O*-β-D-葡萄糖苷(批号:MUST-200920,纯度 99.8%)、澳洲茄碱(批号:MUST-20042004,纯度 99.6%)、澳洲茄边碱(批号:MUST-19102621,纯度 99.4%)、对香豆酸(批号:MUST-20050603,纯度 99.9%)购于成都曼思特生物科技有限公司,贝母辛(批号:B20082,纯度 98%)、亚油酸(批号:B21421,纯度 98%)、鸟嘌呤(批号:B20906,纯度 98%)、次黄嘌呤(批号:B20211,纯度 98%)购于上海源叶生物科技有限公司,芦丁(批号:100080-201409,纯度 99.9%)、精氨酸(批号:140685-201707,纯度 99.9%)、脯氨酸(批号:140677-201507,纯度 99.9%)、硬脂酸(批号:190032-201001,纯度 99.9%)购于中国食品药品检定研究院。甲酸(美国 Sigma-Aldrich 公司),甲醇和乙腈(德国 Merck 公司)。

1.2 混合对照品溶液的制备

精密称取 16 种对照品适量,用甲醇溶解定容,配制成对照品储备液。取各对照品储备液 10 μL,用甲

醇稀释定容,配制成各成分质量浓度约为 10 μg/mL 的混合对照品溶液,过 0.22 μm 的微孔滤膜。

1.3　样品前处理

QYSLD 样品溶液的制备:称取黄芪 30 g、玉竹 10 g、天龙 6 g、地龙 6 g、龙葵 20 g、白花蛇舌草 20 g、薏苡仁 20 g、泽漆 6 g、莪术 10 g、川贝母 6 g。加入 1 340 mL 水,浸泡 1 h,武火煮沸后文火煎 1.5 h,滤过;残渣加入 1 072 mL 水,武火煮沸,文火煎 40 min,滤过;合并两次煎煮液浓缩至 1 g/mL。取 2.5 mL 浓缩液与 7.5 mL 95% 乙醇混合,涡旋混匀,避光放置 12 h,取上清液减压离心挥干,残渣加甲醇振摇溶解,定容至 50 mL,摇匀,用 0.22 μm 微孔滤膜过滤后装入进样小瓶。

空白溶液的制备:除不加药物外,其他步骤与 QYSLD 样品溶液相同。

1.4　液相条件

色谱柱:Waters ACQUITY UPLC BEH C_{18} 柱(100 mm×2.1 mm, 1.7 μm),柱温:35 ℃,流动相:A 为 0.1%(v/v)甲酸水溶液、B 为乙腈,流速:0.2 mL/min。梯度洗脱程序:0~7 min, 3%B~15%B; 7~11 min, 15%B; 11~21 min, 15%B~25%B; 21~26 min, 25%B~35%B; 26~36 min, 35%B~55%B; 36~45 min, 55%B~73%B; 45~51 min, 73%B~85%B; 51~56 min, 85%B~95%B; 56~61 min, 95%B; 61~62 min, 95%B~3%B; 62~65 min, 3%B。进样量:2 μL。

1.5　质谱条件

离子源为电喷雾电离(ESI)源,分别采用正、负离子模式检测,实时校正液为亮氨酸脑啡肽。离子源温度 120 ℃;扫描范围为 m/z 50~1 200,毛细管电压 3.0 kV(正离子模式)、2.5 kV(负离子模式);锥孔电压 40 kV;脱溶剂温度 350 ℃;脱溶剂气体流速:600 L/h。MS^E 低碰撞能量为 6 eV, MS^E 高碰撞能量为 20~35 eV。

1.6　QYSLD 化学成分数据库的建立

通过检索 CNKI、Medline、PubMed、Chemicalbook 和 ChemSpider 等数据库,收集、整理 QYSLD 中十味中药所含化学成分的信息(包括 351 种化学成分名称,分子式及结构式),结构式使用 ChemDraw 绘制,文件格式保存为 mol 格式。

1.7　数据分析

将 MS^E 低碰撞能量(获得准分子离子或加合离子精确质量数)、高碰撞能量(设置碰撞能量区间,获得特征碎片离子、中性丢失等二级质谱信息)所采集到的 QYSLD 样品溶液、空白溶液质谱信息和 1.6 节下建立的 QYSLD 化学成分库均导入 UNIFI 平台。UNIFI 阈值设定:二维检测最小峰面积为 200; 三维检测的低、高碰撞能量下峰强度分别设置为 500 和 150;保留时间偏差为±0.1 min;精确质量数偏差为 $\pm5\times10^{-6}$;正离子模式下,选择 $[M+H]^+$ 和 $[M+Na]^+$ 为准分子离子或加合离子;负离子模式下,选择 $[M-H]^-$ 和 $[M+HCOO]^-$。在上述阈值的过滤条件下对待测成分的离子峰进行自动辨识、校正并输出结构信息。然后结合准分子离子、碎片离子的精确质量数及部分对照品对化合物结构进行确认(具体结果见表 1)。

2　结果与讨论

2.1　QYSLD 化学成分分析

利用 UPLC-QTOF-MS 中的 MS^E 模式采集空白溶液、QYSLD 样品溶液及混合对照品溶液在正、负离子模式下的质谱信息,得到各自的总离子流色谱图(见图 1)。根据 1.7 节下的数据分析方法对 QYSLD 中的化学成分进行定性分析,共识别出 166 种化合物。其中皂苷类 22 种,生物碱类 13 种,黄酮类 27 种,萜类 32 种,氨基酸类 20 种,苯丙素类 16 种,有机酸类 9 种,甾醇类 6 种,蒽醌类 6 种,其他类 15 种。通过对照品确认了其中 16 种成分,具体结果见表 1。

2.2　各类化合物的鉴定与分析

2.2.1　皂苷类

多数皂苷类成分至少含有 1 个糖链,在高碰撞能量下,其主要的裂解模式为糖基连续性丢失[33]。检测到的化合物中有 15 种甾体皂苷类成分和 7 种三萜皂苷类成分,主要分布于黄芪和玉竹中。以化合物 125(表 1 中序号,下同)为例进行解析,低碰撞能量下,检测到化合物 125(t_R = 29.11 min)加合离子 m/z 829.462 8 $[M+HCOO]^-$,高碰撞能量下接连损失葡萄糖基(Glc, 162 Da)和木糖基(Xyl, 132 Da),产生 m/z 621.402 7 $[M-H-Glc]^-$ 和 m/z 489.356 4 $[M-H-Glc-Xyl]^-$ 碎片离子,随后 C-17 支链断裂,再脱去 H_2O(18 Da)产生 m/z 329.233 9 $[M-H-Glc-Xyl-C_8H_{16}O_3]^-$ 碎片离子,m/z 329.233 9 的碎片离子继续丢失 CH_3(15 Da)、O(16 Da),产生 m/z 283.134 5 $[M-H-Glc-Xyl-C_8H_{16}O_3-C_2H_6O]^-$ 碎片离子。经 UNIFI 平台靶向筛查,结合对照品验

表 1　芪玉三龙汤化学成分的质谱数据及鉴定结果

Table 1　MS data and identification results of chemical constituents of Qi-Yu-San-Long decoction (QYSLD)

No.	t_R/min	Formula	Observed m/z	Calculated m/z	Adduct	Mass error/10^{-6}	Ions (m/z)	Compound	Sources	Ref.
1	1.08	$C_{10}H_{14}N_2O_5$	243.0974	243.0981	$[M+H]^+$	−2.8	110.0717, 109.0801	thymidine	c,d	[12,13]
2	1.10	$C_6H_{14}N_4O_2$	175.1192	175.1195	$[M+H]^+$	−1.7	158.0904, 131.1329, 114.1051	L-arginine[1)]	c,d	[12,14]
3	1.27	$C_6H_8O_4$	145.0500	145.0501	$[M+H]^+$	−0.6	130.0526, 86.0618	dimethyl fumarate	b	–
4	1.27	$C_5H_9NO_2$	116.0718	116.0712	$[M+H]^+$	5.1	98.0603, 82.0663	L-proline[1)]	d	[12]
5	1.70	$C_5H_4N_4O$	137.0457	137.0458	$[M+H]^+$	−0.7	119.0356, 110.0353	hypoxanthine[1)]	c,d	[12,14]
6	1.72	$C_5H_4N_4O_2$	153.0407	153.0407	$[M+H]^+$	0	136.0636, 110.0353	xanthine	c	[14]
7	1.74	$C_9H_{11}NO_3$	182.0810	182.0812	$[M+H]^+$	−1.0	165.0541, 164.0798, 138.0551	tyrosine	d	[12]
8	1.79	$C_9H_{12}N_2O_6$	245.0764	245.0774	$[M+H]^+$	−4.0	110.9773, 97.9692	uridine	c,d	[12,13]
9	1.81	$C_4H_4N_2O_2$	113.0345	113.0351	$[M+H]^+$	−5.3	97.0277, 84.0447	uracil	d	[12]
10	1.81	$C_{15}H_{22}O_4$	289.1428	289.1416	$[M+Na]^+$	4.1	267.0068, 251.0321, 249.0064	zedoalactone A	i	[15]
11	2.09	$C_{10}H_{13}N_5O_4$	268.1047	268.1040	$[M+H]^+$	2.6	152.0571, 107.0488	2′-deoxyguanosine	d	[12]
12	2.10	$C_5H_5N_5$	136.0620	136.0623	$[M+H]^+$	−2.2	119.0378, 91.0560	adenine	d	[12]
13	2.11	$C_{16}H_{22}O_{11}$	389.1090	389.1084	$[M-H]^-$	1.5	341.1052, 227.1015, 211.0550	deacetyl asperulosidic acid	f	[16]
14	2.12	$C_{18}H_{18}O_4$	299.1291	299.1283	$[M+H]^+$	2.6	269.0891, 251.0442	(6αR, 11αR)-3,9-dimethoxy-10-hydroxypterocarpan	a	[17]
15	2.20	$C_5H_5N_5O$	152.0572	152.0564	$[M+H]^+$	5.2	135.0302, 110.0353	guanine[1)]	d	[12]
16	2.20	$C_{10}H_{13}N_5O_5$	284.0989	284.0995	$[M+H]^+$	−2.1	269.0881, 150.0652, 135.0302	guanosine	f	[16]
17	2.24	$C_{10}H_{12}N_4O_5$	269.0886	269.0886	$[M+H]^+$	0	291.0698, 135.0302	inosine	d	[12]
18	2.26	$C_6H_6O_3$	127.0387	127.0395	$[M+H]^+$	−6.2	110.0347, 98.0964, 82.0340	5-hydroxymethylfurfural	b	[18]
19	2.42	$C_6H_{13}NO_2$	132.1016	132.1025	$[M+H]^+$	−6.8	115.0512, 88.0391	leucine	d	[12]
20	2.50	$C_7H_{10}N_2O_2$	155.0813	155.0821	$[M+H]^+$	−5.1	138.0545, 127.0416, 110.0589	cyclo(gly-pro)	c	[13]
21	3.24	$C_5H_6N_2O_2$	127.0508	127.0508	$[M+H]^+$	0	110.0696, 109.0673, 86.0941	thymine	c	[14]
22	3.50	$C_9H_{11}NO_2$	166.0862	166.0868	$[M+H]^+$	−3.6	120.0808, 89.0390	phenylalanine	d	[12]
23	4.31	$C_{17}H_{24}O_{11}$	449.1308	449.1295	$[M+HCOO]^-$	2.8	403.1223, 373.1110, 223.0584	deacetyl asperulosidic acid methyl ester	f	[16]
24	4.43	$C_{17}H_{24}O_{11}$	449.1308	449.1295	$[M+HCOO]^-$	2.8	403.1223, 373.1150, 341.1052	scandoside methyl ester	f	[16]
25	4.77	$C_9H_7NO_2$	162.0562	162.0555	$[M+H]^+$	4.3	144.0438, 146.0571, 130.0643	3,8-diol-quinoline	c	[13]
26	4.87	$C_{17}H_{24}O_{11}$	449.1308	449.1295	$[M+HCOO]^-$	2.8	403.1181, 241.0694, 191.0329	gardenoside	f	[19]
27	4.94	$C_9H_7NO_2$	162.0562	162.0555	$[M+H]^+$	4.3	144.0438, 128.0606	3,5-diol-quinoline	c	[13]
28	5.10	$C_{17}H_{24}O_{11}$	403.1223	403.1246	$[M-H]^-$	−5.7	241.0694, 223.0615, 165.0542	5-hydroxylarborescoside	f	[16]
29	5.18	$C_{17}H_{24}O_{11}$	403.1223	403.1246	$[M-H]^-$	−5.7	241.0694, 223.0584, 165.0516	epi-5-hydroxylarborescoside	f	[16]
30	5.78	$C_9H_6O_4$	179.0337	179.0344	$[M+H]^+$	−3.9	163.0384, 145.0581	esculetin	f	[20]
31	5.78	$C_{11}H_{10}O_5$	223.0601	223.0601	$[M+H]^+$	0	205.0314, 191.0338	isofraxidin	h	[21]
32	5.93	$C_{17}H_{16}O_6$	339.0833	339.0845	$[M+Na]^+$	−3.5	317.0545, 257.0316	pendulone	a	[17]
33	6.79	$C_{19}H_{28}O_{11}$	477.1596	477.1608	$[M+HCOO]^-$	−2.5	269.1004, 239.0550, 195.0660	diffusoside A	f	[20]
34	6.92	$C_{18}H_{20}O_5$	315.1252	315.1232	$[M-H]^-$	6.3	299.1238, 287.1312, 284.1270	7-*O*-methylisomucronulatol	a	[17]
35	7.02	$C_{18}H_{22}O_{11}$	413.1094	413.1084	$[M-H]^-$	2.4	397.1013, 267.0792, 251.0546	asperuloside	f	[22]

表 1　（续）
Table 1　(Continued)

No.	t_R/min	Formula	Observed m/z	Calculated m/z	Adduct	Mass error/10^{-6}	Ions (m/z)	Compound	Sources	Ref.
36	7.23	$C_9H_8O_4$	181.0493	181.0501	$[M+H]^+$	−4.4	165.0544, 163.0358, 137.0212	caffeic acid	a	[23]
37	7.61	$C_9H_{10}O_5$	199.0595	199.0606	$[M+H]^+$	−5.5	183.1154, 181.0499	ethyl gallate	h	[21]
38	8.70	$C_{18}H_{16}O_6$	327.0876	327.0869	$[M-H]^-$	2.1	311.0935, 309.0828, 283.0588	4,4′-dihydroxy-α-truxillic acid	f	[20]
39	8.71	$C_{27}H_{30}O_{17}$	625.1422	625.1410	$[M-H]^-$	1.9	445.0767, 300.0269	quercetin-3-*O*-(2-*O*-glucopyranosyl)-β-D-glucopyranside	f	[20]
40	8.83	$C_{27}H_{30}O_{17}$	625.1422	625.1410	$[M-H]^-$	1.9	445.0767, 300.0269	heliosin	h	[21]
41	9.20	$C_{16}H_{20}O_{10}$	371.0979	371.0978	$[M-H]^-$	0.2	353.0983, 309.0756, 225.0708	deacetyl asperuloside	f	[20]
42	9.37	$C_9H_8O_3$	163.0388	163.0395	$[M-H]^-$	−4.2	119.0492, 89.0385	*E*-*p*-coumaric acid[1)]	f	[16]
43	9.43	$C_9H_8O_3$	165.0549	165.0552	$[M+H]^+$	−1.8	147.0454, 119.0601	4-coumaricacid	f	[16]
44	9.46	$C_{15}H_{22}O_4$	267.1590	267.1596	$[M+H]^+$	−2.2	231.1383, 216.1706	curcolactone	i	[15]
45	9.56	$C_{11}H_{18}N_2O_2$	211.1437	211.1441	$[M+H]^+$	−1.8	196.0955, 167.0721	cyclo(pro-leu)	c	[13]
46	9.70	$C_{45}H_{73}NO_{18}$	960.4877	960.4804	$[M+HCOO]^-$	7.6	914.4787, 768.4251, 752.4225	(3β,12β,22α,25*R*)-3,12-dihydroxy-siprosol-5-en-27-hydroxymethyl-12-*O*-β-D-glucopyranosyl-(1-2)-[*O*-α-L-rhamnopyranosyl-(1-2)]-*O*-β-D-glucopyranoside	e	[24]
47	10.08	$C_{15}H_{10}O_6$	287.0550	287.0556	$[M+H]^+$	−2.0	271.1732, 255.0665	kaempferol	f,h	[20,21]
48	10.27	$C_{27}H_{30}O_{16}$	609.1451	609.1456	$[M-H]^-$	−0.8	462.1776, 300.0198	rutin[1)]	a,f,h	[21-23]
49	10.49	$C_{10}H_8O_4$	193.0502	193.0501	$[M+H]^+$	0.5	178.0284, 177.0578, 161.0264	scopoletin	f	[22]
50	10.69	$C_9H_8O_3$	209.0458	209.0450	$[M+HCOO]^-$	3.8	163.0383, 147.0433	*Z*-*p*-coumaric acid	f	[16]
51	11.07	$C_{11}H_{16}O_3$	197.1173	197.1178	$[M+H]^+$	−2.5	219.0999, 179.1085	loliolide	f	[19]
52	11.10	$C_{22}H_{22}O_{10}$	491.1189	491.1190	$[M+HCOO]^-$	−0.2	283.0588, 268.0365, 239.0308, 211.0371, 195.0415, 135.0053	calycosin 7-*O*-β-D-glucopyranoside[1)]	a	[17]
53	11.18	$C_{15}H_{20}O_6$	295.1178	295.1182	$[M-H]^-$	−1.3	280.0955, 265.0865, 247.0793	zedoarolide A	i	[15]
54	12.32	$C_{20}H_{24}O_7$	375.1460	375.1444	$[M-H]^-$	4.2	421.1497, 357.1360	7*R*,7′*R*,8*S*,8′*S*-(+)-neo-olivil	f	[19]
55	12.60	$C_{27}H_{45}NO_3$	432.3462	432.3472	$[M+H]^+$	−2.3	414.3373, 271.2059, 253.1429	verticine	j	[25]
56	13.50	$C_{18}H_{18}O_6$	329.1039	329.1025	$[M-H]^-$	4.2	221.0429, 187.0967, 97.0705	(−)-3,3′-bisdemethylpinoresinol	b	[26]
57	13.75	$C_{19}H_{18}O_7$	403.1016	403.1029	$[M+HCOO]^-$	−3.2	357.0986, 341.1022, 309.0751	4′-hydroxy-3,3′,5,7-tetramethoxyflavone	h	[21]
58	14.10	$C_{38}H_{40}O_{21}$	831.2022	831.1984	$[M-H]^-$	4.5	625.1422, 445.0767, 300.0269	quercetin-3-*O*-[2-*O*-(6-*O*-*E*-sinapoyl)-β-D-glueopyranosyl]-β-D-galactopyranoside	f	[22]
59	15.13	$C_{37}H_{38}O_{20}$	801.1902	801.1878	$[M-H]^-$	2.9	625.1422, 445.0767, 300.0269	quercetin-3-*O*-[2-*O*-(6-*O*-*E*-feruloyl)-β-D-glueopyranosyl]-β-D-glucopyranosyl	f	[22]
60	15.31	$C_{35}H_{34}O_{17}$	771.1786	771.1773	$[M+HCOO]^-$	1.6	707.0512, 445.0776, 427.0709	3-*O*-[5′-*O*-*p*-coumaroyl-β-D-apiofuranosyl(1→2)-β-D-glueopyranosyl] rhamnocitrin	a	[23]
61	15.62	$C_{26}H_{32}O_{11}$	519.1891	519.1866	$[M-H]^-$	4.8	501.1735, 489.1754, 357.0930	dehydrodiconiferyl alcohol-9′-*O*-D-glucopyranside	f	[27]
62	15.83	$C_{27}H_{41}NO_3$	428.3181	428.3165	$[M+H]^+$	3.7	410.3031, 393.2835, 114.0920	peimisine[1)]	j	[25]
63	16.21	$C_{27}H_{45}NO_5$	464.3351	464.3376	$[M+H]^+$	−5.3	448.3407, 446.3248, 430.3299	pingpeimine A	j	[25]
64	16.50	$C_9H_{16}O_4$	187.0967	187.0970	$[M-H]^-$	−1.6	171.0998, 169.0850, 143.1048	azelaic acid	a	[17]

表 1 （续）
Table 1 （Continued）

No.	t_R/min	Formula	Observed m/z	Calculated m/z	Adduct	Mass error/10^{-6}	Ions (m/z)	Compound	Sources	Ref.
65	17.29	$C_{26}H_{30}O_{13}$	549.1608	549.1608	$[M-H]^-$	0	595.1710, 369.0942, 225.1107, 206.9696, 178.9747, 134.8650	*E*-6-*O*-*p*-coumaroyl scandoside methyl ester	f	[22]
66	17.36	$C_9H_6O_2$	147.0439	147.0446	$[M+H]^+$	−4.7	129.0335, 121.0280, 103.0541	coumarin	e	[24]
67	17.36	$C_{11}H_8O_3$	189.0541	189.0546	$[M+H]^+$	−2.6	147.0454, 145.0278, 101.0388	3-acetylcoumarin	e	[24]
68	17.62	$C_{27}H_{43}NO_3$	430.3319	430.3321	$[M+H]^+$	−0.4	412.3240, 394.3108	peiminine	j	[25]
69	18.39	$C_{27}H_{32}O_{14}$	579.1706	579.1714	$[M-H]^-$	−1.3	549.1752, 417.1201, 399.1101	*E*-6-*O*-*p*-feruloyl scandoside methyl ester	f	[22]
70	18.50	$C_{30}H_{48}O_6$	549.3442	549.3427	$[M+HCOO]^-$	2.7	503.3367, 485.3242	cycloasgenin A	a	[23]
71	18.67	$C_{19}H_{20}O_6$	389.1229	389.1236	$[M+HCOO]^-$	−1.7	343.1170, 327.1250, 311.0924	(±)5,7-dihydroxy-3-(2-hydroxy-4-methoxybenzyl)-6,8-dimethyl chroman-4-one	b	[18]
72	18.81	$C_{26}H_{30}O_{13}$	549.1608	549.1608	$[M-H]^-$	0	387.1055, 369.0981	*Z*-6-*O*-*p*-coumaroyl scandoside methyl ester	f	[22]
73	19.01	$C_{51}H_{82}O_{26}$	1155.5100	1155.5071	$[M+HCOO]^-$	2.5	109.5069, 947.4662, 785.6918	26-*O*-*β*-D-glucopyranosyl-(25*S*)-furster-5-ene-3*β*, 22,26-triol-3-*O*-*β*-D-glucopyranosyl-(1→2)-[*β*-D-xylopyranose-(1→3)]-*β*-D-glucopyranosyl(1→4)*β*-D-galactopyranoside	b	[18]
74	19.78	$C_{52}H_{84}O_{26}$	1123.5152	1123.5173	$[M-H]^-$	−1.8	961.4740, 637.1315	26-*O*-*β*-D-glucopyranosyl-22-*O*-methyl-(25*S*)-furster-5-ene-3*β*, 26-diol-3-*O*-*β*-D-glucopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-glucopyranosyl(1→4)*β*-D-galactopyranoside	b	[18]
75	19.80	$C_{27}H_{43}NO_3$	430.3319	430.3321	$[M+H]^+$	−0.4	412.3240, 394.3148	imperialine	j	[25]
76	20.61	$C_{18}H_{18}O_6$	331.1178	331.1176	$[M+H]^+$	0.6	316.1674, 301.0702	6-methyl-4′,5,7-trihydroxy-8-methoxyhomoisoflavone	b	[18]
77	20.90	$C_{19}H_{24}O_4$	315.1598	315.1596	$[M-H]^-$	0.6	299.0919, 268.0399, 239.0308	16*α*-hydroxyandrost-4-ene-3,17,19-trione	a	–
78	21.51	$C_{15}H_{20}O_4$	265.1442	265.1440	$[M+H]^+$	0.7	287.1259, 229.1200, 199.1086	zedoalactone E	i	[15]
79	21.99	$C_{16}H_{12}O_5$	283.0588	283.0606	$[M-H]^-$	−6.3	239.0340, 211.0371, 135.0076	calycosin[1)]	a	[23]
80	22.00	$C_{16}H_{12}O_5$	283.0602	283.0606	$[M-H]^-$	−1.4	268.0365, 251.0331	2-hydroxy-1,3-dimethoxy anthraquinone	f	[20]
81	22.05	$C_{14}H_8O_3$	225.0542	225.0552	$[M+H]^+$	−4.4	209.0576, 197.0594, 169.0641	1-hydroxyanthraquinone	f	[22]
82	22.29	$C_{45}H_{73}NO_{16}$	884.5015	884.5008	$[M+H]^+$	0.7	738.4464, 722.4485, 576.3915, 414.3373, 271.2059, 253.1949	solasonine[1)]	e	[28]
83	22.31	$C_{27}H_{43}NO_2$	414.3363	414.3372	$[M+H]^+$	−2.1	399.3335, 396.3255, 381.3139	solasodine	e	[24]
84	22.42	$C_{21}H_{20}O_6$	369.1338	369.1333	$[M+H]^+$	1.3	351.1237, 337.1079	curcumin	i	[15]
85	22.97	$C_{17}H_{14}O_6$	313.0711	313.0712	$[M-H]^-$	−0.3	298.0459, 283.0209, 267.0283	2′,3′-dihydroxy-7,4′-dimethoxyisoflavone	a	[23]
86	23.03	$C_{17}H_{18}O_5$	301.1072	301.1076	$[M-H]^-$	−1.3	286.0828, 271.0597	(3*R*)-*δ*-methoxyvestitol	a	[17]
87	23.03	$C_{17}H_{14}O_6$	315.0865	315.0869	$[M+H]^+$	−1.2	299.0561, 283.0624, 268.0386	kumatakenin	a	[17]
88	23.10	$C_{45}H_{73}NO_{15}$	868.5061	868.5053	$[M+H]^+$	0.9	850.4937, 722.4472, 576.3894	*α*-solanine	e	[24]
89	23.14	$C_{45}H_{73}NO_{15}$	868.5035	868.5053	$[M+H]^+$	−2.0	722.4485, 576.3915, 414.3373, 396.3286, 271.2059, 253.1949	solamargine[1)]	e	[28]
90	23.20	$C_{15}H_{22}O_9$	391.1217	391.1240	$[M+HCOO]^-$	−5.8	327.1246, 165.0568, 121.0265	aucubin	f	–
91	23.66	$C_{20}H_{18}O_5$	339.1229	339.1232	$[M+H]^+$	−0.8	307.0974, 303.0525, 149.0245	demethoxycurcumin	i	[15]
92	23.81	$C_{15}H_{22}O_3$	251.1642	251.1647	$[M+H]^+$	−1.9	223.1090, 221.0691	(1*S*,10*S*),(4*S*,5*S*)-germacrone-1(10),4-diepoxide	i	[15]

表 1 （续）
Table 1 （Continued）

No.	t_R/min	Formula	Observed *m/z*	Calculated *m/z*	Adduct	Mass error/10^{-6}	Ions (*m/z*)	Compound	Sources	Ref.
93	23.86	$C_{15}H_{10}O_4$	255.0648	255.0657	$[M+H]^+$	−3.5	209.0589, 181.0524	2-hydroxymethy-1-hydroxy anthraquinone	f	[20]
94	24.83	$C_{21}H_{20}O_{13}$	525.0883	525.0880	$[M+HCOO]^-$	0.5	461.1809, 299.0538	myricetin 3-*β*-D-glucopyranoside	h	[21]
95	24.95	$C_{39}H_{63}NO_{11}$	722.4485	722.4479	$[M+H]^+$	0.8	704.4357, 576.3915, 558.3787	solasurine	e	[24]
96	25.00	$C_{47}H_{78}O_{19}$	991.5170	991.5114	$[M+HCOO]^-$	5.6	945.5103, 915.4443, 885.4560	astragaloside V	a	[23]
97	25.22	$C_{39}H_{62}O_{15}$	769.4011	769.4016	$[M-H]^-$	−0.6	815.4083, 623.3426, 461.2894	soladulcoside A	e	[29]
98	25.59	$C_{17}H_{16}O_6$	315.0859	315.0869	$[M-H]^-$	−3.1	299.0919, 191.0326	5,7-dihydroxy-3-(2,4-dihydroxy)-6-methyl chroman-4-one	b	[18]
99	25.62	$C_{27}H_{32}O_{13}$	609.1838	609.1825	$[M+HCOO]^-$	2.1	563.3176, 417.1536, 409.0696	*E*-6-*O*-*p*-methoxy cinnamoyl scandoside methyl ester	f	[20]
100	25.88	$C_{21}H_{26}O_6$	373.1664	373.1657	$[M-H]^-$	1.8	313.1432, 149.0574	hexahydrocurcumin	i	[15]
101	25.93	$C_9H_8O_2$	149.0596	149.0603	$[M+H]^+$	−4.6	133.0648, 131.0490, 105.0699	cinnamic acid	f	[20]
102	26.14	$C_{27}H_{42}O_3$	415.3208	415.3207	$[M+H]^+$	0.2	397.3150, 367.2215	diosgenin	b	[18]
103	26.19	$C_{16}H_{12}O_6$	299.0547	299.0556	$[M-H]^-$	−3.0	284.0309, 268.0953	santal	a	[30]
104	26.42	$C_{15}H_{20}O_2$	233.1534	233.1542	$[M+H]^+$	−3.4	217.1242, 203.1070, 185.0959	curcumafuranol	i	[15]
105	26.95	$C_{39}H_{62}O_{13}$	739.4246	739.4269	$[M+H]^+$	−3.1	577.3882, 431.0850, 415.3227	3*β*-hydroxy-(25*S*)-spirosteroid-1-*O*-*α*-L-rhamnopyranosyl (1→2)-*β*-D-galactopyranoside	b	[18]
106	27.06	$C_{15}H_{18}O_3$	247.1326	247.1334	$[M+H]^+$	−3.2	229.1224, 199.0732	zedoarol	i	[15]
107	27.20	$C_9H_{14}O_3$	169.0850	169.0865	$[M-H]^-$	−8.8	153.0840, 123.0792, 109.0624	ningpogenine	b	[18]
108	27.37	$C_{15}H_{20}O_4$	263.1291	263.1283	$[M-H]^-$	3.0	233.1134, 227.1293	zedoarofuran	i	[15]
109	27.38	$C_{15}H_{22}O$	219.1742	219.1749	$[M+H]^+$	−3.1	240.1457, 203.1431, 161.1323	bisacumol	i	[15]
110	27.70	$C_{15}H_{10}O_3$	237.0545	237.0552	$[M-H]^-$	−2.9	161.0591, 144.0316	3-hydroxyflavone	a	[23]
111	27.70	$C_{15}H_{10}O_4$	255.0652	255.0657	$[M+H]^+$	−1.9	240.0419, 239.0375, 237.0203	2,7-dihydroxy-3-methylanthraquinone	f	[20]
112	27.93	$C_{47}H_{78}O_{19}$	991.5170	991.5114	$[M+HCOO]^-$	5.6	945.5103, 915.4567, 783.4444	astragaloside VII	a	[23]
113	27.95	$C_{27}H_{42}O_4$	431.3147	431.3161	$[M+H]^+$	−3.2	413.3037, 398.3493, 383.1681	3*β*,14*α*-dihydroxy-(25*S*)- spirost-5-ene	b	[18]
114	27.96	$C_{18}H_{34}O_5$	329.2339	329.2333	$[M-H]^-$	1.8	311.2234, 293.2112, 285.2042	9,12,13-trihydroxy-10-octadecenoic acid	f	[20]
115	28.01	$C_{15}H_{10}O_8$	317.0297	317.0303	$[M-H]^-$	−1.8	299.1340, 174.0004	myricetin	b	[18]
116	28.03	$C_5H_{11}NO_2$	118.0860	118.0868	$[M+H]^+$	−6.7	100.1119, 85.0292, 83.0496	valine	d	[12]
117	28.16	$C_{16}H_{12}O_4$	267.0650	267.0657	$[M-H]^-$	−2.6	252.0413, 224.0436	2-hydroxy-3-methoxy-6-methylanthraquinone	f	[20]
118	28.20	$C_{16}H_{12}O_4$	269.0808	269.0814	$[M+H]^+$	−2.2	254.0563, 253.0495, 237.0544	formononetin	a	[23]
119	28.28	$C_{27}H_{43}NO$	398.3434	398.3423	$[M+H]^+$	2.7	368.2259, 353.2303, 351.1874	solanidine	e	[24]
120	28.59	$C_{15}H_{20}O_2$	233.1536	233.1542	$[M+H]^+$	−2.5	203.1433, 189.1301, 159.1134	(5*R*,6*R*,7*α*R)-5-isopropenyl-3,6-dimethyl-6-vinyl-5,6,7,7*α*-tetrahydro-4H-benzofuran-2-one	i	[15]
121	28.71	$C_{17}H_{14}O_5$	299.0909	299.0919	$[M+H]^+$	−3.3	284.0672, 283.0565	5-hydroxy-6,7-dimethoxylflavone	h	[21]
122	28.72	$C_{30}H_{48}O_6$	549.3710	549.3427	$[M+HCOO]^-$	5.1	503.3356, 485.3249	cycloorbigeinn B	a	[23]
123	28.81	$C_{17}H_{16}O_5$	301.1082	301.1076	$[M+H]^+$	1.9	286.0830, 283.0975, 268.0720	6-methyl-4′,5,7-trihydroxyhomoisoflavone	b	[18]
124	28.88	$C_{41}H_{66}O_{13}$	767.4564	767.4582	$[M+H]^+$	−2.3	737.3598, 605.4071, 473.3648	cycloorbicoside C	a	[23]

表 1 （续）
Table 1 （Continued）

No.	t_R/min	Formula	Observed m/z	Calculated m/z	Adduct	Mass error/10^{-6}	Ions (m/z)	Compound	Sources	Ref.
125	29.11	$C_{41}H_{68}O_{14}$	829.4628	829.4586	$[M+HCOO]^-$	5.0	783.4558, 621.4027, 489.3564, 329.2339, 283.1345	astragaloside IV[1)]	a	[23]
126	29.55	$C_{15}H_{16}O_4$	259.0970	259.0970	$[M-H]^-$	0	244.0747, 188.0130, 172.9893	linderane	i	[15]
127	29.73	$C_{23}H_{28}O_7$	415.1749	415.1757	$[M-H]^-$	−1.9	355.1545, 313.1432, 295.1345	magnolin	g	–
128	30.43	$C_{43}H_{70}O_{15}$	871.4705	871.4691	$[M+HCOO]^-$	1.6	765.4443, 735.3339, 603.3862	astragaloside II[1)]	a	[23]
129	31.41	$C_{43}H_{70}O_{15}$	871.4705	871.4691	$[M+HCOO]^-$	1.6	765.4443, 735.3339, 603.3862	isoastragaloside II	a	[23]
130	31.76	$C_{15}H_{10}O_3$	237.0545	237.0552	$[M-H]^-$	−2.9	223.0370, 209.0591, 195.0415	2-hydroxy-3-methylanthraquinone	f	[20]
131	31.80	$C_{16}H_{26}O_3$	311.1860	311.1858	$[M+HCOO]^-$	0.6	249.1874, 247.1694	methylzedoarondiol	i	[15]
132	31.80	$C_{15}H_{10}O_3$	239.0702	239.0708	$[M+H]^+$	−2.5	223.0335, 221.0593, 208.0510	2-hydroxy-6-methylanthraquinone	f	[20]
133	32.32	$C_{15}H_{22}O_2$	235.1692	235.1698	$[M+H]^+$	−2.5	257.1507, 203.1441, 159.1170	13-hydroxygermacrone	i	[15]
134	32.50	$C_{15}H_{30}O_2$	287.2214	287.2222	$[M+HCOO]^-$	−2.7	195.1013, 181.1191, 167.1058	pentadecanoic acid	d	[12]
135	33.26	$C_{30}H_{48}O_5$	533.3482	533.3478	$[M+HCOO]^-$	0.7	487.3421, 469.3290	cycloalpigenin A	a	[23]
136	33.44	$C_{45}H_{72}O_{16}$	913.4828	913.4797	$[M+HCOO]^-$	3.3	867.4761, 825.4601, 807.4461	astragaloside I[1)]	a	[23]
137	33.48	$C_{15}H_{18}O_3$	247.1336	247.1334	$[M+H]^+$	0.8	269.1151, 229.1223, 214.0984	curcolone	i	[15]
138	33.48	$C_{15}H_{16}O_2$	229.1239	229.1229	$[M+H]^+$	4.3	214.0984, 199.0752, 185.0595	curzeone	i	[15]
139	34.46	$C_{45}H_{72}O_{16}$	913.4827	913.4797	$[M+HCOO]^-$	3.2	867.4761, 807.4519	isoastragaloside I	a	[23]
140	34.50	$C_{27}H_{28}N_2O_4$	445.2115	445.2127	$[M+H]^+$	−2.6	467.1935, 385.1888, 293.2242	aurantiamide acetate	f	[20]
141	35.77	$C_{45}H_{72}O_{16}$	867.4761	867.4742	$[M-H]^-$	2.1	705.2188, 543.2976, 397.1870	dioscin	b	[18]
142	37.73	$C_{15}H_{22}O_2$	235.1679	235.1698	$[M+H]^+$	−8.0	209.1567, 179.0920	isocurcumenol	i	[15]
143	39.40	$C_{29}H_{43}N_3O_6$	528.3068	528.3079	$[M-H]^-$	−2.0	303.2325, 242.0775, 224.0681	[(2*R*,3*S*,4*S*,5*R*)-5-(4-amino-2-oxopyrimidin-1(2*H*)-yl)-3,4-dihydroxytetrahydrofuran-2-yl]methyl (5*Z*,8*Z*,11*Z*,14*Z*)-icosa-5,8,11,14-tetraenoate	d	–
144	39.47	$C_{28}H_{47}NO_3$	468.3441	468.3454	$[M+Na]^+$	−2.7	450.3255, 435.3379, 431.2525	puqienine C	j	[25]
145	39.64	$C_{15}H_{22}O_2$	235.1693	235.1698	$[M+H]^+$	−2.1	219.1369, 204.1387, 174.0919	neoprocurcumenol	i	[15]
146	40.02	$C_{29}H_{44}O_2$	469.3310	469.3318	$[M+HCOO]^-$	−1.7	423.3251, 407.2923, 393.2780	(22*E*,24*R*)-stigmasta-4,22-dien-3,6-dione	i	[31]
147	40.23	$C_{25}H_{43}N_3O_6$	480.3074	480.3092	$[M-H]^-$	−3.7	464.3180, 255.2317	*N*-[1-[(2*R*,3*R*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-2-oxopyrimidin-4-yl]hexadecanamide	d	–
148	41.07	$C_{25}H_{43}N_3O_6$	480.3074	480.3092	$[M-H]^-$	−3.7	255.2317, 224.0681	1-*β*-D-arabinofuranosylcytosine 5′-palmitoyl ester	d	–
149	42.09	$C_{26}H_{52}NO_7P$	566.3451	566.3458	$[M+HCOO]^-$	−1.2	281.2474, 242.0775	1-(11*Z*-octadecenoyl)-glycero-3-phosphocholine	g	–
150	44.67	$C_{27}H_{47}N_3O_6$	508.3406	508.3387	$[M-H]^-$	3.7	283.2619, 242.0775	1-(*β*-D-arabinofuranosyl)-4-(octadecanoylamino)pyrimidin-2(1*H*)-one	d	–
151	45.56	$C_{27}H_{47}N_3O_6$	508.3406	508.3387	$[M-H]^-$	3.7	283.2619, 224.0681	1-(3′-*O*-stearoyl-*β*-D-arabinofuranosyl)-cytosine	d	–
152	47.41	$C_{28}H_{44}O_3$	427.3204	427.3212	$[M-H]^-$	−1.8	383.3303, 355.2972	ergosterol peroxide	b	[26]
153	47.48	$C_{30}H_{48}O_3$	455.3528	455.3525	$[M-H]^-$	0.6	501.3584, 409.3054, 383.3313	ursolic acid	f	[20]
154	47.50	$C_{30}H_{48}O_3$	457.3651	457.3682	$[M+H]^+$	−6.7	439.3601, 411.3648, 301.1428	oleanic acid	f	[20]

表 1　(续)
Table 1　(Continued)

No.	t_R/min	Formula	Observed m/z	Calculated m/z	Adduct	Mass error/10^{-6}	Ions (m/z)	Compound	Sources	Ref.
155	47.53	$C_{15}H_{22}$	203.1795	203.1800	$[M+H]^+$	−2.4	188.1522, 173.1322, 159.1168	*α*-curcumene	c,i	[13,15]
156	47.55	$C_{15}H_{24}$	205.1951	205.1956	$[M+H]^+$	−2.4	190.1671, 175.1480, 161.1320	*δ*-elemene	c,i	[13,15]
157	48.20	$C_{28}H_{46}O_3$	429.3362	429.3369	$[M-H]^-$	−1.6	411.3229, 283.2619, 229.1971	cerevisterol	b	[18]
158	49.72	$C_{20}H_{32}O_2$	303.2325	303.2324	$[M-H]^-$	3.2	259.2421, 217.1901	arachidonic acid	d	[12]
159	50.30	$C_{18}H_{32}O_2$	279.2318	279.2324	$[M-H]^-$	−2.1	261.2204, 221.1519, 205.1931	linoleic acid[1)]	g	[32]
160	53.31	$C_{18}H_{34}O_2$	281.2474	281.2481	$[M-H]^-$	2.4	259.2421, 241.2187, 205.1953	oleic acid	g	[32]
161	56.02	$C_{16}H_{32}O_2$	255.2317	255.2324	$[M-H]^-$	−2.7	237.2246, 211.1679	palmitic acid	a,d	[12,23]
162	56.50	$C_{18}H_{36}O_2$	283.2619	283.2643	$[M-H]^-$	−8.4	255.2284, 253.2186, 239.2948	stearic acid[1)]	c,d	[12,13]
163	57.65	$C_{28}H_{46}O$	399.3609	399.3627	$[M+H]^+$	−4.5	383.3289, 381.3206, 369.3143	(22*E*,24*R*)-ergosta-7,22-dien-3*β*-ol	b	[26]
164	57.68	$C_{28}H_{46}O$	399.3610	399.3627	$[M+H]^+$	−4.2	339.2884, 309.2783	ergost-4-en-3-one	b	[26]
165	60.83	$C_{29}H_{48}O$	413.3775	413.3783	$[M+H]^+$	−1.9	397.3517, 359.3033, 309.2783	stigmasterol	b	[18]
166	62.72	$C_{37}H_{58}O_{14}$	725.3722	725.3748	$[M-H]^-$	−3.5	579.2893, 417.0092	3*β*,14*α*-dihydroxy-(25*S*)-spirost-5-ene-3-*O*-*β*-D-glucopyranosyl (1→4)-*O*-*β*-D-galactopyranoside	b	[18]

1) compound identified by standard; a: Astragali Radix; b: Polygonati Odorati Rhizoma; c: Scolopendra; d: Pheretima; e: *Solanum nigrum* L.; f: *Hedyotis diffusa* Willd.; g: Coicis Semen; h: *Euphorbia helioscopia* L.; i: Curcumae Rhizoma; j: Fritillariae Cirrhosae Bulbus; −: not reported.

证,确定该化合物为黄芪甲苷,裂解途径如图 2 所示。其他皂苷类成分以类似方式进行解析。

2.2.2　生物碱类

生物碱类化合物主要来源于 QYSLD 中龙葵和川贝母。龙葵中的生物碱大多数为糖苷生物碱。以图 1 中化合物 82(t_R = 22.29 min)为例,在正离子模式、低碰撞能量下,检测到准分子离子 m/z 884.501 5 $[M+H]^+$,在高碰撞能量下连续脱去鼠李糖基(Rha, 146 Da)、葡萄糖基(Glc, 162 Da)、半乳糖基(Gal, 162 Da),形成 m/z 为 738.446 4、576.391 5和 414.337 3 的碎片离子;通过 N 规则,可判断m/z 414.337 3 的苷元离子为含 1 个 N 原子的生物碱[34],推测该苷元为澳洲茄胺;此外,澳洲茄胺 E 环断裂产生m/z 271.205 9 和m/z 253.194 9 的特征碎片离子。由此推测化合物 82 是以澳洲茄胺为苷元,以 Rha、Glc、Gal 为糖基的澳洲茄碱,这一结果经对照品的 t_R 和 MS^E 数据予以验证,具体裂解信息见图 3。

其他糖苷生物碱的裂解方式多与澳洲茄碱类似,在正离子模式下产生准分子离子峰$[M+H]^+$或$[M+Na]^+$,糖链中的糖基连续性丢失形成苷元,苷元环在高碰撞能量下断裂产生特征碎片离子[34,35]。根据此裂解规律,结合 UNIFI 靶向筛查结果,推测化合物 88、89 和 95 分别为 *α*-茄碱、澳洲茄边碱和澳茄新碱。

川贝母生物碱在正离子模式下有准分子离子$[M+H]^+$,在高碰撞能量下常脱去一分子 H_2O(18 Da)产生碎片离子。以化合物 62(t_R = 15.83 min)为例,$[M+H]^+$为m/z 428.318 1 的准分子离子,脱 H_2O 产生m/z 410.303 1 的碎片离子,经对照品比对后,鉴定该化合物为贝母辛。另推测化合物 55、68 和 75 分别为贝母素甲、贝母素乙和西贝母碱[36]。

2.2.3　黄酮类

黄芪、玉竹、白花蛇舌草和泽漆均含有黄酮类化合物。以化合物 52 为例,在负离子模式下产生加合离子峰$[M+HCOO]^-$, m/z为 491.118 9,高碰撞能量下产生 m/z 283.058 8 $[M-H-Glc]^-$、m/z 268.036 5 $[M-H-Glc-CH_3]^-$、m/z 239.030 8 $[M-H-Glc-CH_3-CHO]^-$、m/z 211.037 1 $[M-H-Glc-CH_3-CHO-CO]^-$、m/z 195.041 5 $[M-H-Glc-CH_3-CHO-CO-O]^-$、$m/z$ 135.005 3 $[M-H-Glc-CH_3-CHO-CO-C_6H_4]^-$的碎片离子,推测其为毛蕊异黄

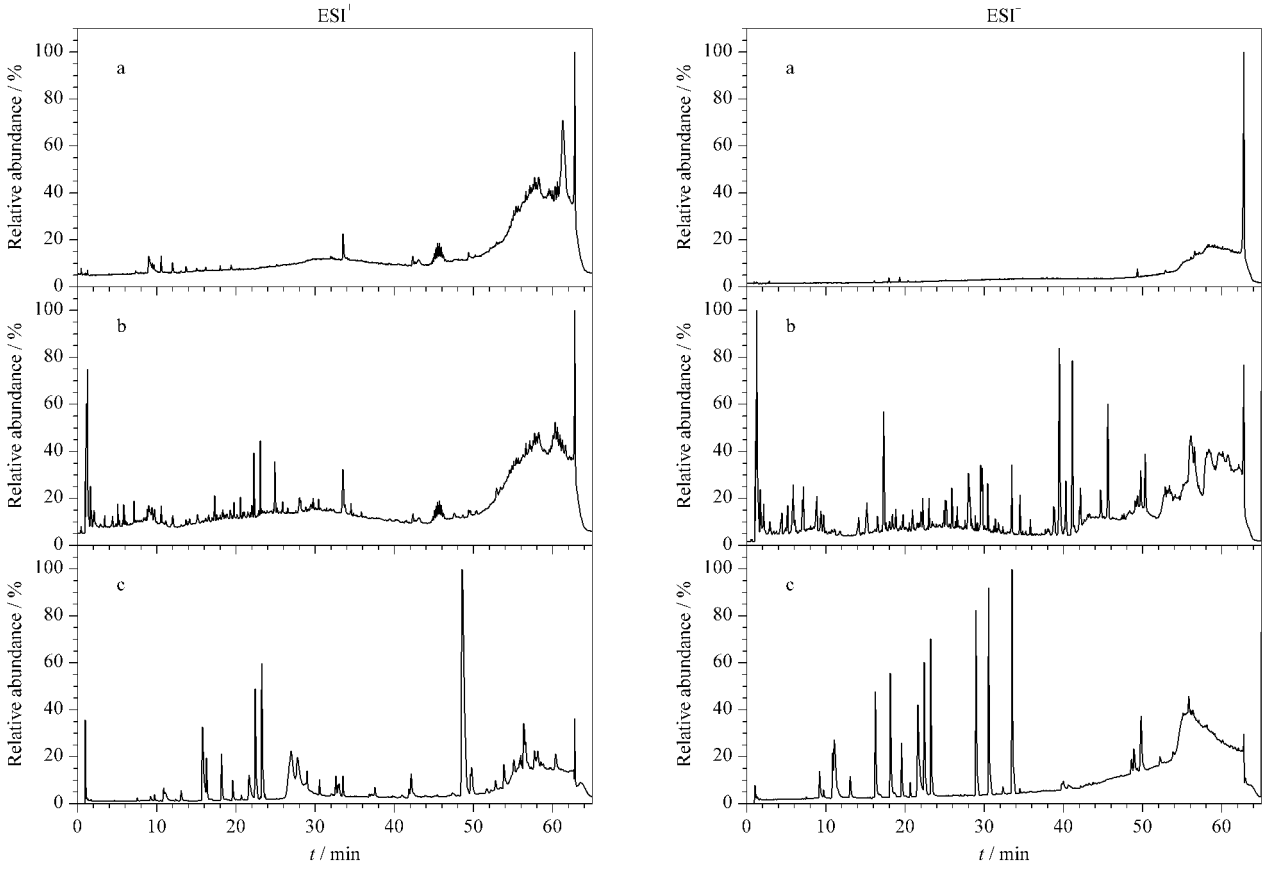

图 1 （a）空白溶液、（b）QYSLD 样品及（c）16 种混合对照品的总离子流色谱图

Fig. 1 TIC chromatograms of (a) blank solution, (b) QYSLD sample and (c) 16 mixed references

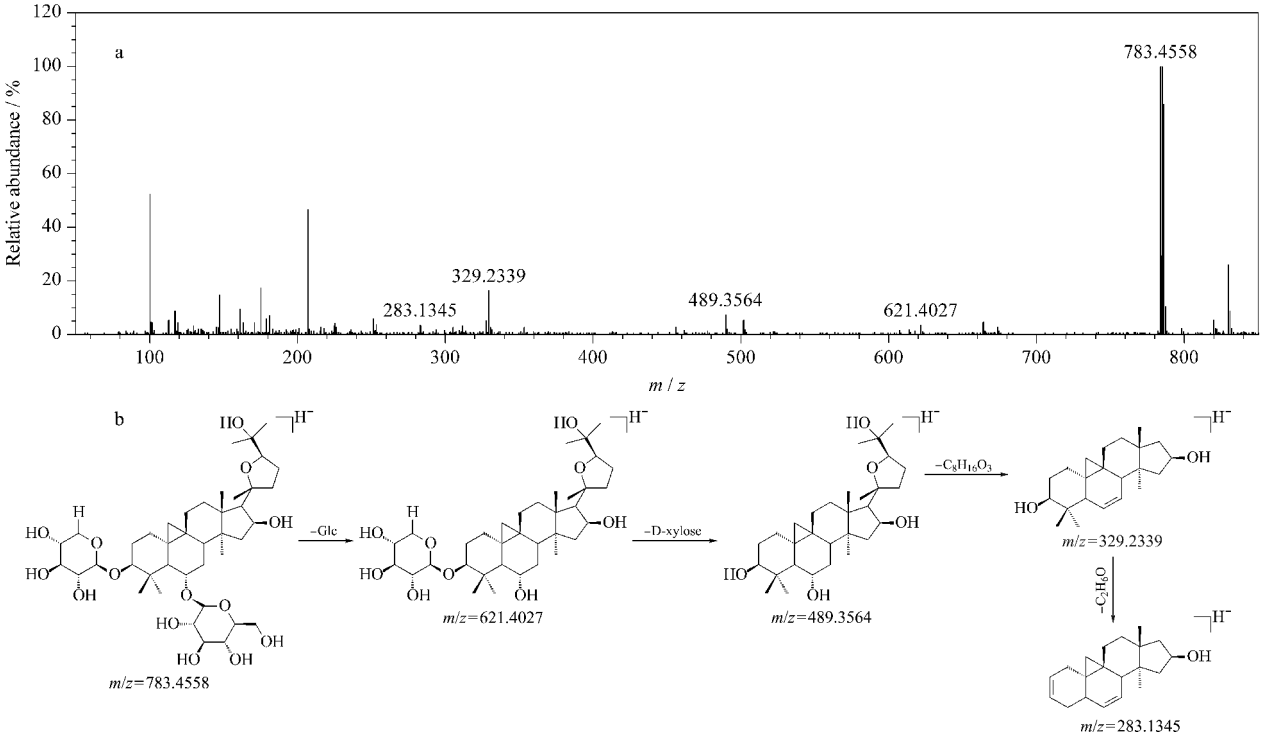

图 2 ESI⁻模式下黄芪甲苷的（a）质谱图及（b）裂解途径

Fig. 2 (a) Mass spectrum and (b) fragmentation pathway of astragaloside IV in ESI⁻ mode

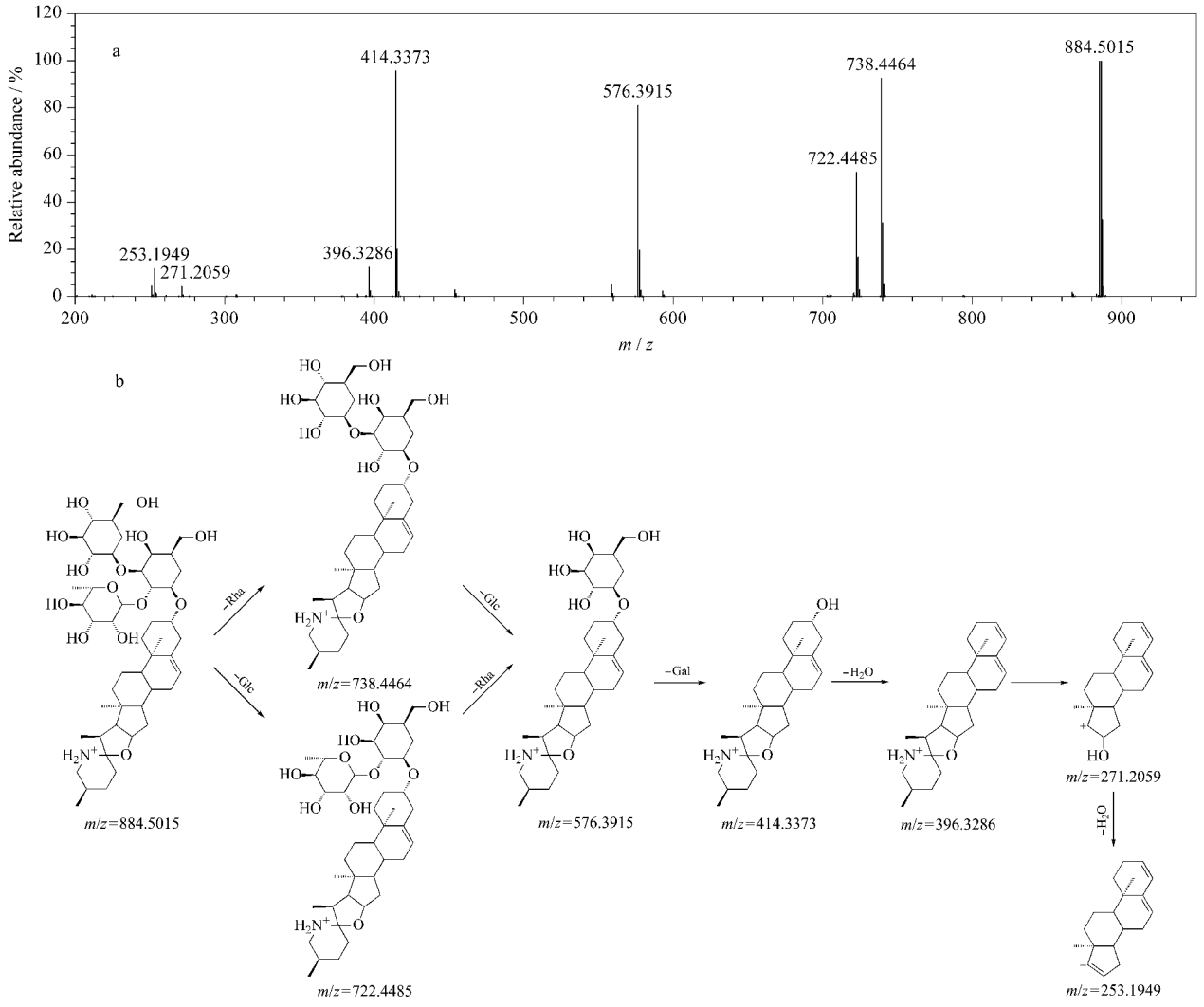

图 3　ESI⁺模式下澳洲茄碱的(a)质谱图及(b)裂解途径

Fig. 3　(a) Mass spectrum and (b) fragmentation pathway of solasonine in ESI⁺mode

酮-7-*O*-*β*-D-葡萄糖苷[17],具体裂解信息见图 4。由文献[37]可知,黄酮苷在负离子模式下信号较强,在高碰撞能量下易脱去糖基,其苷元进一步丢失 H_2O(18 Da)、CH_3(15 Da)产生特征碎片离子。依据上述裂解规律,结合 UNIFI 平台靶向筛查结果,推测化合物 48、79、118 分别为芦丁、毛蕊异黄酮、芒柄花素[23](具体质谱信息见表 1),其中化合物 48 和 79 经对照品予以确认。

2.2.4　萜类

萜类化合物共识别出 32 种,其中 17 种属于倍半萜类(来源于莪术),15 种属于环烯醚萜类(来源于白花蛇舌草)。环烯醚萜类 C-1 位常为羟基,且多与糖基结合成苷。环烯醚萜苷类成分裂解所需碰撞能量较小,易产生碎片离子[22],典型特征是吡喃环上易发生 Glc(162 Da)的中性丢失,苷元母核结构上常见 H_2O(18 Da)、CO(28 Da)和 CO_2(44 Da)的后续丢失。如化合物 65,在低、高碰撞能量下均可观察到准分子离子峰 $[M-H]^-$,*m/z* 为 549.160 8,以及特征碎片离子*m/z* 369.094 2 $[M-H-Glc-H_2O]^-$、*m/z* 225.110 7 $[M-H-Glc-H_2O-coumaroyl]^-$、*m/z* 206.969 6 $[M-H-Glc-H_2O-coumaroyl-H_2O]^-$、*m/z* 178.974 7 $[M-H-Glc-H_2O-coumaroyl-H_2O-CO]^-$、*m/z* 134.865 0 $[M-H-Glc-H_2O-coumaroyl-H_2O-CO-CO_2]^-$,推测该化合物为反式-6-*O*-对香豆酰鸡屎藤苷甲酯。化合物 137 属于倍半萜类,在低碰撞能量下观察到准分子离子是*m/z* 247.133 6 $[M+H]^+$,计算分子式为 $C_{15}H_{18}O_3$,在高碰撞能量下产生*m/z* 229.122 3 $[M+H-H_2O]^+$、*m/z* 214.098 4 $[M+H-H_2O-CH_3]^+$、

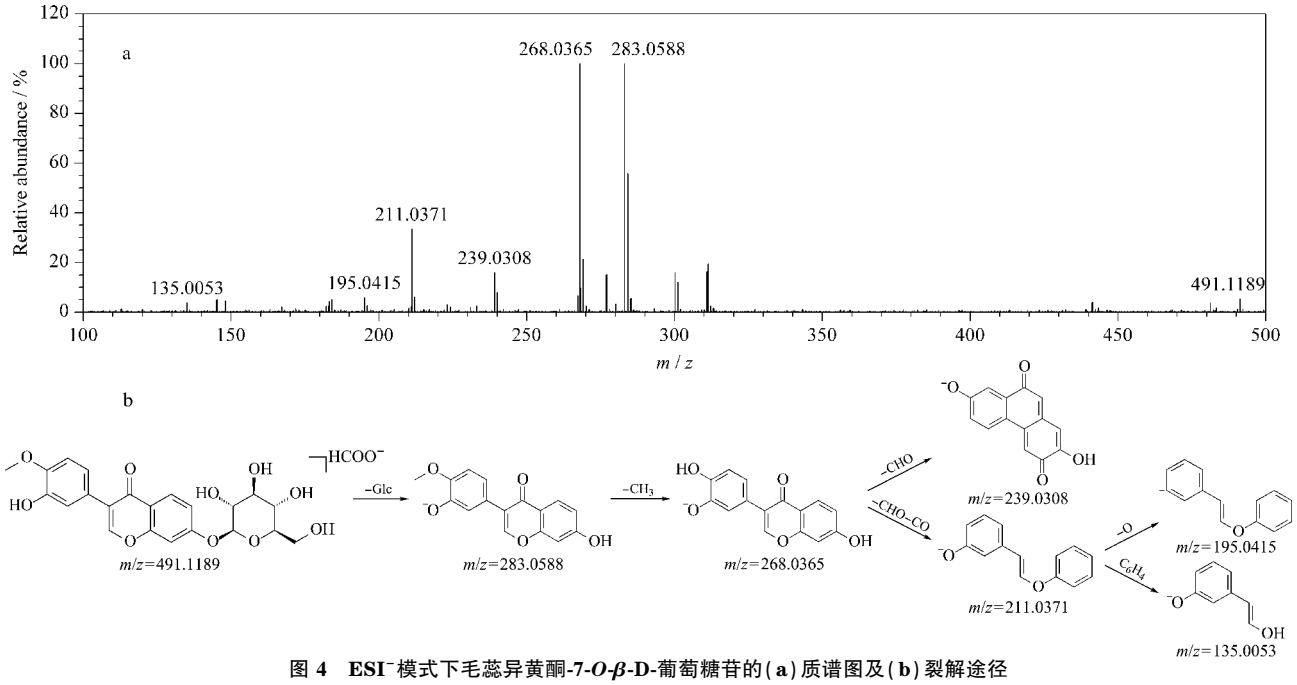

图 4 ESI⁻模式下毛蕊异黄酮-7-*O*-*β*-D-葡萄糖苷的(a)质谱图及(b)裂解途径

Fig. 4 (a) Mass spectrum and (b) fragmentation pathway of calycosin 7-*O*-*β*-D-glucopyranoside in ESI⁻mode

m/z 199.075 2 $[M+H-H_2O-2CH_3]^+$的碎片离子，推测该化合物为姜黄醇酮[38]。

2.2.5 苯丙素类

共鉴别出 16 种苯丙素类化合物。以化合物 42 为例，其在低碰撞能量下准分子离子为 *m/z* 163.038 8 $[M-H]^-$，在高碰撞能量下脱去 CO_2(44 Da)、C_2H_6(30 Da)产生 *m/z* 119.049 2 和 *m/z* 89.038 5 的碎片离子，经对照品确认该化合物为对香豆酸。依据此裂解规律推导化合物 36、43、66 和 101 可能是咖啡酸、4-羟基香豆素、香豆素和肉桂酸。

2.2.6 氨基酸类

此类化合物主要存在于天龙和地龙中。由文献[12]可知，在正离子模式下，氨基酸类化合物的裂解规律主要是脱去 NH_3(17 Da)和 CO_2(44 Da)。以化合物 2(t_R=1.10 min)为例，低碰撞能量下产生准分子离子峰$[M+H]^+$，*m/z* 为 175.119 2，通过 Masslynx 软件推导其化学式为 $C_6H_{14}N_4O_2$，高碰撞能量下接连丢失 NH_3 和 CO_2，形成特征碎片离子 *m/z* 158.090 4 $[M+H-NH_3]^+$和*m/z* 114.105 1 $[M+H-NH_3-CO_2]^+$，经对照品确认该化合物为精氨酸。根据裂解方式及对照品的质谱信息指认化合物 4、5 和 15 分别为脯氨酸、次黄嘌呤和鸟嘌呤。

2.2.7 有机酸类

有机酸类化合物裂解规律一般是脱去 H_2O(18 Da)、CO_2(44 Da)和 CH_3(15 Da)等小分子基团。化合物 162 准分子离子为*m/z* 283.261 9 $[M-H]^-$，高碰撞能量下产生*m/z* 253.218 6 $[M-H-2CH_3]^-$、*m/z* 239.294 8 $[M-H-CO_2]^-$的碎片离子，符合上述裂解规律，该化合物为硬脂酸并经对照品验证。依据上述有机酸裂解规律，结合 UNIFI 平台靶向筛查结果，化合物 159、160 和 161 依次推测为亚油酸、油酸和棕榈酸。

2.2.8 其他类

其他类主要是 QYSLD 中含量种类较少、响应值较小的化合物。通过 UNIFI 靶向筛查，并结合文献报道[13,15,18-21]，共推测出 15 种化合物(依次为化合物 18、25、27、37、38、54、107、120、140、143、147～151)，具体质谱信息见表 1。

3 结论

本研究运用 UPLC-QTOF-MS 的 DIA 技术(MS^E 模式)结合靶向筛查方法对 QYSLD 中复杂的化学成分进行快速、全面的分析，结果从 QYSLD 中共识别出 166 个化学成分，其中 16 个成分经过对照品验证。本研究所建立的方法能够快速、可靠的表征 QYSLD 中的化学成分，为该复方的药效物质及质量控制研究奠定了基础，同时也为其他中药(复方)所含化学成分的快速解析提供方法参考。但 UNIFI 平台靶向筛查方法也有不足，如无法区分准

分子离子和二级碎片离子均相似的同分异构体。因此,需通过 t_R、精确准分子离子质量、碎片离子质量、文献信息及对照品的色谱、质谱信息等对 UNIFI 平台靶向筛查的可疑化合物进行复核,以排除假阳性结果。

参考文献:

[1] Tong J B, Gao Y T, Fan C L, et al. Journal of New Chinese Medicine, 2018, 50(4): 146
童佳兵, 高雅婷, 范春雷, 等. 新中医, 2018, 50(4): 146

[2] Tong J B, Zhang X X, Han M X, et al. China Journal of Traditional Chinese Medicine and Pharmacy, 2018, 33(5): 2014
童佳兵, 张星星, 韩明向, 等. 中华中医药杂志, 2018, 33(5): 2014

[3] Zhang X X, Li Z G. China Journal of Traditional Chinese Medicine and Pharmacy, 2017, 32(12): 5358
张星星, 李泽庚. 中华中医药杂志, 2017, 32(12): 5358

[4] Cheng J C, Tong J B, Zhu J, et al. Chinese Journal of Experimental Traditional Medical Formulae, 2020, 26(9): 29
程建超, 童佳兵, 朱洁, 等. 中国实验方剂学杂志, 2020, 26(9): 29

[5] Wu H, Chen Y, Li Q L, et al. J Chromatogr B Analyt Technol Biomed Life Sci, 2018, 1102/1103: 23

[6] Wu H, Wang L N, Zhan X, et al. Phytomedicine, 2020, 76: 153259

[7] Li T, Xu X, Chang A Q, et al. Chinese Journal of Chromatography, 2020, 38(5): 554
李婷, 许霞, 常安琪, 等. 色谱, 2020, 38(5): 55

[8] Müller F, Kolbowski L, Bernhardt O M, et al. Mol Cell Proteomics, 2019, 18(4): 786

[9] Wu H, Chen Y, Li Z G, et al. R Soc Open Sci, 2018, 5(9): 181143

[10] Fu J, Wu H, Wu H, et al. J Pharm Biomed Anal, 2019, 170: 305

[11] López A, Dualde P, Yusà V, et al. Talanta, 2016, 160: 547

[12] Zhang Y, Dong W T, Huo J H, et al. Chinese Traditional and Herbal Drugs, 2017, 48(2): 252
张玉, 董文婷, 霍金海, 等. 中草药, 2017, 48(2): 252

[13] Ji L L, Lü S W, Yang Z X. Special Wild Economic Animal and Plant Research, 2020, 42(4): 75
汲丽丽, 吕邵娃, 杨志欣. 特产研究, 2020, 42(4): 75

[14] Zhang Q, Zhu Y H, Wen H M, et al. Chinese Journal of Experimental Traditional Medical Formulae, 2017, 23(14): 57
张琪, 朱叶华, 文红梅, 等. 中国实验方剂学杂志, 2017, 23(14): 57

[15] Xiao C K. Chinese Journal of Experimental Traditional Medical Formulae, 2012, 18(21): 339
肖长坤. 中国实验方剂学杂志, 2012, 18(21): 339

[16] Li S S, Jiang H X, Lin Z T, et al. J Chromatogr A, 2015, 1424: 37

[17] Wang Y M, Liu L, Ma Y K, et al. Molecules, 2019, 24(22): 4064

[18] Zhang J, Wang Y Z, Yang W Z, et al. China Journal of Chinese Materia Medica, 2019, 44(10): 1989
张娇, 王元忠, 杨维泽, 等. 中国中药杂志, 2019, 44(10): 1989

[19] Ma H, Li F L, Wang F, et al. Journal of Chinese Medicinal Materials, 2016, 39(1): 98
马河, 李方丽, 王芳, 等. 中药材, 2016, 39(1): 98

[20] Chen R, He J Y, Tong X L, et al. Molecules, 2016, 21(6): 710

[21] Wang L, Ni M, Yin D L, et al. Chinese Traditional Patent Medicine, 2014, 36(9): 1944
王莉, 倪明, 尹德龙, 等. 中成药, 2014, 36(9): 1944

[22] Liu E H, Zhou T, Li G B, et al. J Sep Sci, 2012, 35(2): 263

[23] Li X X, Qu L, Dong Y Z, et al. Molecules, 2014, 19(11): 18850

[24] Li L, Zheng Z F, Sun J Y, et al. Chinese Traditional and Herbal Drugs, 2016, 47(6): 1016
李玲, 郑重飞, 孙敬勇, 等. 中草药, 2016, 47(6): 1016

[25] Wang Y F, Gu Z Y, He C H. Northwest Pharmaceutical Journal, 2015, 30(4): 436
王云飞, 顾政一, 何承辉. 西北药学杂志 2015, 30(4): 436

[26] Yin W, Tao A L, Liu J Q, et al. Natural Product Research and Development, 2014, 26(7): 1034
尹伟, 陶阿丽, 刘金旗, 等. 天然产物研究与开发, 2014, 26(7): 1034

[27] Yao Z H, Zhou X Q, Wen L R, et al. Journal of Chinese Medicinal Materials, 2016, 39(4): 737
姚志红, 周先强, 温丽荣, 等. 中药材, 2016, 39(4): 737

[28] Wang J, Jin Y B, Wang T J, et al. Chinese Journal of Chromatography, 2015, 33(8): 809
王珏, 金一宝, 王铁杰, 等. 色谱, 2015, 33(8): 809

[29] Tuan Anh H L, Tran P T, Thao D T, et al. Biomed Res Int, 2018: 3120972

[30] Liu Y, Du J, Shen Y H. Chinese Journal of Experimental Traditional Medical Formulae, 2017, 23(18): 222
刘洋, 杜婧, 沈颜红. 中国实验方剂学杂志, 2017, 23(18): 222

[31] Chen J J, Tsai T H, Liao H R, et al. Molecules, 2016, 21(10): 1385

[32] Tian H X, Zheng X X, Hu D, et al. Guizhou Agricultural Sciences, 2017, 45(7): 82
田洪星, 郑晓霞, 胡蝶, 等. 贵州农业科学, 2017, 45(7): 82

[33] Zhao J, Qin Z X, Peng B, et al. Journal of Chinese Mass Spectrometry Society, 2017, 38(1): 97
赵静, 秦振娴, 彭冰, 等. 质谱学报, 2017, 38(1): 97

[34] Lelario F, De Maria S, Rivelli A R, et al. Toxins (Basel), 2019, 11(4): 230

[35] Yuan B, Byrnes D R, Dinssa F F, et al. J Food Sci, 2019, 84(2): 235

[36] Mohammat A, Yili A, Aisa H A. Molecules, 2017, 22(5): 719

[37] Chi Y M, Li Y, Zhang Y, et al. Chinese Journal of Chromatography, 2013, 31(9): 838
池玉梅, 李瑶, 张瑜, 等. 色谱, 2013, 31(9): 838

[38] Wang Y, Guo L P, Huang L Q, et al. China Pharmacy, 2013, 24(35): 3338
王颖, 郭兰萍, 黄璐琦, 等. 中国药房, 2013, 24(35): 3338