# UPLC-Q Exactive-Orbitrap MS法同时测定牛黄降压丸中15种胆酸类成分的含量

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**摘要**: 采用超高效液相色谱-四级杆/静电场轨道阱高分辨质谱法 (UPLC-Q Exactive-Orbitrap MS) 进行牛黄降压丸中15种成分 (牛磺胆酸、7-酮-3$\alpha$,12-$\alpha$-羟基胆烷酸、甘氨胆酸、3-氧代脱氧胆酸、牛磺鹅去氧胆酸、3$\alpha$-羟基-7-氧代-5$\beta$-胆烷酸、猪胆酸、牛磺脱氧胆酸钠、猪去氧胆酸、胆酸、甘氨鹅脱氧胆酸、甘氨脱氧胆酸、牛磺石胆酸钠、鹅去氧胆酸、去氧胆酸) 的含量测定。色谱条件: 采用Thermo Fisher Scientfic Bremen HYPERSIL GOLD (100 mm × 2.1 mm, 1.9 μm) 色谱柱, 以0.1%甲酸水−甲醇为流动相, 进行梯度洗脱; 质谱采用加热电喷雾离子源 (HESI) 负离子模式, 平行反应监测 (PRM) 扫描模式测定数据。结果表明, 15种胆酸类成分在各自的质量浓度范围内具有良好的线性关系 ($r \geqslant 0.999\ 0$), 加样回收率均在93.7%～105.2%之间 ($n = 9$)。采用建立好的方法测定15批样品的含量, 结果表明不同批次牛黄降压丸中胆酸类成分的含量差异较大。本研究为牛黄降压丸的全面质量控制提供究提供思路及参考。

**关键词**: UPLC-Q Exactive-Orbitrap MS; 牛黄降压丸; 胆酸; 含量测定

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# Simultaneous determination of fifteen components in Niuhuang Jiangya Pills by UPLC-Q Exactive-Orbitrap MS

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**Abstract**: An ultra performance liquid chromatography-quadrupole/electrostatic field orbitrap high resolution mass spectrometry (UPLC-Q Exactive-Orbitrap MS) method for the simultaneous determination of 15 compounds (taurocholic acid, 7-keto-3$\alpha$,12-$\alpha$-dihydroxycholanic acid, glycocholic acid, 3-oxo-7$\alpha$,12$\alpha$-hydroxy-5$\beta$-cholanoic acid, taurochenodeoxycholic acid, 3$\alpha$-hydroxy-7-oxo-5$\beta$-cholanic acid, hyocholic acid, sodium taurodeoxycholate, hyodeoxycholic acid, cholic acid, glycochenodeoxycholic acid, glycodeoxycholic acid, taurolithocholic acid sodium salt, chenodeoxycholic acid, deoxycholic acid) in Niuhuang Jiangya Pills was established. The separation was performed on a Thermo Fisher Scientfic Bremen HYPERSIL GOLD C18 column (100 mm × 2.1 mm, 1.9 μm). Methanol and water (containing 0.1% formic acid)were adopted as the mobile phase by gradient elution. MS detection was performed with multiple reaction monitoring mode. The results showed that fifteen compounds had a good linearity within their respective concentration ranges ($r > 0.999\ 0$). The average recovery rates were 93.7%−105.2% ($n = 9$). The established method was used to determine the content of 15 batches of samples, and the results showed that the content of cholic acid was quite different. The present study provides an important reference for the overall quality control of Niuhuang Jiangya Pills.

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**Key words**: UPLC-Q Exactive-Orbitrap MS; Niuhuang Jiangya Pill; cholic acid; quantitative analysis

牛黄降压丸是经典降压古方, 收载于《中华人民共和国药典》2020年版一部[1], 由羚羊角、珍珠、水牛角浓缩粉、人工牛黄、冰片、白芍等14味中药组成。牛黄降压丸具有清心化痰、平肝安神之效, 用于心肝火旺、痰热壅盛所致的头晕目眩、头痛失眠、烦躁不安; 高血压病见上述证候者。现代研究表明[2,3], 牛黄降压丸能够明显改善原发性高血压患者的临床症状, 降压作用显著, 不良反应小; 牛黄降压胶囊降压疗效确切, 降压平稳、长效, 对高血压引起的心血管并发症有一定的防治作用。《中华人民共和国药典》2020版一部以白芍中芍药苷的含量、黄芩中黄芩苷的含量、冰片中龙脑的含量为牛黄降压丸的定量测定指标, 牛黄为方中主药, 具有清心、解热、开窍、定惊、化痰等作用, 仅以TLC法进行了定性鉴别。人工牛黄中主要含有胆红素类、胆汁酸类、氨基酸类等成分, 胆汁酸类是人工牛黄的主要活性成分, 与牛黄的解热镇痛、抗炎、利胆保肝、降压和镇咳等药理作用有密切关系[4-6]。目前尚未见同时测定牛黄降压丸中多个胆酸类成分含量的文献报道。牛黄降压丸为中药复方制剂, 具有成分多的特点, 采用HPLC法进行检测分离难且分析时间长。而液质联用技术已广泛应用于中药领域的定性、靶向定量分析和质量控制[7-10]。其中超高效液相色谱 (UPLC) 具有快速分离化合物的能力, 高分辨质谱 (HRMS) 具有选择性好、灵敏度高、专属检测能力强等特点, 二者联合使用[11-13], 可产生用于辅助结构推测的二级质谱信息。在UPLC-Q Exactive-Orbitrap MS中, 四极杆对母离子具有高选择性, 轨道离子阱对精确质量数具有高分辨性, 样品的基质效应小, 可以实现复杂中药定性和定量分析。因此, 本研究采用超高效液相色谱−四级杆/静电场轨道阱高分辨质谱 (UPLC-Q Exactive-Orbitrap MS) 法同时对牛黄降压丸中15种胆酸类成分进行含量测定, 为牛黄降压丸及其相关制剂的质量控制提供分析基础。

## 材料与方法

**仪器** DIONEX UITIMATE 3000超高效液相色谱系统串联四级杆−静电轨道阱质谱仪 (美国赛默飞世尔公司); MILLI-Q Direct16超纯水制备仪 (美国Millipore公司); CPA225D十万分之一电子天平 (德国Sartorius公司); XM-400UHP小美静音超声仪 (昆山小美超声仪器有限公司)。

**样品与试剂** 甘氨胆酸 (批号: Y08A9E57765)、3-氧代脱氧胆酸 (批号: N07GY167164)、牛磺鹅去氧胆酸 (批号: Y09S8K43540)、3$\alpha$-羟基-7-氧代-5$\beta$-胆烷酸 (批号: N30GB169735)、猪去氧胆酸 (批号: S27M8I36899)、胆酸 (批号: T16J8Q28663)、甘氨鹅脱氧胆酸 (批号: Y29M9K57235)、甘氨脱氧胆酸 (批号: Y27M9E56969)、鹅去氧胆酸 (批号: Z01011LA14)、去氧胆酸 (批号: S05N6I5476)、脱氢胆酸 (批号: S30J9I64022)、7-酮-3$\alpha$,12-$\alpha$-二羟基胆烷酸 (批号N30GB169735), 经HPLC测定, 纯度均在98%以上; 牛磺胆酸 (批号: B15J11J118403, 纯度 ⩾ 95%)、牛磺石胆酸钠 (批号: Y06N9K74072, 纯度 ⩾ 97%), 以上对照品均购自上海源叶生物科技有限公司。牛磺脱氧胆酸钠 (批号: B26J9J53831, 经HPLC测定, 纯度 ⩾ 95%, 美国Sigma-Aldrich公司); 猪胆酸 (批号A1110816, 纯度 ⩾ 98%, 美国Cayman公司)。甲醇为LC-MS级 (赛默飞世尔公司), 甲酸为HPLC级 (上海阿拉丁生化科技股份有限公司), 其余试剂均为分析纯。收集了2个厂家共15批牛黄降压丸 (S1～S5: 批号分别为5450363、4900016、4900010、4900013、5450358, 天津中新药业集团股份有限公司达仁堂制药厂生产; S6～S15, 批号分别为: 20015693、20015696、19012368、21011836、18010021、20010473、20015692、20015701、21013349、20015565, 北京同仁堂科技发展股份有限公司制药厂生产)。

**色谱条件** 色谱柱: Thermo Fisher Scientfic Bremen HYPERSIL GOLD (100 mm × 2.1 mm, 1.9 μm), 以含0.1%甲酸水溶液 (A)−甲醇 (B) 为流动相, 线性梯度洗脱: 0～0.5 min, 20% B; 0.5～1.5 min, 20%～70% B; 1.5～7 min, 70%～61% B; 7～8 min, 61% B; 8～14 min, 61%～80% B; 14～15 min, 80%～85% B; 15～16 min, 85%～90% B; 16～17.5 min, 90%～20% B; 17.5～19 min, 20% B, 流速: 0.2 mL·min$^{-1}$, 柱温为40 ℃, 进样量2 μL。

**质谱条件** 采用加热电喷雾离子源 (HESI) 负离子模式: 喷雾电压: 3.0 kV (−), 毛细管温度: 325 ℃, 辅助气加热温度: 350 ℃, 鞘气流速: 45 arb, 辅助气流速: 10 arb, 吹扫气体流速: 1 arb, 质谱测定数据采用PRM扫描模式, 各待测成分及内标物的质谱优化参数见表1。

**内标溶液及混合对照品溶液的配制** 精密称取脱氢胆酸 (内标物) 适量, 加甲醇溶解, 配制为单一对照品母液, 并精密量取内标母液适量, 逐级稀释为20 ng·mL$^{-1}$的内标溶液。

精密称取牛磺胆酸、7-酮-3$\alpha$,12-$\alpha$-羟基胆烷酸、甘氨胆酸、3-氧代脱氧胆酸、牛磺鹅去氧胆酸、3$\alpha$-羟基-7-氧代-5$\beta$-胆烷酸、猪胆酸、牛磺脱氧胆酸钠、猪去氧胆

**Table 1** MS parameters for 15 analytes and 1 internal standards

| Compound | Molecular formula | $t_R$/min | Parent ion (*m/z*) | Daughter ion (*m/z*) | Collision energy/eV |
|---|---|---|---|---|---|
| IS (dehydrocholic acid) | $C_{24}H_{34}O_5$ | 5.29 | 401.23 | 249.14 | 36 |
| Taurocholic acid | $C_{30}H_{53}NO_7S$ | 7.72 | 514.28 | 79.56 | 124 |
| 7-Keto-3*α*,12-*α*-dihydroxycholanic acid | $C_{24}H_{38}O_5$ | 7.92 | 405.26 | 123.08 | 45 |
| Glycocholic acid | $C_{26}H_{43}NO_6$ | 9.85 | 464.30 | 74.02 | 41 |
| 3-Oxo-7*α*,12*α*-hydroxy-5*β*-Cholanoic acid | $C_{24}H_{38}O_5$ | 11.11 | 405.26 | 271.10 | 45 |
| Taurochenodeoxycholic acid | $C_{26}H_{45}NO_6S$ | 12.33 | 498.28 | 79.95 | 100 |
| 3*α*-Hydroxy-7-oxo-5*β*-cholanic acid | $C_{24}H_{38}O_4$ | 12.79 | 389.26 | 389.26 | 10 |
| Hyocholic acid | $C_{24}H_{40}O_5$ | 12.90 | 407.28 | 407.28 | 10 |
| Sodium taurodeoxycholate | $C_{26}H_{44}NNaO_6S$ | 13.44 | 498.29 | 79.95 | 100 |
| Hyodeoxycholic acid | $C_{24}H_{40}O_4$ | 14.06 | 391.28 | 391.28 | 10 |
| Cholic acid | $C_{24}H_{40}O_5$ | 14.38 | 407.28 | 407.28 | 10 |
| Glycochenodeoxycholic acid | $C_{26}H_{43}NO_5$ | 14.45 | 448.30 | 74.02 | 40 |
| Glycodeoxycholic acid | $C_{26}H_{45}NO_6$ | 15.14 | 448.30 | 74.02 | 40 |
| Taurolithocholic acid sodium salt | $C_{26}H_{44}NNaO_5S$ | 16.04 | 482.29 | 79.95 | 96 |
| Chenodeoxycholic acid | $C_{24}H_{40}O_4$ | 17.04 | 391.28 | 391.28 | 10 |
| Deoxycholic acid | $C_{24}H_{40}O_4$ | 17.28 | 391.28 | 391.28 | 10 |

酸、胆酸、甘氨鹅脱氧胆酸、甘氨脱氧胆酸、牛磺石胆酸钠、鹅去氧胆酸、去氧胆酸对照品适量, 加甲醇溶解, 配制为单一对照品母液。精密量取各对照品母液适量, 加甲醇定容, 摇匀, 配制成浓度分别为7 999.2、416.5、5 175.8、187.3、497.5、3 484.5、8 757.6、1 944.7、1 698.6、9 020.3、193.3、1 542.2、148.9、635.9、2 629.7 ng·mL$^{-1}$的混合对照品溶液。取混合对照品溶液适量, 按1∶1比例加入内标溶液后进样分析。

**供试品溶液的制备** 取牛黄降压丸0.10 g, 精密称定, 置于锥形瓶中, 精密加入甲醇10.00 mL, 称定重量, 超声 (100 W, 40 kHz) 处理30 min, 放冷至室温, 用甲醇补足减失的重量, 摇匀, 用0.22 μm微孔滤膜过滤, 取续滤液适量, 置于量瓶中, 加入甲醇稀释至刻度, 得供试品溶液。取供试品溶液适量, 按1∶1比例加入内标溶液后进样分析。

**专属性考察** 取空白溶液、对照品溶液、供试品溶液适量进样分析, 比较色谱图。

**线性回归方程及定量限考察** 准确移取混合对照品溶液, 用甲醇逐级稀释, 配制成不同浓度梯度的对照品溶液, 按上述色谱条件及质谱条件进行测定, 得到对照品峰面积 (Ai)、内标物峰面积 (As), 用对照品/内标物的峰面积之比 (Ai/As) 作为纵坐标, 对照品的质量浓度作为横坐标, 进行线性回归计算; 以信噪比3和10计算最低检测限 (LOD) 和最低定量限 (LOQ)。

**精密度试验** 取混合对照品母液适量, 分别配制低、中、高三个浓度梯度, 当天连续进样6次测定其日内精密度; 连续测定3天, 计算日间精密度。

**重复性试验** 取同一批牛黄降压丸 (S6), 按照供试品溶液制备方法平行制备6份供试品溶液, 按上述色谱条件和质谱条件进行测定, 记录样品与内标的峰面积比值, 计算15种成分含量及其RSD。

**稳定性试验** 取同一份供试品溶液 (S6), 室温下于0、2、4、6、8、12、24和48 h, 按色谱条件及质谱条件进行测定, 记录各成分峰面积并计算其RSD值。

**加样回收率试验** 精密称取同一批次 (S6) 已知含量的牛黄降压丸9份, 每3份为1组, 分别精密加入低、中、高3个浓度水平的混合对照品溶液 (约为目标成分含量50%、100%、150%), 按照供试品溶液方法制备, 按色谱条件和质谱条件进行测定, 记录各成分峰面积, 计算各成分的加样回收率和RSD。

**含量测定** 取15批牛黄降压丸样品, 按照供试品溶液方法制备, 按色谱条件和质谱条件进行样品中15种待测成分的同时测定, 记录峰面积并计算样品含量。

## 结果

### 1 方法学考察

**1.1 专属性考察** 空白溶液, 对照品溶液及供试品溶液中的各成分的PRM色谱图见图1。15种成分峰形良好, 该方法专属性良好。

**1.2 线性回归方程及定量限考察** 15种成分在各自检测范围内$r > 0.999$, 表明线性关系良好。回归方程、线性范围、检测限、定量限结果见表2。

**1.3 精密度试验** 低、中、高三个浓度梯度对照品溶液的日内精密度和日间精密度RSD均 ≤ 5% (表3), 表明仪器精密度较好。

**1.4 重复性试验** 6份供试品溶液中15种成分含量的RSD均小于5% (表3), 表明该方法重现性良好。

**1.5 稳定性试验** 供试品溶液 (S6) 在0、2、4、6、8、12、24和48 h测定的峰面积的RSD均小于5% (表3)。表明供试品溶液在48 h内稳定性良好。

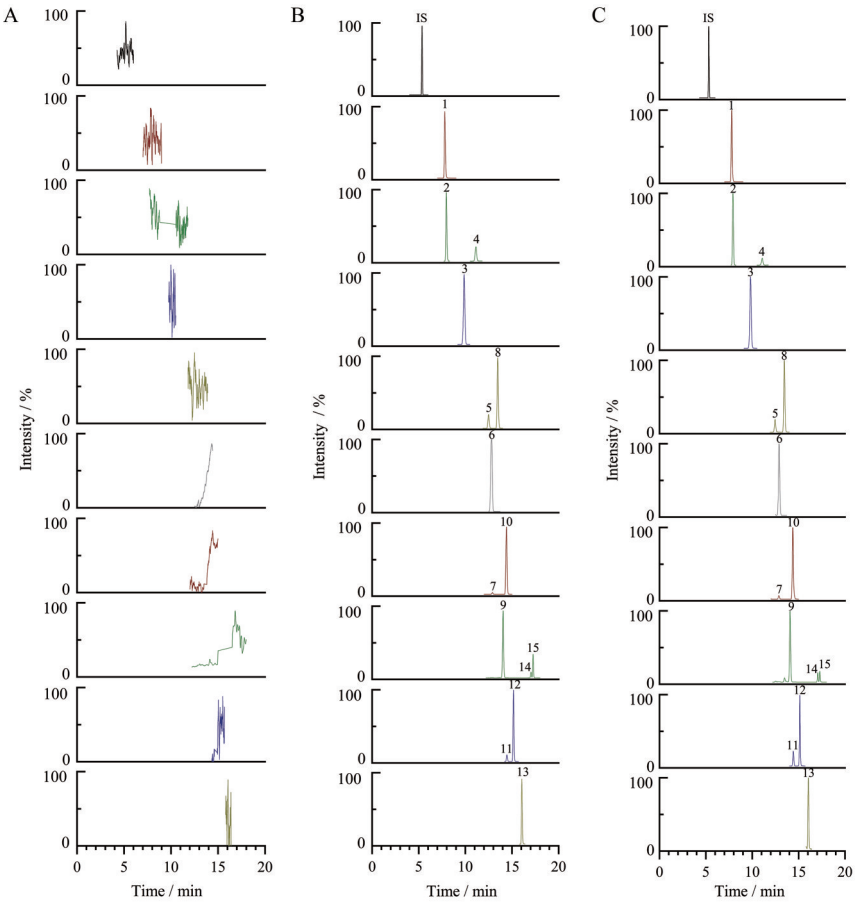


**Figure 1** PRM chromatograms of blank solution (A), reference substance (B) and samples (C). IS: Dehydrocholic acid; 1: Taurocholic acid; 2: 7-Keto-3$\alpha$,12-$\alpha$-dihydroxycholanic acid; 3: Glycocholic acid; 4: 3-Oxo-7$\alpha$,12$\alpha$-hydroxy-5$\beta$-cholanoic acid; 5: Taurochenodeoxycholic acid; 6: 3$\alpha$-Hydroxy-7-oxo-5$\beta$-cholanic acid; 7: Hyocholic Acid; 8: Sodium taurodeoxycholate; 9: Hyodeoxycholic acid; 10: Cholic acid; 11: Glycochenodeoxycholic acid; 12: Glycodeoxycholic acid; 13: Taurolithocholic acid sodium salt; 14: Chenodeoxycholic acid; 15: Deoxycholic acid

**Table 2** Regression equations, linear range, LOD and LOQ of 15 analytes

| Compound | Regression equation | Linear range/ng·mL$^{-1}$ | $r$ | LOD/ng·mL$^{-1}$ | LOQ/ng·mL$^{-1}$ |
|---|---|---|---|---|---|
| Taurocholic acid | $Y$ = 0.318 5$X$ + 14.957 | 25.46−3 997.32 | 0.999 9 | 0.17 | 0.51 |
| 7-Keto-3$\alpha$,12-$\alpha$-dihydroxycholanic acid | $Y$ = 0.105 9$X$ + 0.207 5 | 3.98−205.91 | 0.999 5 | 0.72 | 2.16 |
| Glycocholic acid | $Y$ = 0.557 5$X$ + 19.486 | 42.44−2 525 | 0.999 7 | 0.17 | 0.50 |
| 3-Oxo-7$\alpha$,12$\alpha$-hydroxy-5$\beta$-cholanoic acid | $Y$ = 0.164 4$X$ + 0.122 6 | 1.80−93.03 | 0.999 7 | 0.36 | 1.07 |
| Taurochenodeoxycholic acid | $Y$ = 0.498 6$X$ + 0.831 2 | 4.84−242.34 | 0.999 8 | 0.17 | 0.51 |
| 3$\alpha$-Hydroxy-7-oxo-5$\beta$-cholanic acid | $Y$ = 0.012 6$X$ + 0.391 7 | 47.48−1 716.39 | 0.999 5 | 2.41 | 7.23 |
| Hyocholic acid | $Y$ = 0.002$X$ + 0.127 2 | 136−4 241.81 | 0.999 5 | 3.00 | 9.01 |
| Sodium taurodeoxycholate | $Y$ = 0.593 2$X$ + 0.034 3 | 23.77−954.86 | 0.999 6 | 0.67 | 2.01 |
| Hyodeoxycholic acid | $Y$ = 3.873 1$X$ + 81.839 | 6.90−840.41 | 0.999 1 | 1.03 | 3.08 |
| Cholic acid | $Y$ = 0.089 9$X$ + 9.461 5 | 70.88−4 469.69 | 0.999 7 | 1.06 | 3.17 |
| Glycochenodeoxycholic acid | $Y$ = 0.842 6$X$ + 0.835 3 | 1.75−95.46 | 0.999 7 | 0.17 | 0.52 |
| Glycodeoxycholic acid | $Y$ = 0.741 5$X$ + 7.683 3 | 15.54−760.59 | 0.999 8 | 0.07 | 0.20 |
| Taurolithocholic acid sodium salt | $Y$ = 0.786 5$X$ + 0.334 5 | 1.42−72.88 | 0.9998 | 0.09 | 0.26 |
| Chenodeoxycholic acid | $Y$ = 1.084 6$X$ + 9.218 3 | 8.88−310.40 | 0.999 6 | 0.17 | 0.52 |
| Deoxycholic acid | $Y$ = 1.676 9$X$ + 22.648 | 12.97−1 305.79 | 0.999 4 | 0.07 | 0.21 |

**1.6 加样回收率试验** 15种化学成分的样品回收率为93.7%～105.2%, RSD均≤5.0% (表4), 表明该方法的准确度良好。

## 2 样品含量测定

取15批牛黄降压丸样品, 按照供试品溶液制备样品溶液, 按色谱条件和质谱条件对15种待测成分进行

**Table 3** The result of precision, stability and repeatability (%)

| Compound | RSD | | | | | | RSD ($n$ = 6) | |
|---|---|---|---|---|---|---|---|---|
| | Intraday precision ($n$ = 6) | | | Interday precision ($n$ = 3) | | | | |
| | $C_{low}$ | $C_{mid.}$ | $C_{high}$ | $C_{low}$ | $C_{mid.}$ | $C_{high}$ | Repeatability | Stability |
| Taurocholic acid | 2.9 | 2.1 | 3.0 | 2.4 | 1.3 | 3.2 | 1.0 | 4.2 |
| 7-Keto-3$\alpha$,12-$\alpha$-dihydroxycholanic acid | 4.3 | 1.3 | 3.0 | 1.2 | 0.3 | 2.6 | 2.1 | 4.6 |
| Glycocholic acid | 4.4 | 2.9 | 3.1 | 4.1 | 4.0 | 3.8 | 1.5 | 3.9 |
| 3-Oxo-7$\alpha$,12$\alpha$-hydroxy-5$\beta$-cholanoic acid | 3.1 | 2.6 | 2.1 | 1.1 | 0.2 | 1.9 | 2.8 | 4.8 |
| Taurochenodeoxycholic acid | 4.4 | 4.4 | 3.1 | 5.0 | 3.7 | 3.8 | 1.5 | 2.9 |
| 3$\alpha$-Hydroxy-7-oxo-5$\beta$-cholanic acid | 2.5 | 2.2 | 1.1 | 2.5 | 2.4 | 0.3 | 1.8 | 4.6 |
| Hyocholic acid | 3.5 | 3.1 | 1.4 | 3.3 | 2.0 | 2.0 | 2.8 | 3.6 |
| Sodium taurodeoxycholate | 3.0 | 1.5 | 4.1 | 3.6 | 1.2 | 3.3 | 4.1 | 3.1 |
| Hyodeoxycholic acid | 1.5 | 2.4 | 2.1 | 1.1 | 1.9 | 3.0 | 2.5 | 2.8 |
| Cholic acid | 2.6 | 2.6 | 3.4 | 3.0 | 1.5 | 3.8 | 2.4 | 2.9 |
| Glycochenodeoxycholic acid | 1.9 | 1.0 | 1.3 | 1.4 | 0.3 | 0.5 | 2.1 | 3.7 |
| Glycodeoxycholic acid | 2.1 | 1.2 | 2.8 | 2.9 | 1.1 | 3.1 | 2.3 | 4.0 |
| Taurolithocholic acid sodium salt | 4.9 | 2.4 | 2.9 | 3.1 | 1.3 | 1.8 | 1.2 | 1.4 |
| Chenodeoxycholic acid | 1.9 | 1.8 | 2.4 | 1.8 | 1.2 | 2.4 | 1.9 | 4.0 |
| Deoxycholic acid | 1.6 | 2.6 | 2.5 | 0.7 | 1.5 | 0.4 | 1.5 | 4.8 |

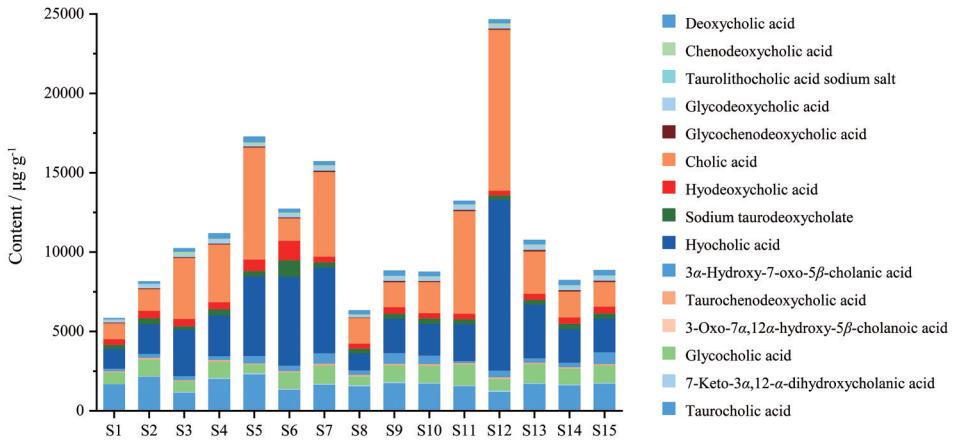


**Figure 2** Determination of bile acid content in different batches of Niuhuang Jiangya pills

了同时测定, 记录峰面积并计算样品含量 (表5, 图2)。

## 讨论

本研究曾采用高效液相色谱-蒸发光散射检测器法 (HPLC-ELSD) 测定牛黄降压丸中多种胆酸类成分的含量, 但因复方制剂成分比较复杂, 杂质峰对待测成分干扰较大, 且制剂中部分胆酸类化合物的含量较低, 无法同时进行15种胆酸类成分的检测。因此本研究参考文献[14-16]方法, 采用超高效液相色谱-四级杆/静电场轨道阱高分辨质谱法 (UPLC-Q Exactive-Orbitrap MS) 在短时间内对15种胆酸类化合物进行分离检测, 该方法灵敏度更高、专属性更强、结果更准确。

研究结果表明不同厂家、不同批次的牛黄降压丸中各成分的含量有所不同。各成分的含量范围分别为牛磺胆酸 (1 167.9～2 316.26 μg·g$^{-1}$)、7-酮-3$\alpha$,12$\alpha$-羟基胆烷酸 (20.00～91.59 μg·g$^{-1}$)、甘氨胆酸 (533.81～1 316.19 μg·g$^{-1}$)、3-氧代脱氧胆酸 (7.22～30.6 μg·g$^{-1}$)、牛磺鹅去氧胆酸 (59.68～95.52 μg·g$^{-1}$)、3$\alpha$-羟基-7-氧代-5$\beta$-胆烷酸 (118.16～747.8 μg·g$^{-1}$)、猪胆酸 (1 075.27～10 770.48 μg·g$^{-1}$)、牛磺脱氧胆酸钠 (195.1～1 041.01 μg·g$^{-1}$)、猪去氧胆酸 (302.74～1 230.95 μg·g$^{-1}$)、胆酸 (1002.86～10141.43 μg·g$^{-1}$)、甘氨鹅脱氧胆酸 (27.97～87.16 μg·g$^{-1}$)、甘氨脱氧胆酸 (121.13～287.72 μg·g$^{-1}$)、牛磺石胆酸钠 (2.3～5.64 μg·g$^{-1}$)、鹅去氧胆酸 (21.72～175.27 μg·g$^{-1}$)、去氧胆酸 (34.62～316.87 μg·g$^{-1}$), 各胆酸类成分的含量差异较大。目前, 国家已公开人工牛黄中贝斯素的配方为牛胆粉和牛磺酸组成, 但不同厂家在生产人工牛黄时所涉及到的生产工艺、原料配比可能存在不小的差异[17]。石岩等[18]曾对不同生产厂家生产的人工牛黄中的甘氨胆酸钠、胆酸、猪去氧胆酸、鹅去氧胆酸和去氧胆酸的含量进行比对后发现, 只有2家生产的人工牛黄差异较小, 认为可能是2家企业工艺和原料等较相似的原因, 而其他5家均有较大差异。此外, 2020版中国药典对于人工

**Table 4** Recoveries of targeted compounds in samples. $n = 9$, $\bar{x} \pm s$

| Compound | Original content/μg | Added content/μg | Detected content/μg | Recovery/% | RSD/% |
|---|---|---|---|---|---|
| Taurocholic acid | 67.58 | 33.79 | 100.4 ± 1.19 | 97.1 | 3.6 |
| | | 67.58 | 132.75 ± 1.905 | 96.4 | 2.9 |
| | | 101.37 | 164.2 ± 3.404 | 95.3 | 3.5 |
| 7-Keto-3*α*,12*α*-dihydroxycholanic acid | 3.15 | 1.58 | 4.7 ± 0.036 | 98.3 | 2.3 |
| | | 3.15 | 6.31 ± 0.107 | 99.9 | 3.4 |
| | | 4.73 | 7.78 ± 0.165 | 97.9 | 3.6 |
| Glycocholic acid | 51.50 | 25.75 | 76.58 ± 0.732 | 97.4 | 2.9 |
| | | 51.50 | 100.52 ± 0.573 | 95.2 | 1.2 |
| | | 77.24 | 131.05 ± 1.008 | 103.0 | 1.3 |
| 3-Oxo-7*α*,12*α*-hydroxy-5*β*-cholanoic acid | 0.77 | 0.38 | 1.17 ± 0.014 | 103.7 | 3.5 |
| | | 0.77 | 1.52 ± 0.033 | 102.1 | 4.3 |
| | | 1.15 | 1.9 ± 0.059 | 98.2 | 5.0 |
| Taurochenodeoxycholic acid | 3.73 | 1.87 | 5.58 ± 0.044 | 99.1 | 2.4 |
| | | 3.73 | 7.57 ± 0.121 | 102.9 | 3.2 |
| | | 5.60 | 9.14 ± 0.226 | 96.6 | 4.2 |
| 3*α*-Hydroxy-7-oxo-5*β*-cholanic acid | 16.96 | 8.48 | 25.29 ± 0.394 | 98.2 | 3.5 |
| | | 16.96 | 34.82 ± 0.289 | 105.2 | 4.7 |
| | | 25.45 | 42.09 ± 1.301 | 98.7 | 1.6 |
| Hyocholic acid | 69.89 | 34.94 | 103.77 ± 0.467 | 97.0 | 1.4 |
| | | 69.89 | 135.98 ± 1.091 | 94.6 | 1.7 |
| | | 104.83 | 177.61 ± 4.921 | 102.8 | 4.6 |
| Sodium taurodeoxycholate | 13.01 | 6.51 | 19.42 ± 0.205 | 98.5 | 3.2 |
| | | 13.01 | 26.16 ± 0.538 | 101.1 | 4.1 |
| | | 19.52 | 33.25 ± 0.791 | 103.7 | 3.9 |
| Hyodeoxycholic acid | 15.39 | 7.69 | 22.98 ± 0.063 | 98.6 | 0.8 |
| | | 15.39 | 30.36 ± 0.701 | 97.3 | 4.7 |
| | | 23.08 | 38.52 ± 0.292 | 100.2 | 1.3 |
| Cholic acid | 70.69 | 35.35 | 105.49 ± 0.511 | 98.4 | 1.5 |
| | | 70.69 | 139.28 ± 0.665 | 97.0 | 1.0 |
| | | 106.04 | 173.49 ± 2.843 | 96.9 | 2.8 |
| Glycochenodeoxycholic acid | 3.25 | 1.62 | 4.85 ± 0.004 | 98.7 | 0.3 |
| | | 3.25 | 6.46 ± 0.104 | 99.1 | 3.2 |
| | | 4.87 | 8.09 ± 0.205 | 99.5 | 4.2 |
| Glycodeoxycholic acid | 10.85 | 5.43 | 16.43 ± 0.243 | 102.9 | 4.3 |
| | | 10.85 | 21.62 ± 0.546 | 99.2 | 5.0 |
| | | 16.28 | 26.22 ± 0.375 | 94.4 | 2.4 |
| Taurolithocholic acid sodium salt | 0.18 | 0.09 | 0.26 ± 0.003 | 99.3 | 3.7 |
| | | 0.18 | 0.35 ± 0.007 | 99.9 | 4.3 |
| | | 0.26 | 0.42 ± 0.006 | 93.7 | 2.3 |
| Chenodeoxycholic acid | 4.03 | 2.01 | 5.94 ± 0.037 | 95.0 | 1.9 |
| | | 4.03 | 8.26 ± 0.103 | 105.1 | 2.4 |
| | | 6.04 | 10.17 ± 0.29 | 101.7 | 4.7 |
| Deoxycholic acid | 9.7 | 4.85 | 14.46 ± 0.192 | 98.1 | 4.0 |
| | | 9.7 | 19.61 ± 0.385 | 102.1 | 3.9 |
| | | 14.55 | 24.5 ± 0.379 | 101.7 | 2.6 |

牛黄中所采用的牛胆粉仅写明由牛胆汁加工而成, 并未对其来源及加工方法有明确规定。曾有研究表明经冷冻干燥所得的自制牛胆粉中甘氨胆酸、甘氨去氧胆酸、牛磺胆酸和牛磺去氧胆酸等胆汁酸成分的含量普遍高于市售牛胆粉[19]。因此推断可能是由于不同来源的牛胆粉等原料质量参差不齐, 或因人工牛黄加工、贮藏方式等差异导致其胆酸类成分差异较大, 从而影响了牛黄降压丸中各成分含量的稳定性。为了保证牛黄降压丸制剂的质量稳定性, 药品生产企业除了严格控制制剂生产工艺外, 还应确保人工牛黄原料药材的质量稳定, 并对牛黄降压丸中的胆酸类成分进行质量控制。

综上所述, 本研究建立UPLC-Q Exactive-Orbitrap MS法对牛黄降压丸方中君药牛黄的15个胆酸类成分同时进行质量控制, 该方法专属性强、快速灵敏、可用于牛黄降压丸多成分的同时定量分析, 为药企制剂生产、牛黄降压丸质量标准的改进提供科学依据。

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**Table 5** Results of content determination of various constituents in Niuhuang Jiangya Tablets. $n = 3$

| Compound | Content/μg·g$^{-1}$ | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 | S13 | S14 | S15 |
| Taurocholic acid | 1 701.46 | 2 167.88 | 1 167.90 | 2 031.69 | 2 316.26 | 1 351.64 | 1 662.81 | 1 561.94 | 1 766.15 | 1 736.18 | 1 577.02 | 1 222.16 | 1 721.89 | 1 627.81 | 1 733.08 |
| 7-Keto-3$\alpha$,12$\alpha$-dihydroxycholanic acid | 20.00 | 38.74 | 63.56 | 65.77 | 91.59 | 63.08 | 69.85 | 84.61 | 61.82 | 58.69 | 50.78 | 81.46 | 58.65 | 65.44 | 64.08 |
| Glycocholic acid | 728.42 | 1052.55 | 658.97 | 1 029.25 | 533.81 | 1 029.93 | 1 155.55 | 555.27 | 1 063.13 | 1 064.52 | 1 316.19 | 731.75 | 1 178.33 | 988.71 | 1 070.89 |
| 3-Oxo-7$\alpha$,12$\alpha$-hydroxy-5$\beta$-cholanoic acid | 7.22 | 21.79 | 10.73 | 7.41 | 6.27 | 15.38 | 15.79 | 30.60 | 8.35 | 14.70 | 7.73 | 27.92 | 11.72 | 8.34 | 8.36 |
| Taurochenodeoxycholic acid | 67.51 | 89.94 | 59.68 | 94.11 | 66.68 | 74.62 | 94.69 | 64.79 | 92.33 | 95.52 | 88.76 | 76.54 | 87.11 | 94.32 | 92.54 |
| 3$\alpha$-Hydroxy-7-oxo-5$\beta$-cholanic acid | 155.59 | 241.30 | 248.17 | 240.99 | 460.25 | 339.28 | 655.52 | 276.03 | 670.18 | 535.83 | 118.16 | 423.99 | 280.91 | 280.80 | 747.80 |
| Hyocholic acid | 1 229.51 | 1 861.00 | 2 943.29 | 2 558.02 | 5 027.51 | 5 590.93 | 5 390.96 | 1 075.27 | 2 158.80 | 2 001.19 | 2 314.97 | 10 770.48 | 3 372.67 | 2 119.30 | 2 132.02 |
| Sodium taurodeoxycholate | 273.64 | 380.35 | 195.10 | 402.01 | 330.52 | 1 041.01 | 336.65 | 295.55 | 322.63 | 338.49 | 317.43 | 264.45 | 313.86 | 324.39 | 313.78 |
| Hyodeoxycholic acid | 358.00 | 475.61 | 463.35 | 437.79 | 724.54 | 1 230.95 | 353.72 | 314.77 | 410.35 | 336.78 | 348.22 | 302.74 | 372.34 | 405.56 | 422.57 |
| Cholic acid | 1 002.86 | 1 362.44 | 3 847.96 | 3 646.58 | 7 064.64 | 1 413.81 | 5 335.54 | 1 630.35 | 1 576.33 | 1 956.47 | 6 465.91 | 10 141.43 | 2 680.69 | 1 634.87 | 1 558.71 |
| Glycochenodeoxycholic acid | 40.56 | 57.66 | 45.64 | 42.73 | 53.05 | 64.91 | 79.34 | 27.97 | 78.78 | 65.03 | 87.16 | 67.55 | 84.55 | 75.54 | 78.70 |
| Glycodeoxycholic acid | 177.64 | 248.91 | 163.03 | 254.78 | 131.77 | 217.04 | 256.07 | 121.13 | 266.03 | 242.13 | 287.72 | 190.89 | 280.26 | 250.97 | 264.24 |
| Taurolithocholic acid sodium salt | 2.30 | 3.25 | 2.55 | 4.58 | 3.77 | 3.51 | 5.53 | 3.32 | 5.40 | 5.19 | 5.37 | 4.58 | 5.09 | 5.64 | 5.56 |
| Chenodeoxycholic acid | 21.72 | 32.99 | 175.27 | 55.42 | 127.65 | 80.52 | 85.04 | 61.13 | 52.95 | 54.65 | 48.52 | 125.35 | 57.80 | 62.29 | 64.15 |
| Deoxycholic acid | 34.62 | 93.37 | 172.30 | 287.59 | 316.87 | 194.03 | 202.70 | 208.35 | 278.74 | 235.73 | 177.64 | 207.64 | 238.17 | 276.80 | 287.07 |
| Total/μg·g$^{-1}$ | 5 821.05 | 8 127.79 | 10 217.51 | 11 158.73 | 17 255.18 | 12 710.64 | 15 699.75 | 6 311.08 | 8 811.99 | 8 741.11 | 13 211.58 | 24 638.93 | 10 744.02 | 8 220.75 | 8 843.55 |

导文章选题、项目总体设计、审阅文章。

**利益冲突**: 不存在利益冲突。

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