

基于“性状-化学成分”关联性分析的三七质量评价新策略

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摘要: 传统的中药材商品规格主要依据性状等感官指标划分为不同的等级, 作为优质优价的依据, 与现行标准是否吻合、是否能够反映药材的内在质量, 尚缺乏系统评价。三七为常用、大宗药材, 目前市场上仅根据头数(支数/500 g)划分为8个等级, 但与《中华人民共和国药典》规定的以三七总皂苷(3种皂苷之和)含量为指标的标准不相关。本研究采用超高效液相色谱-四级杆-飞行时间串联质谱(UPLC-Q-TOF-MS/MS)并结合质谱分子网络对不同头数三七皂苷类成分进行快速鉴定, 共表征64种皂苷成分。通过正交偏最小二乘判别分析(OPLS-DA)筛选出与三七头数相关的17个皂苷差异化合物; 采用高效液相色谱法(HPLC)对不同头数三七中5种主要皂苷类成分R₁、Rb₁、Rg₁、Rd、Re进行含量测定, 相关性分析结果表明Rd、R₁是差异皂苷中VIP值最大的皂苷类成分, 与三七头数呈显著负相关($P < 0.05$)。基于36批三七样品测定结果, 采用Rd/三七总皂苷(TPNS)比值(>0.08)为指数, 可将三七划分为20~60头(优选)与80~200头(统货)两个等级。本研究基于“性状-化学分析”相结合的理念, 整合非靶向定性分析与定量测定方法, 为三七品质评价提供新的科学依据和策略。

关键词: 三七; 等级; 品质评价; 高分辨质谱法; 皂苷

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A new strategy for quality evaluation of *Panax notoginseng* based on the correlation between macroscopic characteristics and chemical profiling

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Abstract: The traditional commodity specifications of Chinese medicinal materials are mainly divided into different grades based on macroscopic characteristics. As the basis for high quality and good price, there is still a lack of systematic evaluation on whether they are consistent with the current standards and whether they can reflect the internal quality of medicinal material. *Panax notoginseng* is a commonly used, large consumption of Chinese medicinal material. At present, it is divided into 8 grades in the market based on "Tou" (the number of crude drug / 500 g), but it is not related to the standard of total saponins of *Panax notoginseng* (the sum of three saponins) in

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Chinese Pharmacopoeia. In this study, ultra-performance liquid chromatography-quadrupole-time of flight mass spectrometry (UPLC-Q-TOF-MS/MS) coupled with mass spectrometry molecular network were used for the rapid identification of saponins of *Panax notoginseng* with different "Tou" and a total of 64 saponins were identified. Seventeen saponins related to "Tou" were screened by orthogonal partial least squares discriminant analysis (OPLS-DA). The content of five saponins R_1 , R_{b_1} , R_{g_1} , R_d , and R_e in *Panax notoginseng* with different "Tou" was determined by high performance liquid chromatography (HPLC). The results of correlation analysis showed that R_d and R_1 with the largest VIP values among the differential saponins, which significantly negatively correlated with "Tou" ($P < 0.05$). Based on the determination results of 36 batches of samples, using R_d /Total *Panax notoginseng* saponins (TPNS) ratio (> 0.08) as the index, *Panax notoginseng* can be divided into two grades: 20–60 "Tou" (superior) and 80–200 "Tou" (qualified). Based on the concept of "macroscopic characteristics and chemical profiling", this study integrates the non-targeted analysis and quantitative determination methods to provide a new strategy for quality evaluation of *Panax notoginseng*.

Key words: *Panax notoginseng*; grade; quality evaluation; high-resolution mass spectrometry; saponin

传统的药材质量评价方法,以“辨状论质”为主,而其科学内涵研究较浅,市场中药材常“以大者为佳”,有研究表明^[1,2]化学成分与药材大小呈负相关或不相关,其性状及内在成分的相关性还需进一步探讨。三七为五加科人参属植物三七 *Panax notoginseng* (Burk.) F. H. Chen (PN) 的干燥根及根茎,常用于散瘀止血、消肿定痛^[3],广泛应用于心脑血管系统疾病、各种出血症的临床预防和治疗^[4]。三七中含有皂苷、氨基酸、黄酮、多糖等成分,其中皂苷为其主要药效成分,《中华人民共和国药典》(2020年版第一部)规定三七药材含三七皂苷 R_1 、人参皂苷 R_{g_1} 、 R_{b_1} 的总量应不少于 5%^[5]。三七为药食同源中药,在临床、制药、保健等方面均有广泛的使用,仅在《中华人民共和国药典》中就有 111 种含三七的中成药,因此市场对三七的需求量大。另一方面,近年来市场流通的三七商品规格等级混乱、质量参差不齐,严重影响了其临床药效。因此建立科学、合理的等级标准和品质评价方法是确保三七优质优价和临床疗效的关键。

三七传统经验鉴别以“身干、个大、体重、质坚实”为佳^[6]。国家医药管理局和卫生部制定的《七十六种商品规格标准》(1984)中将三七按头数(支/500 g)分为 11 个等级^[7],中华中医药学会发布的《中药材商品规格等级标准汇编》(2019)将三七药材简化为 8 个等级^[8]。Liu 等^[9]对三七商品规格等级标准进行了修订,但未发掘出外观性状与指标成分含量的相关性。因此,亟待建立一种外观性状和内在成分相关联的三七质量评价方法。

本研究基于三七不同头数等级,采用 UPLC-Q-TOF-MS/MS 非靶向定性分析、高效液相色谱定量测定,结合多元统计分析,挖掘外观性状(头数)与皂苷类成分含量的潜在关联性^[10],从而为三七质量评价、实现优质优价提供科学依据。

材料与方法

仪器 Triple TOF 5600⁺ Q-TOF-MS/MS 高分辨飞行时间质谱联用仪 (AB Sciex, Framingham, MA, 美国); SHIMADZU SIL-30A Lite 高效液相色谱仪 (Shimadzu Corporation, Kyoto, 日本); 安捷伦公司 Agilent 1290 超高效液相色谱仪 (四元泵 1290 Flexible Pump, 进样器 1290 Vial Sampler, 柱温箱 1290 MCT, 检测器 1290 DAD FS, 美国); Sartorius BSA 124S-CW 电子分析天平 (北京赛多利斯仪器有限公司); SCQ-5201 数控超声波清洗器 (上海声彦超声波仪器有限公司); YB-3000A 型多功能粉碎机 (永康市速锋工贸有限公司); SL-300 型高速多功能粉碎机 (浙江省永康市松青五金厂)。

对照品与试剂 三七皂苷 R_1 (批号: PRF15011610)、人参皂苷 R_{g_1} (批号: PRF10031209)、 R_e (批号: PRF10022728)、 R_{b_1} (批号: PRF10031805)、 R_d (批号: PRF8060521), 购于成都普瑞法科技开发有限公司, 纯度大于 98%。甲醇 (分析醇, 国药集团化学试剂有限公司); 乙腈和甲酸 (色谱纯, Fisher 公司, 美国); 超纯水由 Milli-Q 超纯水系统制备 (Millipore, 美国)。

样品 供研究的 36 批三七药材 (表 1), 主要产地为云南省, 生长年限为 3~4 年, 其性状与现行《中华人民共和国药典》规定一致, 均为正品三七, 具有较好的代表性。经上海标准化研究中心王峥涛教授收集鉴定, 标本保存在上海中药标准化研究中心。

对照品储备液制备 取三七皂苷 R_1 对照品、人参皂苷 R_{g_1} 对照品、人参皂苷 R_e 对照品、人参皂苷 R_{b_1} 对照品及人参皂苷 R_d 对照品适量, 精密称定, 加甲醇分别制成每 1 mL 含对照品 5 mg 的溶液。

供试品溶液制备 取三七药材粉末 0.5 g (过四号筛), 精密称定, 置于 50 mL 具塞锥形瓶中, 精密加入 70% 甲醇 20 mL, 称定重量, 超声处理 (功率 250 W, 频

Table 1 Sample information of *Panax notoginseng*. Tou: The number of crude drug/500 g

Sample number	Market grade	Origin	Sample number	Market grade	Origin
SQ-1	20 Tou	Kunming, Yunnan	SQ-19	80 Tou	Yunnan
SQ-2	20 Tou	Kunming, Yunnan	SQ-20	80 Tou	Yunnan
SQ-3	20 Tou	Kunming, Yunnan	SQ-21	80 Tou	Yunnan
SQ-4	20 Tou	Qujing, Yunnan	SQ-22	80 Tou	Yunnan
SQ-5	20 Tou	Yunnan	SQ-23	80 Tou	Yunnan
SQ-6	20 Tou	Yunnan	SQ-24	80 Tou	Yunnan
SQ-7	40 Tou	Chuxiong, Yunnan	SQ-25	120 Tou	Wenshan, Yunnan
SQ-8	40 Tou	Yuxi, Yunnan	SQ-26	120 Tou	Yuxi, Yunnan
SQ-9	40 Tou	Yunnan	SQ-27	120 Tou	Kunming, Yunnan
SQ-10	40 Tou	Kunming, Yunnan	SQ-28	120 Tou	Yunnan
SQ-11	40 Tou	Qujing, Yunnan	SQ-29	120 Tou	Yunnan
SQ-12	40 Tou	Yunnan	SQ-30	120 Tou	Yunnan
SQ-13	60 Tou	Wenshan, Yunnan	SQ-31	200 Tou	Honghe, Yunnan
SQ-14	60 Tou	Kunming, Yunnan	SQ-32	200 Tou	Yuxi, Yunnan
SQ-15	60 Tou	Chuxiong, Yunnan	SQ-33	200 Tou	Kunming, Yunnan
SQ-16	60 Tou	Yunnan	SQ-34	200 Tou	Yunnan
SQ-17	60 Tou	Yunnan	SQ-35	200 Tou	Yunnan
SQ-18	60 Tou	Yunnan	SQ-36	200 Tou	Kunming, Yunnan

率 100 kHz) 60 min, 放冷, 再次称定重量, 用 70% 甲醇补足减失的重量, 摇匀, 过 0.22 μm 微孔滤膜。

鉴定分析色谱条件 色谱柱为 Waters ACQUITY HSS T3 色谱柱 (100 mm \times 2.1 mm, 1.8 μm), Waters ACQUITY HSS T3 预柱 (5 mm \times 2.1 mm, 1.8 μm); 流动相为乙腈 (A) 和 0.1% 甲酸水溶液 (B), 梯度洗脱程序为 0~2 min, 15%~30% A; 2~8 min, 30%~35% A; 8~10 min, 35%~42% A; 10~15 min, 42%~44% A; 15~21 min, 44%~55% A。柱温为 45 $^{\circ}\text{C}$, 流速为 0.4 mL \cdot min $^{-1}$, 进样量为 3 μL ^[11]。

含量测定色谱条件 参照《中华人民共和国药典》第一部三七项下的高效液相色谱法测定。色谱柱为 Waters CORTECS C18 (150 mm \times 4.6 mm, 2.7 μm); 流动相为乙腈 (A)–纯水 (B), 梯度洗脱程序为 0~12 min, 19% A; 12~60 min, 19%~36% A。流速为 0.6 mL \cdot min $^{-1}$, 进样量为 10 μL , 检测波长为 203 nm。

质谱条件 电喷雾离子源 (ESI), 负离子检测模式, 采集使用 DIA 模式扫描, 质荷比采集范围 (m/z 100~1 250); 毛细管电压 4 500 V; 离子源温度 500 $^{\circ}\text{C}$; 气帘气 35 psi; 去簇电压 80 V; 碰撞能为 60 eV^[11]。

数据处理及统计分析 采用 PeakView 软件 (AB SCIEX, MA, USA) 将质谱数据的化合物信息包括保留时间, 一级和二级质谱信息与课题组自建数据库进行初步比对分析^[11]。进一步采用 Proteo Wizard 软件将质谱数据转化为 mzML 格式, 采用 Fill zilla 软件将数据传输至全球天然产物分子网络 (GNPS) 网站, 建立质谱网络对皂苷类成分进行快速的注释、鉴定和可视化, 采用 Cytoscape 3.9.1 软件对分子网络图谱进行美化。采用 Progenesis QI 软件 (Waters Corporation,

Milford, MA, 美国) 处理原始质谱数据, 对不同样品的谱图进行峰对齐、峰提取以及峰匹配, 将其离子强度归一化, 最终生成包含保留时间 (t_R)、质荷比 (m/z)、归一化离子强度的三维离子图。导入 SIMCA-P14.1 软件进行偏最小二乘法判别分析 (PLS-DA) 和正交偏最小二乘判别分析 (OPLS-DA), 获得不同等级三七样本之间的总体代谢差异和组内样本之间的变异性大小。基于有监督模式下的 OPLS-DA 分析, 依据变量重要性投影 (VIP) 值筛选出与三七等级相关的差异化合物信息。五种皂苷含量数据采用 Origin 2022 (OriginLab, 美国)、GraphPad Prism 9.0 (GraphPad Software, 美国) 软件用于统计作图, 组间比较用 t 检验分析, 若 $P < 0.05$ 则表示差异有统计学意义。

结果

1 三七中皂苷类成分的鉴定表征

人参皂苷根据结构的不同主要分为达玛烷型、齐墩果酸型和奥克梯隆型。其中达玛烷型人参皂苷占比最高, 根据羟基的不同取代位点又可以划分为人参二醇型皂苷 (PPD 型) 和人参三醇型皂苷 (PPT 型)。首先采用不同结构类型的人参皂苷对照品采集其特征诊断碎片离子, 推导其质谱裂解行为, 然后根据人参皂苷的质谱裂解规律进行三七样品中未知皂苷成分的鉴定。

如 PPD 型人参皂苷 Rb_1 , 其 C-3 位和 C-20 位各连接有两个葡萄糖, 分子量 m/z 1 108.602 9。在人参皂苷 Rb_1 对照品一级质谱图中可以观察到 (图 1-A1) m/z 1 107.592 4 ($[\text{M}-\text{H}]^-$) 的准分子离子峰、 m/z 1 153.599 8 ($[\text{M}+\text{HCOO}]^-$) 加合离子峰和 m/z 599.299 0 ($[\text{M}/2+\text{HCOO}-\text{H}]^{2-}$) 双电荷离子峰。从人参皂苷 Rb_1 的二级

质谱图(图 1-A2)可以看出, 人参皂苷 R_b 准分子离子丢失葡萄糖基 (-Glc, 162 Da) 产生 m/z 945.546 4 ($[M-Glc-H]^-$) 碎片离子, 丢失葡萄糖-葡萄糖二糖基 (-2Glc, 324 Da) 产生 m/z 783.494 6 ($[M-2Glc-H]^-$) 的碎片离子, 同时低质量端的 m/z 323.096 6 验证了侧链的两个葡萄糖残基共同断裂。碎片离子 m/z 783.494 6 ($[M-2Glc-H]^-$) 丢失侧链的葡萄糖基 (-Glc, 162 Da) 产生 m/z 621.435 9 ($[M-3Glc-H]^-$) 的碎片离子, 丢失葡萄糖-葡萄糖二糖基 (-2Glc, 324 Da) 产生 m/z 459.381 3 ($[M-4Glc-H]^-$) 碎片离子, 其中 m/z 459 碎片离子为 PPD 型皂苷的特征诊断离子。

三七皂苷 R_1 属于 PPT 型皂苷, 是三七区别于人参和西洋参的标志性皂苷。三七皂苷 R_1 对照品的一级质谱图中可以观察到(图 1-B1) m/z 931.527 6 ($[M-H]^-$) 的准分子离子峰和 m/z 977.533 5 ($[M+HCOO]^-$) 加合离子峰。二级质谱图(图 1-B2)中, 三七皂苷 R_1 准分子离子丢失木糖基 (-Xyl, 132 Da) 产生 m/z 799.487 1 ($[M-Xyl-H]^-$) 碎片离子, 丢失葡萄糖基 (-Glc, 162 Da) 产生

m/z 769.476 5 ($[M-Glc-H]^-$) 的碎片离子。碎片离子 m/z 769.476 5 ($[M-Glc-H]^-$) 丢失葡萄糖-木糖二糖基 (-Glc-Xyl, 294 Da) 产生 m/z 475.379 8 ($[M-2Glc-Xyl-H]^-$) 碎片离子, m/z 475 碎片离子该碎片离子 PPT 型皂苷的特征诊断离子。

为进一步表征三七中的皂苷成分, 本文建立了可视化质谱分子网络对皂苷类成分进行快速的注释, 其中可以发现三七中皂苷类成分特征碎片离子为 m/z 475 和 m/z 459。不同皂苷之间分子量 m/z 差值以 18、162、146 Da 居多, 对比各化合物结构结合质谱分子网络发现(图 2), 橙色箭头为其相连节点丢失一分子鼠李糖基 (-Rha, 146 Da), 紫色箭头为其相连节点丢失一分子葡萄糖基 (-Glc, 162 Da), 青色箭头为其相连节点丢失一分子丙二酰基 (-Mal, 86 Da), 蓝色箭头为其相连节点丢失一分子水 (-H₂O, 18 Da), 红色箭头为其相连节点丢失一分子甲氧基 (-CH₃O, 30 Da), 已鉴别节点呈蓝色, 未鉴别节点呈绿色, 见图 2。最后共从三七样品中鉴定表征了 64 个皂苷类成分, 如表 2, 总离子流色谱图见图 3。

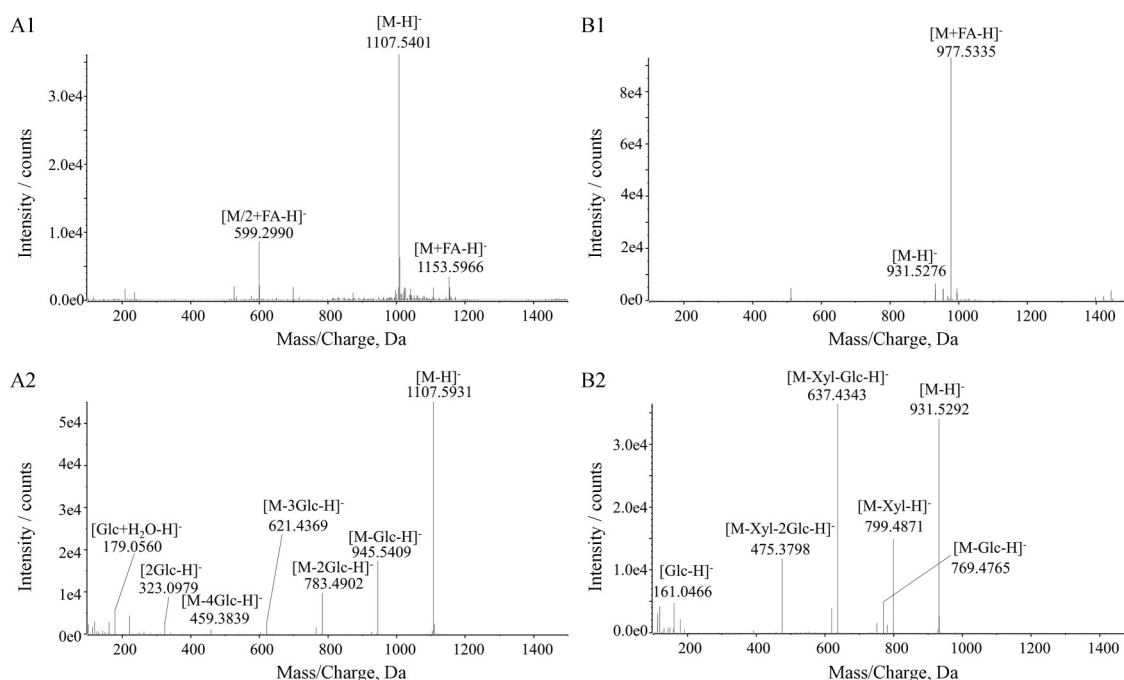


Figure 1 Mass spectra of representative saponins. A: Ginsenoside R_b ; B: Notoginsenoside R_1 . 1: MS¹; 2: MS². FA: Formic acid; -Glc: Glucosyl; -Xyl: Xylosyl

Table 2 Identification of saponins in *Panax notoginseng*

No.	t_R /min	Measured m/z	Theory m/z	Proposed formula	Mass error (ppm)	MS/MS fragment	Identification
1	3.56	1 007.545 7 [M+HCOO] ⁻	1 007.542 7 [M+HCOO] ⁻	C ₄₈ H ₈₂ O ₁₉	2.98	961.544 3; 799.490 0; 637.434 5; 475.379 2; 323.097 7; 221.068 6; 119.033 6	Notoginsenoside M
2	4.03	977.536 7 [M+HCOO] ⁻	977.532 2 [M+HCOO] ⁻	C ₄₇ H ₈₀ O ₁₈	4.60	931.529 1; 799.486 9; 637.433 5; 619.424 3; 475.380 0; 179.056 9; 161.046 3	Notoginsenoside R_1
3	4.19	979.543 9 [M+HCOO] ⁻	979.547 8 [M+HCOO] ⁻	C ₄₇ H ₈₂ O ₁₈	-3.98	933.544 1; 801.506 9; 638.452 2; 475.391 5	Notoginsenoside Ft ₂

Continued

No.	t_R /min	Measured m/z	Theory m/z	Proposed formula	Mass error (ppm)	MS/MS fragment	Identification
4	4.36	991.552 5 [M+HCOO] ⁻	991.547 8 [M+HCOO] ⁻	C ₄₈ H ₈₂ O ₁₈	4.74	945.546 5; 783.494 0; 637.434 6; 475.380 8; 391.286 9; 179.057 1; 101.024 8	Ginsenoside Re
5	4.38	845.493 3 [M+HCOO] ⁻	845.489 9 [M+HCOO] ⁻	C ₄₂ H ₇₂ O ₁₄	4.02	799.487 8; 637.435 1; 475.380 9; 391.285 6; 161.045 9; 101.024 8	Ginsenoside Rg ₁
6	5.38	1 005.533 0 [M+HCOO] ⁻	1 005.527 1 [M+HCOO] ⁻	C ₄₈ H ₈₀ O ₁₉	5.87	959.528 4; 797.476 2; 635.420 6; 473.367 8; 161.046 3	Notoginsenoside G
7	5.43	815.483 7 [M+HCOO] ⁻	815.479 3 [M+HCOO] ⁻	C ₄₁ H ₇₀ O ₁₃	5.40	769.477 1; 607.423 4; 475.380 8; 391.293 8; 179.056 9; 161.045 4	20(S)-Sanchirrhoside A ₄
8	5.64	1 007.542 9 [M+HCOO] ⁻	1 007.542 7 [M+HCOO] ⁻	C ₄₈ H ₈₂ O ₁₉	0.20	961.541 7; 799.485 9; 637.433 7; 553.215 8; 475.381 0; 161.047 1	Notoginsenoside N
9	5.73	1 167.583 5 [M+HCOO] ⁻	1 167.579 9 [M+HCOO] ⁻	C ₅₄ H ₉₀ O ₂₄	3.08	1 121.575 5; 959.528 0; 797.475 7; 635.426 5; 473.367 0; 323.114 3; 179.057 6	Notoginsenoside B
10	5.81	815.483 6 [M+HCOO] ⁻	815.479 3 [M+HCOO] ⁻	C ₄₁ H ₇₀ O ₁₃	5.27	769.479 8; 637.437 0; 619.423 1; 475.382 2; 161.045 8; 149.045 1	20(S)-Sanchirrhoside A ₃
11	5.87	887.499 7 [M+HCOO] ⁻	887.500 5 [M+HCOO] ⁻	C ₄₄ H ₇₄ O ₁₅	-0.90	841.490 6; 781.473 8; 637.434 3; 619.424 6; 475.376 2; 391.282 9	6-O-Acetyl-ginsenoside Rg ₁ isomer
12	6.00	815.481 6 [M+HCOO] ⁻	815.479 3 [M+HCOO] ⁻	C ₄₁ H ₇₀ O ₁₃	2.82	637.434 7; 553.334 2; 475.378 0; 161.047 6	Ginsenoside F ₃ /F ₅ isomer
13	6.09	887.506 2 [M+HCOO] ⁻	887.500 5 [M+HCOO] ⁻	C ₄₄ H ₇₄ O ₁₅	6.42	841.500 7; 781.478 7; 637.435 2; 619.425 7; 475.384 7; 161.046 3	6-O-Acetyl-ginsenoside Rg ₁
14	6.11	887.506 6 [M+HCOO] ⁻	887.500 5 [M+HCOO] ⁻	C ₄₄ H ₇₄ O ₁₅	6.87	841.497 9; 781.478 1; 637.435 2; 475.379 9; 391.299 1; 179.055 3; 161.045 9	6-O-Acetyl-ginsenoside Rg ₁ isomer
15	7.79	1 417.680 0 [M+HCOO] ⁻	1 417.685 2 [M+HCOO] ⁻	C ₆₄ H ₁₀₈ O ₃₁	-3.67	1 371.681 6; 1 239.648 5; 1 077.602 5; 619.820 6; 553.296 4; 459.382 6; 161.046 3	Notoginsenoside D
16	8.07	845.493 2 [M+HCOO] ⁻	845.489 9 [M+HCOO] ⁻	C ₄₂ H ₇₂ O ₁₄	3.90	799.490 6; 637.435 1; 475.382 5; 391.286 5; 161.044 5	20(S)-Ginsenoside Rf
17	8.48	1 005.529 9 [M+HCOO] ⁻	1 005.527 1 [M+HCOO] ⁻	C ₄₈ H ₈₀ O ₁₉	2.78	959.526 9; 797.477 5; 635.416 4; 473.363 2; 221.070 7; 113.024 9	Notoginsenoside G isomer
18	8.66	845.493 5 [M+HCOO] ⁻	845.489 9 [M+HCOO] ⁻	C ₄₂ H ₇₂ O ₁₄	4.26	799.490 6; 637.432 9; 475.379 6; 323.100 0; 221.067 1; 179.056 3	20-(R)-Ginsenoside Rf
19	8.96	815.481 4 [M+HCOO] ⁻	815.479 3 [M+HCOO] ⁻	C ₄₁ H ₇₀ O ₁₃	2.58	769.478 9; 637.435 6; 619.424 5; 475.380 9; 391.286 6; 161.045 9	Notoginsenoside R ₂
20	9.22	1 195.616 3 [M+HCOO] ⁻	1 195.611 2 [M+HCOO] ⁻	C ₅₆ H ₉₄ O ₂₄	4.27	1 149.613 6; 1 107.602 6; 1 089.592 4; 945.550 6; 783.496 7; 459.381 6; 179.056 8	6-O-Acetyl-ginsenoside Rb ₁
21	9.56	1 417.685 3 [M+HCOO] ⁻	1 417.685 2 [M+HCOO] ⁻	C ₆₄ H ₁₀₈ O ₃₁	0.07	1 239.643 4; 1 107.601 4; 945.548 9; 783.493 2; 637.432 5; 475.379 4	Notoginsenoside T
22	9.64	1 285.648 0 [M+HCOO] ⁻	1 285.642 9 [M+HCOO] ⁻	C ₅₉ H ₁₀₀ O ₂₇	3.97	783.495 0; 637.440 3; 459.345 0; 391.285 9; 161.046 3; 101.024 9	Notoginsenoside Fa
23	9.75	1 195.608 3 [M+HCOO] ⁻	1 195.611 2 [M+HCOO] ⁻	C ₅₆ H ₉₄ O ₂₄	-2.43	1 149.601 2; 1 107.592 4; 1 089.582 6; 945.541 8; 459.381 7; 221.068 0; 179.056 8	6-O-Acetyl-ginsenoside Rb ₁ isomer
24	9.84	857.490 5 [M+HCOO] ⁻	857.489 9 [M+HCOO] ⁻	C ₄₃ H ₇₂ O ₁₄	0.70	811.487 4; 769.478 6; 637.434 4; 475.379 7; 391.278 3; 161.044 9	20(S)-Sanchirrhoside A ₂
25	9.91	1 151.586 5 [M+HCOO] ⁻	1 151.585 0 [M+HCOO] ⁻	C ₅₄ H ₉₀ O ₂₃	1.30	1 105.584 4; 943.540 1; 781.476 4; 619.432 9; 457.375 4; 323.093 3; 179.056 4	5,6-Didehydroginsenoside Rb ₁
26	9.92	829.497 7 [M+HCOO] ⁻	829.495 0 [M+HCOO] ⁻	C ₄₂ H ₇₂ O ₁₃	3.25	783.493 8; 637.434 1; 475.379 9; 391.284 2; 101.025 0	20(S)-Ginsenoside Rg ₂
27	9.93	1 137.609 7 [M+HCOO] ⁻	1 137.605 7 [M+HCOO] ⁻	C ₅₄ H ₉₂ O ₂₂	3.52	1 091.607 6; 929.549 7; 767.501 3; 605.445 2; 161.047 2	Notoginsenoside I
28	10.15	683.439 9 [M+HCOO] ⁻	683.437 1 [M+HCOO] ⁻	C ₃₆ H ₆₂ O ₉	4.10	637.436 5; 475.379 6; 391.285 7; 101.024 1	20(S)-Ginsenoside Rh ₁
29	10.34	815.484 0 [M+HCOO] ⁻	815.479 3 [M+HCOO] ⁻	C ₄₁ H ₇₀ O ₁₃	5.76	769.479 2; 475.385 8; 311.097 5; 293.088 7; 149.044 9	Ginsenoside F ₃ /F ₅ isomer
30	10.45	1 153.604 6 [M+HCOO] ⁻	1 153.600 6 [M+HCOO] ⁻	C ₅₄ H ₉₂ O ₂₃	3.47	1 107.597 4; 945.547 5; 783.493 9; 459.384 1; 179.057 0	Ginsenoside Rb ₁
31	10.62	871.513 7 [M+HCOO] ⁻	871.505 6 [M+HCOO] ⁻	C ₄₄ H ₇₄ O ₁₄	9.29	825.504 0; 783.493 3; 637.423 9; 475.386 3	20(S)-6'-O-Acetyl- ginsenoside Rg ₂
32	10.73	1 193.600 5 [M-H] ⁻	1 193.595 5 [M-H] ⁻	C ₅₇ H ₉₄ O ₂₆	4.19	1 149.611 5; 1 107.611 5; 1 089.591 1; 945.543 2; 621.439 1; 179.056 1	Malonyl-ginsenoside Rb ₁

Continued

No.	t_R /min	Measured m/z	Theory m/z	Proposed formula	Mass error (ppm)	MS/MS fragment	Identification
33	10.74	725.449 1 [M+HCOO] ⁻	725.447 6 [M+HCOO] ⁻	C ₃₈ H ₆₄ O ₁₀	2.07	679.427 0; 475.383 3; 101.027 4	20(S)-6'-O-Acetyl-ginsenoside Rh ₁
34	10.78	665.426 8 [M+HCOO] ⁻	665.426 5 [M+HCOO] ⁻	C ₃₆ H ₆₀ O ₈	0.45	619.451 0; 475.379 0; 211.620 0	Ginsenoside Rh ₁₆
35	10.79	1 087.538 7 [M-H] ⁻	1 087.532 5 [M-H] ⁻	C ₅₃ H ₈₄ O ₂₃	5.70	1 087.538 5; 955.503 2; 731.436 7; 551.373 6; 455.364 0; 119.033 0	Stipuleanoside R ₂
36	11.14	955.499 0 [M-H] ⁻	955.490 3 [M-H] ⁻	C ₄₈ H ₇₆ O ₁₉	9.11	793.432 4; 731.457 5; 551.380 4	Ginsenoside Ro
37	11.22	1 193.600 2 [M-H] ⁻	1 193.595 5 [M-H] ⁻	C ₅₇ H ₉₄ O ₂₆	3.94	1 149.610 4; 1 107.602 4; 1 089.591 4; 945.550 5; 783.500 2; 621.436 6; 101.023 8	Malonyl-ginsenoside Rb1 isomer
38	11.29	1 123.594 1 [M+HCOO] ⁻	1 123.590 1 [M+HCOO] ⁻	C ₅₃ H ₉₀ O ₂₂	3.56	1 077.588 7; 945.546 3; 783.496 0; 621.439 1; 149.045 3	Ginsenoside Rc
39	11.39	725.448 6 [M+HCOO] ⁻	725.447 6 [M+HCOO] ⁻	C ₃₈ H ₆₄ O ₁₀	1.38	679.444 0; 637.415 6; 619.421 0	20(R)-6'-O-Acetyl-ginsenoside Rh ₁
40	11.40	1 123.592 8 [M+HCOO] ⁻	1 123.590 1 [M+HCOO] ⁻	C ₅₃ H ₉₀ O ₂₂	2.40	1 077.588 6; 945.543 4; 783.490 5; 621.441 8; 149.045 8	Ginsenoside Rb ₂
41	11.53	925.481 1 [M-H] ⁻	925.479 7 [M-H] ⁻	C ₄₇ H ₇₄ O ₁₈	1.51	793.426 8; 613.376 0; 569.386 7; 149.044 5	Stipuleanoside R ₁
42	11.66	1 123.592 4 [M+HCOO] ⁻	1 123.590 1 [M+HCOO] ⁻	C ₅₃ H ₉₀ O ₂₂	2.05	1 077.588 0; 945.545 2; 915.526 6; 783.505 8; 621.432 8	Ginsenoside Rb ₃
43	11.72	1 163.592 9 [M-H] ⁻	1 163.585 0 [M-H] ⁻	C ₅₆ H ₉₂ O ₂₅	6.79	1 119.606 8; 1 077.588 0 9; 915.531 5; 825.503 6	Malonyl-ginsenoside Rc
44	11.72	989.537 3 [M+HCOO] ⁻	989.532 2 [M+HCOO] ⁻	C ₄₈ H ₈₀ O ₁₈	5.15	943.529 0; 781.477 1; 619.420 9; 457.371 3; 101.025 9	5,6-Didehydroginsenoside Rd
45	11.76	683.441 2 [M+HCOO] ⁻	683.437 1 [M+HCOO] ⁻	C ₃₆ H ₆₂ O ₉	6.00	475.380 7; 391.281 4; 161.047 6; 119.036 0; 113.025 8	Ginsenoside F ₁
46	11.87	857.489 2 [M+HCOO] ⁻	857.489 9 [M+HCOO] ⁻	C ₄₃ H ₇₂ O ₁₄	-0.82	811.481 3; 769.471 3; 637.436 7; 619.425 0; 475.379 6; 391.275 1; 149.050 6; 131.027 5	Sanchirinoside A ₂ isomer
47	11.99	975.552 6 [M+HCOO] ⁻	975.552 9 [M+HCOO] ⁻	C ₄₈ H ₈₂ O ₁₇	-0.31	929.552 8; 767.499 8; 605.442 4; 161.046 5	Vinaginsenoside R ₃
48	12.14	793.437 6 [M-H] ⁻	793.437 5 [M-H] ⁻	C ₄₂ H ₆₆ O ₁₄	0.13	793.441 5; 631.390 0; 569.387 0; 497.370 0; 113.023 1	Chikusetsusaponin Iva
49	12.22	991.552 4 [M+HCOO] ⁻	991.547 8 [M+HCOO] ⁻	C ₄₈ H ₈₂ O ₁₈	4.64	945.546 1; 783.493 8; 765.484 0; 621.440 2; 459.385 5; 161.046 8	Ginsenoside Rd
50	12.44	725.449 8 [M+HCOO] ⁻	725.447 6 [M+HCOO] ⁻	C ₃₈ H ₆₄ O ₁₀	3.03	679.461 1; 637.450 0; 475.382 7; 391.284 2; 161.050 0; 101.024 1	Ginsenoside Rh ₁ isomer
51	12.61	1 031.545 3 [M-H] ⁻	1 031.542 7 [M-H] ⁻	C ₅₁ H ₈₄ O ₂₁	2.52	987.556 3; 945.545 7; 927.533 7; 783.492 7; 621.438 5; 459.385 4; 113.021 2	Malonyl-ginsenoside Rd
52	12.85	1 117.545 1 [M-H] ⁻	1 117.543 1 [M-H] ⁻	C ₅₄ H ₈₆ O ₂₄	1.79	1 085.478 6; 1 029.568 7; 987.565 3; 927.535 5; 765.492 3; 603.993 4; 161.051 6	Malonylfloralginsenosides Rd ₆
53	12.89	1 033.562 6 [M+HCOO] ⁻	1 033.558 4 [M+HCOO] ⁻	C ₅₀ H ₈₄ O ₁₉	4.06	987.600 6; 459.382 5; 119.035 8; 825.508 6	Quinquenoside III
54	12.91	1 031.547 7 [M-H] ⁻	1 031.542 7 [M-H] ⁻	C ₅₁ H ₈₄ O ₂₁	4.85	945.546 4; 783.489 7; 621.436 3; 459.389 5; 221.070 5; 119.033 3	Malonylfloralginsenoside Rd isomer
55	13.23	1 033.556 5 [M+HCOO] ⁻	1 033.558 4 [M+HCOO] ⁻	C ₅₀ H ₈₄ O ₁₉	-1.84	945.547 1; 927.534 9; 783.491 7; 621.437 1; 459.383 9; 119.036 8	Quinquenoside III isomer
56	13.35	991.551 3 [M+HCOO] ⁻	991.547 8 [M+HCOO] ⁻	C ₄₈ H ₈₂ O ₁₈	3.53	945.545 4; 783.495 1; 621.438 2; 459.378 9; 323.098 7; 305.090 1; 179.056 9	Gypenoside XVII
57	13.59	1 031.544 7 [M-H] ⁻	1 031.542 7 [M-H] ⁻	C ₅₁ H ₈₄ O ₂₁	1.94	945.548 3; 783.492 5; 621.438 8; 459.373 2; 113.023 4	Malonylfloralginsenoside Rd isomer
58	13.76	725.449 5 [M+HCOO] ⁻	725.447 6 [M+HCOO] ⁻	C ₃₈ H ₆₄ O ₁₀	2.62	679.441 1; 517.393 8; 161.046 4	6-O-Acetyl-ginsenoside Rh ₁ isomer
59	13.84	961.538 7 [M+HCOO] ⁻	961.537 3 [M+HCOO] ⁻	C ₄₇ H ₈₀ O ₁₇	1.46	915.530 6; 753.482 3; 621.437 1; 537.348 6; 459.389 2; 161.044 7	Notoginsenoside Fe
60	14.43	961.539 2 [M+HCOO] ⁻	961.537 3 [M+HCOO] ⁻	C ₄₇ H ₈₀ O ₁₇	1.98	915.533 9; 783.495 0; 621.438 3; 537.336 9; 459.384 4; 161.045 4	Quinquenoside L ₁₀
61	14.67	961.540 8 [M+HCOO] ⁻	961.537 3 [M+HCOO] ⁻	C ₄₇ H ₈₀ O ₁₇	3.64	915.535 7; 783.494 7; 621.437 8; 459.388 1; 161.045 0	Notoginsenoside Fd

Continued

No.	t_R /min	Measured m/z	Theory m/z	Proposed formula	Mass error (ppm)	MS/MS fragment	Identification
62	17.97	829.496 1	829.495 0	$C_{42}H_{72}O_{13}$	1.33	783.495 3; 621.440 2; 459.384 6; 179.056 5;	Ginsenoside F_2
		$[M+HCOO]^-$	$[M+HCOO]^-$			161.045 5	
63	19.57	829.499 5	829.495 0	$C_{42}H_{72}O_{13}$	5.42	783.491 7; 621.435 2; 459.384 1; 323.099 2;	20(S)-Ginsenoside Rg_3
		$[M+HCOO]^-$	$[M+HCOO]^-$			179.057 2	
64	20.05	829.497 6	829.495 0	$C_{42}H_{72}O_{13}$	3.13	783.494 1; 621.436 6; 459.382 2; 323.100 4;	20(R)-Ginsenoside Rg_3
		$[M+HCOO]^-$	$[M+HCOO]^-$			179.056 1	

2 基于 UPLC-Q-TOF-MS/MS 代谢组学的不同三七等级差异代谢物分析

使用 Progenesis QI 软件对不同头数三七的原始质谱数据进行预处理, 然后将其导入 SIMCA-P 14.1 软件进行多元统计分析。将三七样本按不同头数分为六组进行 PLS-DA 分析, PLS-DA 模型的拟合参数 R^2X (cum) 为 0.32, R^2Y (cum) 为 0.267, Q^2 (cum) 为 0.033 9, 所有样

本均分布于 95% 置信区间内, 数据无异常。从 PLS-DA 分析图可以发现 (图 4A) 20 头、40 头、60 头三七样本聚成一簇, 80 头、120 头、200 头三七样本聚成一簇。为进一步挖掘两组三七样本间的差异成分, 将两者进行 OPLS-DA 分析。OPLS-DA 模型的拟合参数 R^2X (cum) 为 0.333, R^2Y (cum) 为 0.9, Q^2 (cum) 为 0.771, 表明该模型 (图 4B) 具有良好的拟合和预测能力。根据 OPLS-

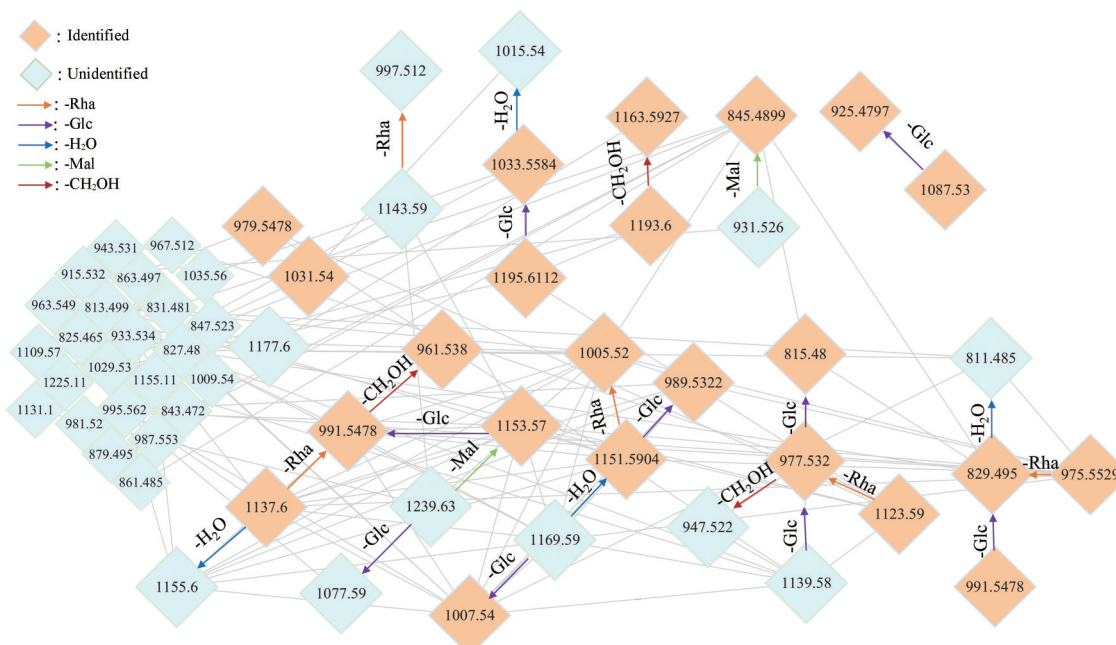


Figure 2 Molecular network of saponins from *Panax notoginseng*. The numbers represent the molecular weight, the identified compounds are the same as Table 2. Rha: Rhamnosyl; Mal: Malonyl

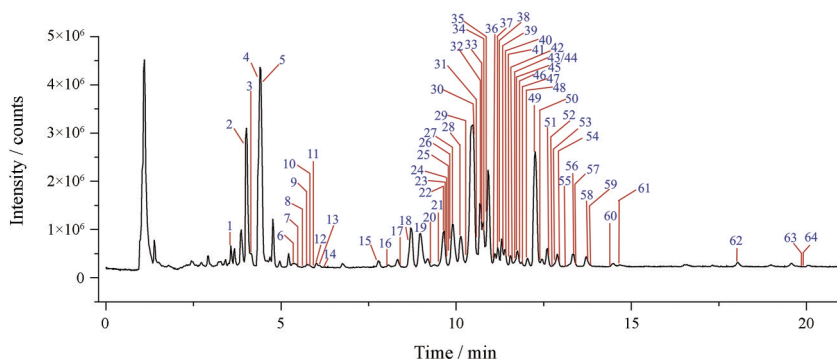


Figure 3 Total ion chromatogram (TIC) of the 70% methanol extract of *Panax notoginseng*. The peak numbers hereby are consistent with those in Table 2

DA分析生成的S-plot图(图4C)进行差异化合物的筛选,其中数据点离原点距离越远,其对三七头数差异贡献程度越大。通过设置VIP值为3,筛选出17个与三七头数相关的差异化合物,分别被鉴定为三七皂苷R₁、人参皂苷Rd等,见表3。另一方面,置换检验结果表明该模型不存在过拟合,实验结果稳定可靠(图4D)。

以VIP值大于10的差异化合物及其响应强度做散点图(图5),人参皂苷Rd、三七皂苷R₁作为指标时,不同等级三七的响应强度之间呈极显著性差异($P < 0.0001$),两个等级之间的样本交叉率小于15%;三七皂苷M作为指标时,不同等级三七的响应强度之间虽呈显著性差异($P < 0.001$),但统货划分线下优选样本

量过多。最终拟选取人参皂苷Rd、三七皂苷R₁作为等级划分指标,后续采取HPLC定量方法对指标成分进一步验证及含量限定。

3 皂苷类成分含量测定

3.1 不同头数三七样品中五种皂苷类成分的含量测定 采用HPLC对不同头数三七(200、120、80、60、40和20头)样本中的皂苷类成分进行含量测定,液相图见图6。含量测定结果显示见表4,样本中三七皂苷R₁含量范围为0.58%~1.31%,平均含量为0.95%。人参皂苷Rg₁含量范围为2.72%~4.49%,平均含量为3.69%。人参皂苷Re含量范围为0.28%~0.71%,平均含量为0.46%。人参皂苷Rb₁的含量范围1.87%~4.10%,平均

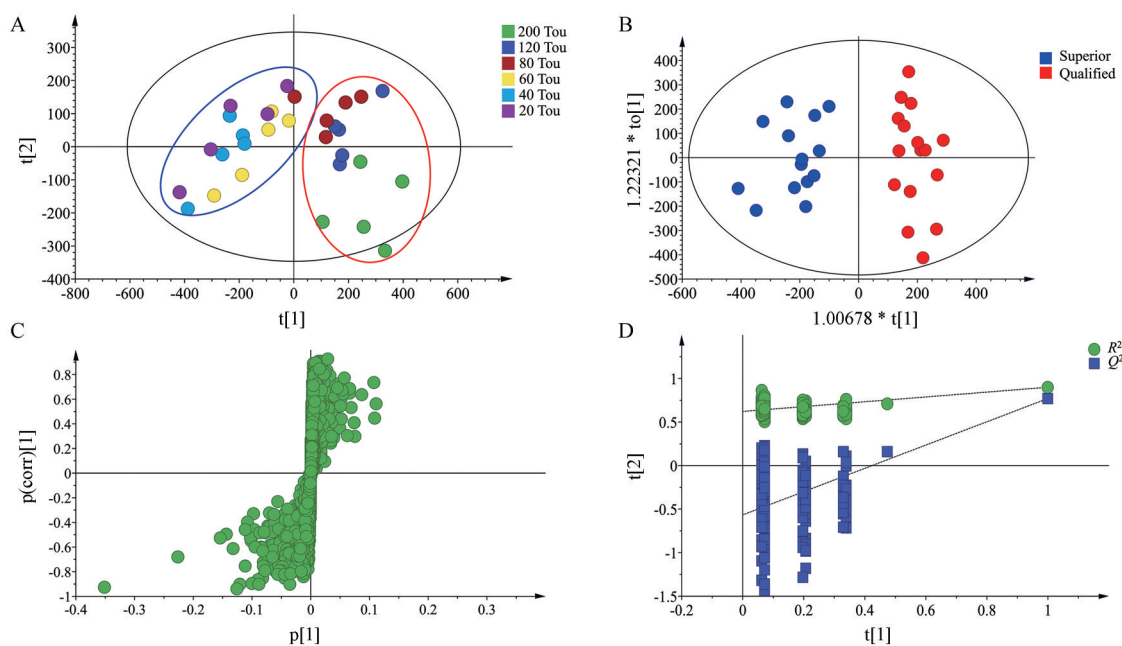


Figure 4 Multivariate statistical analysis based on the correlation between size ("Tou") and characterized saponins of *Panax notoginseng*. A: PLS-DA plot; B: OPLS-DA plot; C: S-plot based on OPLS-DA model; D: Permutation test of OPLS-DA

Table 3 Metabolites with high contributions to different grades of *Panax notoginseng* (VIP > 3, $P < 0.05$)

No.	VIP	<i>m/z</i>	Adduct	Formula	Variation identification
1	24.07	991.544 8	[M+HCOO] ⁻	C ₄₈ H ₈₂ O ₁₈	Ginsenoside Rd
2	16.05	977.530 8	[M+HCOO] ⁻	C ₄₇ H ₈₀ O ₁₈	Notoginsenoside R ₁
3	10.38	1 007.541 0	[M+HCOO] ⁻	C ₄₈ H ₈₂ O ₁₉	Notoginsenoside M
4	9.18	815.478 5	[M+HCOO] ⁻	C ₄₁ H ₇₀ O ₁₃	Notoginsenoside R ₂
5	8.58	1 153.597 0	[M+HCOO] ⁻	C ₅₄ H ₉₂ O ₂₃	Ginsenoside Rb ₁
6	7.09	725.446 3	[M+HCOO] ⁻	C ₃₈ H ₆₄ O ₁₀	20(<i>R</i>)-6'- <i>O</i> -Acetyl-ginsenoside Rh ₁
7	6.86	991.544 4	[M+HCOO] ⁻	C ₄₈ H ₈₂ O ₁₈	Gypenoside XVII
8	5.97	829.492 5	[M+HCOO] ⁻	C ₄₂ H ₇₂ O ₁₃	20(<i>S</i>)-Ginsenoside Rg ₃
9	5.75	961.534 5	[M+HCOO] ⁻	C ₄₇ H ₈₀ O ₁₇	Quinquenoside L ₁₀
10	5.74	683.435 8	[M+HCOO] ⁻	C ₃₆ H ₆₂ O ₉	Ginsenoside F ₁
11	5.36	1 285.639 0	[M+HCOO] ⁻	C ₅₉ H ₁₀₀ O ₂₇	Notoginsenoside Fa
12	5.03	829.494 1	[M+HCOO] ⁻	C ₄₂ H ₇₂ O ₁₃	20(<i>S</i>)-Ginsenoside Rg ₂
13	5.03	683.436 3	[M+HCOO] ⁻	C ₃₆ H ₆₂ O ₉	20(<i>S</i>)-Ginsenoside Rh ₁
14	4.35	1 417.682 0	[M+HCOO] ⁻	C ₆₄ H ₁₀₈ O ₃₁	Notoginsenoside D
15	4.16	1 007.541 0	[M+HCOO] ⁻	C ₄₈ H ₈₂ O ₁₉	Notoginsenoside N
16	3.80	725.446 0	[M+HCOO] ⁻	C ₃₈ H ₆₄ O ₁₀	6- <i>O</i> -Acetyl-ginsenoside Rh ₁
17	3.28	1 123.587 0	[M+HCOO] ⁻	C ₅₃ H ₉₀ O ₂₂	Ginsenoside Rb ₂

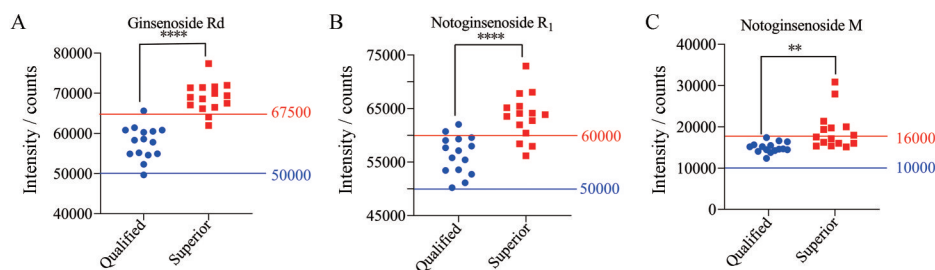


Figure 5 The intensity scatter plot of ginsenosides. A: Ginsenoside Rd; B: Notoginsenoside R₁; C: Notoginsenoside M. The blue line is the minimum line, the red line is the superior and qualified division line. ***P* < 0.01, *****P* < 0.000 1

含量为3.11%。人参皂苷Rd的含量范围0.29%~0.99%，平均含量为0.69%。不同头数三七之间三七总皂苷含量有一定差异，差异较小。

3.2 三七中皂苷类成分含量与头数等级的相关性分析和分级量值的确定 采用Origin软件对三七外观性状指标头数和内在指标成分含量(三七皂苷R₁、人参皂苷Rg₁、Re、Rb₁、Rd)进行皮尔逊相关性分析(图7B)。三七头数与三七皂苷R₁、人参皂苷Rd呈显著负相关(*P* < 0.05)即三七质越重，三七皂苷R₁、人参皂苷

Rd含量越高，而三七头数与人参皂苷Rg₁、Re、Rb₁均无显著相关性。另一方面，差异化合物筛选结果也表明人参皂苷Rd与三七皂苷R₁的VIP值最大，因此两者可作为三七等级划分的指标成分。从皂苷含量柱状图可以发现(图7A)三七皂苷R₁含量趋势为20~60头三七高于80~200头三七，人参皂苷Rd含量趋势为200~60头三七逐渐升高，60~20头三七趋于稳定。基于该趋势，将20~60头三七划分为优选、80~200头三七划分为统货。

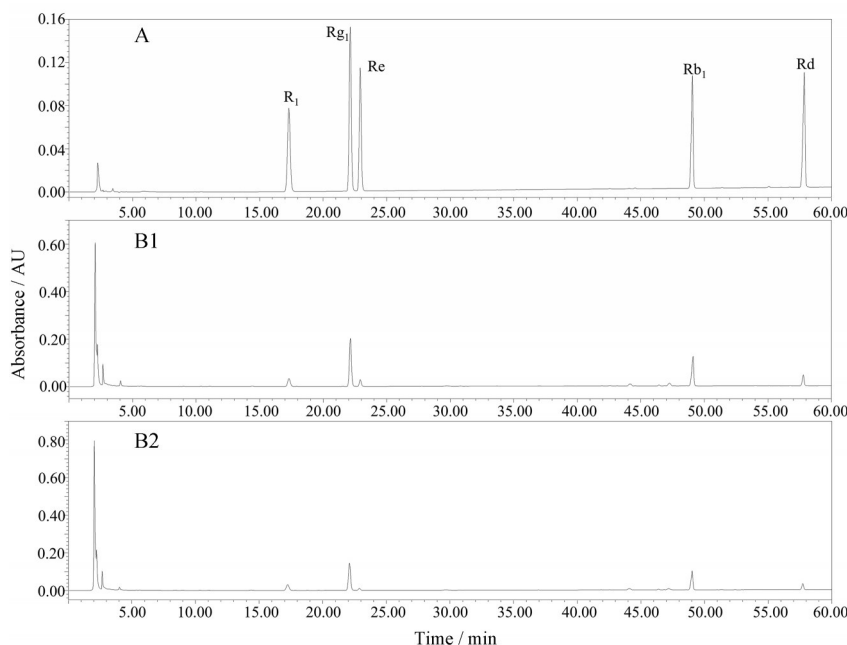


Figure 6 High-performance liquid chromatograms of *Panax notoginseng*. A: Mixed reference substances; B: Samples, 1: 20 Tou; 2: 200 Tou. R₁: Notoginsenoside R₁; Rg₁: Ginsenoside Rg₁; Re: Ginsenoside Re; Rb₁: Ginsenoside Rb₁; Rd: Ginsenoside Rd

Table 4 The content of five representative saponins in *Panax notoginseng*. *n* = 6, $\bar{x} \pm s$ (%). **P* < 0.05, ***P* < 0.01, ****P* < 0.001 vs 200 Tou group. TPNS: Total *Panax notoginseng* saponins (R₁+Rg₁+Re+Rb₁+Rd)

Market grade	R ₁	Rg ₁	Re	Rb ₁	Rd	R ₁ +Rg ₁ +Rb ₁	TPNS
200 Tou	0.86 ± 0.20	3.67 ± 0.64	0.42 ± 0.14	2.95 ± 0.79	0.52 ± 0.14	7.49 ± 1.59	8.42 ± 1.82
120 Tou	0.92 ± 0.11	3.81 ± 0.28	0.48 ± 0.09	3.08 ± 0.37	0.60 ± 0.10	7.81 ± 0.62	8.89 ± 0.70
80 Tou	0.88 ± 0.13	3.87 ± 0.21	0.49 ± 0.08	3.02 ± 0.39	0.65 ± 0.12	7.77 ± 0.60	8.91 ± 0.74
60 Tou	1.04 ± 0.16	3.70 ± 0.38	0.47 ± 0.14	3.30 ± 0.59	0.80 ± 0.14**	8.04 ± 1.06	9.30 ± 1.31
40 Tou	0.93 ± 0.12	3.50 ± 0.49	0.44 ± 0.10	3.03 ± 0.44	0.75 ± 0.11**	7.46 ± 1.01	8.65 ± 1.18
20 Tou	1.10 ± 0.13*	3.59 ± 0.59	0.44 ± 0.07	3.32 ± 0.39	0.82 ± 0.07***	8.01 ± 1.05	9.26 ± 1.17

《中华人民共和国药典》三七项下含量测定规定三七皂苷 R_1 、人参皂苷 Rb_1 、人参皂苷 Rg_1 三种皂苷总量不得少于 5%, 36 批三七样本均符合规定 (图 8A) 但难以三七皂苷 R_1 、人参皂苷 Rb_1 、人参皂苷 Rg_1 三种皂苷总量作为划分指标。以三七皂苷 R_1 、人参皂苷 Rd 为指标成分进行等级划分时, 两种等级之间存在显著差异, 但单以三七皂苷 R_1 含量与不同等级三七作散点图比较发现 (图 8B) 部分统货三七的皂苷含量仍高于优选三七, 以人参皂苷 Rd 含量单作为划分指标与三七皂苷 R_1 划分情况相似 (图 8C)。课题组前期研究发现以皂苷的相对含量比值作为等级量值时, 可对不同生长年限红参样品进行较好的区分^[8]。因此本研究对不同头数三七样品中的皂苷类成分相对含量比值进行了统计分析, 最终以人参皂苷 Rd /三七总皂苷作为限定值, 当限定值为 0.05 时, 所有的样本大于 0.05, 当限定值为 0.08 时, 94.4% 的优选三七大于 0.08, 5.6% 的统货三七大于 0.08 (图 8D)。为了综合评价优选三七与统货三七质量, 建议将人参皂苷 Rd /三七总皂苷限定值定为 0.08。

讨论

三七以个大、质坚体重为佳, 现三七分级标准参考《中药材商品规格等级标准汇编》以三七头数作为分级划分指标。中药材市场因三七头数易客观量化, 均按头数定价售卖, 三七头数作为等级划分是否有科学依

据, 随着现代科技的发展需为其辨状论质提供理论依据^[12,13], 现已有采用三七的味感及药效对三七等级进行评价^[14], 但其不适用于中药材市场流通, 三七市场仍以三七头数为售卖方式, 亟需为其建立“内-外”综合评价方法, 制定科学合理、利于市场流通的等级标准。三七中人参皂苷类成分是其主要药用成分, 其有保护心血管系统的作用, 与三七传统功效活血止血相关性强, 还具有神经保护、抗炎、抗衰老、抗肥胖等作用^[15,16], 具有一定的应用前景。本文研究采用 UPLC-Q-TOF-MS/MS 并结合质谱分子网络对三七人参皂苷类成分进行快速鉴定, 共表征 64 种皂苷成分, 通过 GPNS 分子网络将这些化合物整合为可视化图谱^[17], 为三七中成分表征及质量控制奠定基础。通过 PLS-DA、OPLS-DA 模型分析, 发现不同头数三七之间人参皂苷类成分存在一定差异, 20~60 头三七、80~200 头三七聚为两簇, 同簇间不同头数三七成分差异较小, 可按该规律将 20~60 头三七、80~200 头三七划分为两类, 结合 $VIP > 3$ 、 $P < 0.05$, 筛选出与三七头数相关的 17 个皂苷类差异化化合物, 研究结果可为三七质量评价提供一定的参考。

36 批三七中五种皂苷类成分含量测定结果表明不同头数三七之间有一定差异, 三七皂苷 R_1 、人参皂苷 Rg_1 、人参皂苷 Rb_1 三种皂苷和值的均值为 7.76%, 高于现行质量标准 5%。结合相关性分析 ($P < 0.05$), 筛选出与三七头数相关化合物三七皂苷 R_1 、人参皂苷

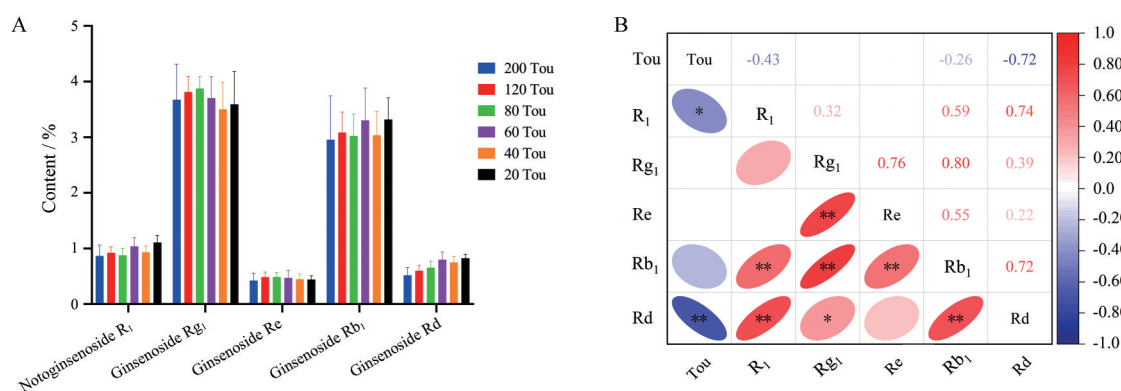


Figure 7 The average content of notoginsenoside R_1 and ginsenoside Rg_1 , Re , Rb_1 , Rd (A); Pearson correlation analysis between size ("Tou") and characterized saponins of *Panax notoginseng* (B). * $P < 0.05$, ** $P < 0.01$

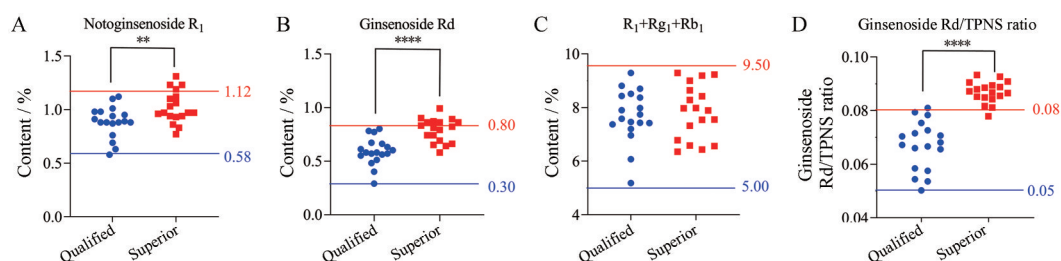


Figure 8 The content scatter plot of ginsenosides. A: Notoginsenoside R_1 ; B: Ginsenoside Rd ; C: Total contents of three ginsenosides; D: Ginsenoside Rd /TPNS. ** $P < 0.01$, **** $P < 0.0001$

Rd, 同时也是非靶向筛选的VIP值最大的差异化合物。20~60头三七中三七皂苷R₁、人参皂苷Rd含量均值为1.02%、0.79% 高于80~200头三七0.89%、0.59%。三七皂苷R₁能改善心血管功能^[18], 人参皂苷Rd能通过调控钙通道抗动脉粥样硬化, 均具有活血化瘀作用^[19], 除此之外, 两种皂苷还有较强的抗炎活性^[20], 因此优选三七两种皂苷的含量更高, 可能具有更高的药理活性。综上, 以三七皂苷R₁、人参皂苷Rd的含量为依据, 将20~60头三七划分为优选, 80~200头三七划分为统货, 分级结果与质谱分类结果一致, 说明该分级方法具有一定的可行性。将三七皂苷R₁、人参皂苷Rd设为质控指标成分, 限定标准为不少于0.76%、0.55%。同时, 以人参皂苷Rd/三七总皂苷作为三七的等级划分量值, 为区分80~200头三七, 建议人参皂苷Rd/三七总皂苷的比值不低于0.08。综上, 本研究基于三七头数采用非靶向定性分析与定量测定相结合的方法对三七质量进行“性状-化学分析”综合分析, 以期对三七等级划分与品质评价提供科学依据和策略。

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