Supporting information

Identification of Metabolites of Liquiritin in Rats by UHPLC-Q-TOF-MS/MS: Metabolic Profiling and Pathway Comparison In Vitro and In Vivo

Xia Zhang, Jintuo Yin, Caijuan Liang, Yupeng Sun, Lantong Zhang*

Department of Pharmaceutical Analysis, School of Pharmacy, Hebei Medical University, Shijiazhuang 050017, P.R. China

* Corresponding author at:
Tel.: +86-311-86266419  E-mail address: zhanglantong@263.net (L. Zhang)

Supplementary identification of in vivo phase I metabolites
**Oxidation reaction**

Metabolites M4, M5 and M6: M4-M6 were observed at retention times of 8.98, 11.42 and 11.94 min with deprotonated molecular ions \([\text{M-H}]^-\) at \(m/z\) 303.0533, 303.0536 and 303.0532, respectively, 114 Da less than the value obtained for deprotonated LQ, which were corresponding to \(\text{C}_{15}\text{H}_{12}\text{O}_{7}\). M4-M6 formed a series of fragment ions at \(m/z\) 285.1340, 259.1548, 241.1436, 153.0916 and 137.0965 due to the loss of \(\text{H}_2\text{O}, \text{CO, O, H}_2\text{O}\) and due to RDA reaction. Moreover, values of cLog P for M4, M5 and M6 were 1.16623, 1.22684 and 1.28623, respectively. So M4, M5 and M6 were tentatively identified according to cLog P values.

Metabolites M15, M16 and M17: M15-M17 were eluted at retention times of 11.52, 13.66 and 13.88 min, respectively, and the mass spectra showed deprotonated molecular ions \([\text{M-H}]^-\) at \(m/z\) 271.0635, 271.0610 and 271.0605, respectively. The masses of the deprotonated M15-M17 were 16 Da higher than the mass of LQ after the loss of \(\text{C}_{6}\text{H}_{10}\text{O}_{5}\), which suggested that hydrolyzed LQ had undergone an oxidation reaction. In the MS² spectra, the fragment ions at \(m/z\) 253.1437, 235.1332, 151.0033 and 119.0505 were observed due to the loss of \(\text{H}_2\text{O}\) and \(\text{H}_2\text{O}\) and due to RDA reaction. The chemical formula of M15-M17 was deduced to be \(\text{C}_{15}\text{H}_{12}\text{O}_{5}\). The cLog P values of M15, M16 and M17 were 1.93694, 2.05485 and 2.44485, respectively, which was used as the basis for assignment of M15-M17.

Metabolites M21, M22: M21 and M22, eluted at 17.00 and 17.65 min, respectively, showed deprotonated molecular ions \([\text{M-H}]^-\) at \(m/z\) 447.1278 and 447.1273, 30 Da higher than that of M0. The MS/MS spectra showed the typical
neutral loss of glucose (162 Da) fragment ion at \( m/z \) 285.1150. The other fragment ions at \( m/z \) 271.0967 \([\text{M-C}_6\text{H}_{10}\text{O}_5\text{-CH}_2\text{-H}]^–\) and 165.0554 (RDA reaction) were detected, which suggested that the occurrence of oxidation and methylation reaction. And the chemical formula of M21 and M22 was \( \text{C}_{22}\text{H}_{24}\text{O}_{10} \). Moreover, the cLog P values of M21 and M22 were 1.01117 and 1.47117, respectively. Therefore, M21 and M22 were identified according to this information.

**Supplementary identification of in vivo phase II metabolites**

Metabolites M37, M38: The metabolites M37 ([M-H] at \( m/z \) 641.1338) and M38 ([M-H] at \( m/z \) 641.1397) were a pair of isomer with retention times of 9.25 and 12.14 min, respectively. The masses of the deprotonated M37 and M38 were 224 Da higher than that of M0, and showed identical fragment ions at \( m/z \) 303.0870 \([\text{M-C}_6\text{H}_{8}\text{O}_6\text{-C}_6\text{H}_{10}\text{O}_5\text{-H}]^–\), 255.0655 \([\text{M-C}_6\text{H}_{8}\text{O}_6\text{-C}_6\text{H}_{10}\text{O}_5\text{-3O-H}]^–\), 135.0080 (RDA reaction) and 119.0504 (RDA reaction), which suggested that oxidation and glucuronide conjugation reaction occurred and that the formula was \( \text{C}_{27}\text{H}_{30}\text{O}_{18} \). According to ChemDraw 12.0, the cLog P values of M37 and M38 were -2.78222 and -2.64222, respectively. According to these evidences, M37 and M38 were assigned.

Metabolites M39, M40: M39 and M40 were separated at 9.67 and 12.55 min with deprotonated molecular ions [M-H] at \( m/z \) 609.1423 and 609.1430, respectively, corresponding to the molecular formula of \( \text{C}_{27}\text{H}_{30}\text{O}_{16} \). The MS/MS spectra showed the typical neutral loss of \( \text{C}_6\text{H}_8\text{O}_6 \) (176 Da), which implied that M39 and M40 were glucuronide conjugation metabolites. Other fragment ions at \( m/z \) 433.1146, 255.0812, 151.0395 and 113.0240 were produced due to successive loss of \( \text{C}_6\text{H}_8\text{O}_6 \), O, \( \text{C}_6\text{H}_{10}\text{O}_5 \)
and due to \textit{RDA} reaction. And they were identified according to the Clog P values of -1.55013 and -1.09013, respectively.

Metabolites M41, M42, M43: M41, M42 and M43, which were eluted at 10.67, 11.45 and 12.00 min, respectively, were characterized as deprotonated molecular ions [M-H]⁻ at \textit{m/z} 351.0164, 351.0163 and 351.0164, respectively. These values were 66 Da lower than the value obtained for M0. The MS/MS spectra showed a number of representative fragment ions at \textit{m/z} 271.0614, 151.0039 and 119.0507 due to loss of SO₃ and due to \textit{RDA} reaction. It was evident that sulfate conjugation occurred and that the molecular formula of M41-M43 was C₁₅H₁₂O₈S. Moreover, M41-M43 were assigned on the basis of cLog P values of 0.361621, 0.821621 and 0.884852, respectively.

Metabolites M48, M49: The mass spectra of M48 and M49 showed deprotonated molecular ions [M-H]⁻ at \textit{m/z} 513.0667 and 513.0677, respectively, which were 96 Da higher than the value obtained for LQ. M48 and M49 were eluted at 12.54 and 13.24 min, respectively, and generated identical secondary fragment ions at \textit{m/z} 433.0558, 255.0665, 151.0393 and 113.0241 by the loss of SO₃, O, and C₆H₁₀O₅ and by \textit{RDA} reaction. These values corresponded to a molecular formula of C₂₁H₂₂O₁₃S. M48 and M49 were identified based on the cLog P values of -1.13483 and -0.674831, respectively.

Metabolite M52: The MS/MS spectrum contained a peak for one metabolite at \textit{m/z} 445.1883, which was 28 Da more than the value obtained for M0. The retention time of M52 was 12.53 min, and the formula of M52 was determined to be C₂₁H₁₈O₁₁
based on the QTOF-MS data. Moreover, fragment ions at \( m/z \) 269.0443 \([M-C_6H_8O_6-H]^-\) and 113.0239 (RDA reaction) were observed, suggesting that M52 was a glucuronide conjugation metabolite.

**Supplementary identification of phase in vitro I metabolites**

Metabolites N5, N6, M7: N5-N7 were eluted at 11.12, 13.60 and 13.83 min and exhibited sharp peaks of deprotonated molecular ions \([M-H]^-\) at \( m/z \) 271.0596, 271.0595 and 271.0592, which were 16 Da higher than the value obtained for hydrolyzed LQ. This observation suggested the occurrence of oxidation of LQ after the loss of C\(_6\)H\(_{10}\)O\(_5\). The corresponding fragment ions were observed at \( m/z \) 253.0474, 151.0016 and 135.0439, which were generated by the loss of H\(_2\)O and by RDA reaction. N5-N7 were isomers and were identified based on their retention times and their cLog P values of 1.93694, 2.05485 and 2.44485, respectively.

**Supplementary identification of in vitro phase II metabolites**

Metabolites N13, N14: On the basis of the deprotonated molecular ions \([M-H]^-\) at \( m/z \) 431.0989 and 431.0985, the molecular formula of N13 and N14 was determined to be C\(_{21}\)H\(_{20}\)O\(_{10}\).\(^{38}\) Moreover, N13 and N14 had retention times of 11.19 and 11.93 min, respectively, and the mass/charge ratio was 176 Da more than that of N9, which implied that N9 had undergone a glucuronide conjugation reaction. The representative fragment ions at \( m/z \) 255.0662, 135.0080 and 113.0242 were produced by the C\(_6\)H\(_8\)O\(_6\) and by RDA reaction. N13 and N14 were identified based on the cLog P values of 0.27429 and 0.55864, respectively.
Fig. S1-2
Fig.S1-3