**Supplementary material**

# Chemical basis underline the bioactivity fortification of licorice by honey-frying due to the natural deep eutectic solvent characteristics of honey

Shasha Kong1#, Pengyue Li1#, Robert Verpoorte2, Jia Wang1, Chao Zhu3, Yuntao Dai1,\* Shilin Chen1

**Author information**

1Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Nanxiaojie 16, 8 Dongzhimennei Ave, 100700 Beijing, China; kss5523@163.com (SK); pyli@icmm.ac.cn (PL); 747053412@qq.com (JW); slchen@icmm.ac.cn (SC)

2Natural Products Laboratory, Institute of Biology, Leiden University, Sylviusweg 72, 2333BE, Leiden, the Netherlands; Verpoorte@chem.leidenuniv.nl (RV)

3College of Medicine & Nursing, Dezhou University, Daxue Xi Lu 566, Decheng District, 253023, Shandong, China; zhuchao830111@163.com (CZ)

#These authors contributed equally to this work

\*Corresponding author Tel: +86-10-64032658. Email: ytdai@icmm.ac.cn

## Method validation of UHPLC-QqQ-MS analysis of licorice decoction

### preparation of samples

***Calibration standard (CS) samples***: The CS solutions were prepared as follows, reference standard stock solutions of liquiritin apioside (**1**), liquiritin (**2**), liquiritigenin (**3**), isoliquiritin apioside (**4**), isoliquiritin (**5**), isoliquiritigenin (**6**), glycyrrhizic acid (**7**), glycyrrhetinic acid (**8**), 2,4-dihydroxyacetophenone (**9**), *D*-fructose (**10**), *D*-glucose (**11**) and 5-hydroxymethylfurfural (**12**) (20 μg/mL, respectively) were dissolved in methanol. A series of mixture working solutions of liquiritin apioside (**1**), liquiritin (**2**), liquiritigenin (**3**), isoliquiritin apioside (**4**), isoliquiritigenin (**6**), glycyrrhetinic acid (**8**), 2,4-dihydroxyacetophenone (**9**) were prepared by diluting stock solution with methanol to the appropriate concentrations: 5, 10, 30, 60, 100, 200, 400 and 800 ng/mL; a series of mixture working solutions of isoliquiritin (**5**), glycyrrhizic acid (**7**) and 5-hydroxymethylfurfural (**12**) were prepared to the appropriate concentrations: 10, 20, 40, 80, 200, 400, 800 and 1000 ng/mL; a series of mixture working solutions of *D*-fructose (**10**) and *D*-glucose (**11**) were prepared to the appropriate concentrations: 40, 80, 100, 200, 400, 800, 1000 and 2000 ng/mL. The working solutions for limit of detections (LODs) and limit of quantifications (LOQs) of analytes were independently prepared by spiking standard solutions with methanol.

***Quality control (QC) samples*:** The QC samples at three concentration levels — 100, 200, 600 ng/mL for all analytes (**1**-**12**) (low, medium, and high levels, respectively) — were prepared in methanol. Meanwhile, the spiked samples at three concentration levels were prepared by spiking standard solutions with licorice decoction.

### Calibration curves, LOD and LOQ

The calibration curve was obtained by plotting the peak area of the compound (y) versus the mass concentration (x) of the reference standard with a 1/x-weighted least-square linear regression algorithm. The LOD of analyte was acquired while the signal-to-noise ratios (S/N) was 3, The LOQ of analyte was defined as the lowest concentration on the calibration curve (S/N = 10).

### Precision, accuracy and stability

The precision of the method was evaluated by determining the twelve analytes of QC samples at low, medium, and high concentrations in six replicates during a single day and by duplicating the experiments on three consecutive days. The intra- and interday precision was represented by relative standard deviations (RSD).

A recovery test was used to evaluate the accuracy of this method. The test was performed by determining the spiked samples and QC samples at three concentration levels. The recovery percentage was calculated by the formula: recovery (%) = (peak area of the analyte in the spiked samples − peak area of the analyte in the licorice decoctions)/peak area of the analyte in the QC samples × 100%.

The stabilities of the analytes were evaluated by analyzing QC samples. The stability was tested by storing the QC samples in the autosampler at 4℃ for 48 h and represented by relative standard deviations (RSD).

### Result

According to Table S3, all the calibration curves of 12 target compounds showed good linearity (*r2* > 0.9956) within the concentration ranges, LODs and LOQs were in the range of 0.8-20 ng/mL, 2-40 ng/mL, respectively. The intraday and interday precision (*n* = 6, RSD%) of the twelve compounds ranged from 1.07% to 8.24% and 1.42% to 7.24%, respectively; the stability data were within the acceptance ranged from 1.54%-8.97% (Table S4). All the results mentioned above indicated that the established method was accurate.

## Method validation of UHPLC-QqQ-MS based pharmacokinetic experiments

### preparation of samples

***Calibration standard (CS) samples***: The CS samples were prepared as follows, reference standard stock solutions of liquiritin apioside (**1**), liquiritin (**2**), liquiritigenin (**3**), and glycyrrhetinic acid (**8**) (20 μg/mL, respectively) were dissolved in methanol, and internal standard (IS) stock solutions of rutin and oleanolic acid (20 μg/mL, respectively) were also dissolved in methanol. The CS samples were obtained by spiking the above stock solutions into blank plasma to yield the final concentration series: 4, 6,10, 40, 80, 200, 400 and 600 ng/mL for liquiritin apioside (**1**); 4, 6, 10, 40, 80, 200, 400 and 600 ng/mL for liquiritin (**2**); 2, 6, 10, 40, 80, 100, 200 and 400 ng/mL for liquiritigenin (**3**); 8, 20, 40, 80, 200, 400, 600 and 800 ng/mL for glycyrrhetinic acid (**8**).

***Quality control (QC) samples*:** The QC samples at three concentration levels — 8, 40, and 400 ng/mL for liquiritin apioside (**1**); 8, 40, and 400 ng/mL for liquiritin (**2**); 4, 20, and 200 ng/mL for liquiritigenin (**3**); 20, 100, and 600 ng/mL for glycyrrhetinic acid (**8**) (low, medium, and high levels, respectively) — were prepared in blank plasma.

### Calibration curves, LOD and LOQ

The calibration curve was determined by plotting the peak area of the compound to IS (y) versus the mass concentration (x) of the reference standard with a 1/x-weighted least-square linear regression algorithm. The LOD of analyte was acquired while the signal-to-noise ratios (S/N) was 3, The LOQ of analyte was defined as the lowest concentration on the calibration curve (S/N = 10).

### Precision and stability

The precision of the method was evaluated by performing six replicate analyses of QC samples at low, medium, and high concentrations for three consecutive days. The intra- and interday precision was represented by RSD.

The stabilities of the four compounds in rat plasma were evaluated by analyzing QC samples. The stability was tested by storing the postpreparative QC samples in the autosampler at 4℃ for 48 h and represented by relative standard deviations (RSD).

### Extraction recovery and matrix effect

The extraction recovery of each analyte was calculated as extraction recovery rate (%) = (peak area of the analyte spiked in blank sample × 100/peak area of the analyte spiked in postpreparative sample). The matrix effect was calculated as matrix effect (%) = (peak areas of the post-extracted standard plasma samples × 100/peak area of the analyte spiked in acetonitrile).

### Result

The calibration curves, LODs and LOQs of the four compounds are shown in Table S5. The calibration curves of all compounds exhibited good linearity with correlation coefficients (*r*2 > 0.9918). The LOQs of liquiritin apioside (**1**), liquiritin (**2**), liquiritigenin (**3**), and glycyrrhetinic acid (**8**) were applicable to the quantitative detection of these compounds in pharmacokinetic studies. The intraday and interday precision (*n* = 6, RSD%) of the four compounds ranged from 4.82% to 10.16% and 5.20% to 10.39%, respectively. The extraction recovery and matrix effect data of the four compounds are summarized in Table S6, these data suggested that the extraction efficiency of the method was in the range of 100±15%, and the matrix effect of all analytes ranged from 95.06% to 104.99%. The results indicate that the matrix effects on all analytes were in an acceptable range. The stability data were within the acceptance ranged from 7.02%-10.45% (Table S6). The results showed that, in rat plasma, the four compounds were stable before quantitation.

Supplementary Tables

Table S1. MS data of the quality marker compounds of different processed licorice determined by UHPLC-Q-Orbitrap.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| No. | T (min) | Formula | [M-H]- (*m/z*) | Error (ppm) | Fragment ion  | Identification |
| Predicted Measured |
| 1 | 6.30 | C26H30O13 | 549.1602 | 549.1608 | 0.56 | 549.16808(100)，255.0660(51.93)，135.0086(59.69)，119.0500(85.92)，91.0188(32.95) | liquiritin apioside\* |
| 2 | 6.41 | C21H22O9 | 417.1180 | 417.1187 | 0.70 | 417.1194(2.49)，255.0661(80.51)，135.0086(25.36)，119.0500(100)，91.0187(17.43) | liquiritin\* |
| 3 | 8.30 | C15H12O4 | 257.0808[M+ H]+ | 257.0808 | -0.025 | 257.0811(82.57)，137.0236(100)，119.0494(16.65)，91.0544(9.61) | liquiritigenin\* |
| 4 | 7.02 | C26H30O13 | 549.1602 | 549.1607 | 0.50 | 549.1606(67.33)，255.0660(64.08)，135.0086(41.14)，119.0500(100)，91.0187(67.33) | isoliquiritin apioside |
| 5 | 7.34 | C21H22O9 | 417.1180 | 417.1185 | 0.58 | 417.1185(34.04)，255.0659(100)，135.0086(33.76)，119.0499(28.23) | isoliquiritin\* |
| 6 | 11.73 | C15H12O4 | 257.0808[M + H]+ | 257.0807 | -0.11 | 257.0810(85.23)，137.0235(100)，119.0493(8.27)，91.0544(10.78) | isoliquiritigenin\* |
| 7 | 13.08 | C42H62O16 | 821.3954 | 821.3957 | 0.33 | 821.3964(100)，351.0566(10.49)，193.0353(5.95) | glycyrrhizic acid\* |
| 8 | 19.52 | C30H46O4 | 471.3468[M + H]+ | 471.3471 | 0.28 | 471.3476(100)，453.3366(0.65) | glycyrrhetinic acid\* |
| 9 | 5.97 | C8H8O3 | 151.0389 | 151.0398 | 0.86 | 151.0398(100)，135.0088(13.32) | 2,4-dihydroxyacetophenone |
| 10 | 1.14 | C6H12O6 | 179.0550 | 179.0558 | 0.83 | 179.0560(17.01)，161.0455(6.92) | *D*-fructose |
| 11 | 0.98 | C6H12O6 | 179.0550 | 179.0558 | 0.81 | 179.0559 (23.84)，161.0455 (12.25) | *D*-glucose |
| 12 | 3.17 | C6H6O3 | 125.0233 | 125.0242 | 0.87 | 125.0241(100)，97.0292(9.38)，81.0343(1.60) | 5-hydroxymethylfurfural\* |
| 13 | 7.66 | C28H32O14 | 591.1708 | 591.1716 | 0.79 | 591.1721(22.26)，549.1626(42.18)，255.0662(60.13)，135.0085(37.30)，119.0500(100) | 6''-*O*-acetylliquiritin apioside |
| 14 | 8.75 | C28H32O14 | 591.1708 | 591.1715 | 0.67 | 591.1715(54.00)，549.1614(6.37)，255.0660(53.26)，135.0086(42.35)，119.0500(100) | 6''-*O*-acetylisoliquiritin apioside |
| 15 | 8.09 | C23H24O10 | 459.1285 | 459.1292 | 0.63 | 459.1289(82.71)，255.0659(100)，135.0087(32.80)，119.0499(40.53) | 6''-*O*-acetylliquiritin |
| 16 | 9.29 | C23H24O10 | 459.1285 | 459.1292 | 0.69 | 459.1295(90.32)，255.0659(100)，135.0086(34.14)，119.0500(42.28) | 6''-*O*-acetylisoliquiritin |
| 17 | 14.21 | C42H62O16 | 821.3954 | 821.3960 | 0.58 | 821.3961(100)，351.0555(12.58) | uralsaponin B |
| 18 | 10.20 | C44H64O18 | 879.4008 | 879.4011 | 0.22 | 879.4016(100)，351.0572(11.74), 7370.3134(1.29) | 22*β*-acetoxylglycyrrhizic acid |

\* Identified by chromatogram of reference substance

Table S2. The MS/MS parameters for eighteen compounds in quantitative analysis with UHPLC-QqQ-MS.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Compounds | Q1 (*m/z*) | Q3 (*m/z*) | Quantitative ion | DP(V) | CE(V) |
| liquiritin apioside (**1**) | 549.1  | 255.2  | √ | -80.0  | -44.0  |
|  | 134.9  |  |  | -60.0  |
| liquiritin (**2**) | 417.1  | 134.9  | √ | -80.0  | -43.0  |
|  | 119.1  |  |  | -60.0  |
| liquiritigenin (**3**) | 255.1  | 134.8  | √ | -80.0  | -21.0  |
|  | 119.1  |  |  | -32.0  |
| isoliquiritin apioside (**4**) | 549.1  | 255.2  | √ | -80.0  | -39.0  |
|  | 134.8  |  |  | -53.0  |
| isoliquiritin (**5**) | 417.1  | 255.2  | √ | -80.0  | -28.0  |
|  | 134.9  |  |  | -39.0  |
| isoliquiritigenin (**6**) | 255.1  | 134.8  | √ | -80.0  | -21.0  |
|  | 119.1  |  |  | -33.0  |
| glycyrrhizic acid (**7**) | 821.3  | 350.9  | √ | -180.0  | -58.0  |
|  | 192.9  |  |  | -60.0  |
| glycyrrhetinic acid (**8**) | 469.3  | 355.3  |  | -80.0  | -62.0  |
|  | 425.4  | √ |  | -53.0  |
| 2,4-dihydroxyacetophenone (**9**) | 151.1  | 109.0  | √ | -80.0  | -22.0  |
|  | 90.7  |  |  | -29.0  |
| *D*-fructose (**10**) | 179.0  | 58.7  | √ | -80.0  | -26.0  |
|  | 89.2  |  |  | -11.0  |
| *D*-glucose (**11**) | 179.0  | 58.9  |  | -80.0  | -24.0  |
|  | 89.2  | √ |  | -11.0  |
| 5-hydroxymethylfurfural (**12**) | 125.1  | 97.0  | √ | -40.0  | -18.0  |
|  | 79.2  |  |  | -23.0  |
| 6''-*O*-acetylliquiritin apioside (**13**) | 591.2  | 255.1  | √ | -80.0  | -45.0  |
|  | 135.0  |  |  | -60.0  |
| 6''-*O*-acetylisoliquiritin apioside (**14**) | 591.2  | 255.1  |  | -80.0  | -40.0  |
|  | 119.1  | √ |  | -60.0  |
| 6''-*O*-acetylliquiritin (**15**) | 459.1  | 255.1  |  | -80.0  | -35.0  |
|  | 135.0  | √ |  | -43.0  |
| 6''-*O*-acetylisoliquiritin (**16**) | 459.1  | 255.1  | √ | -80.0  | -28.0  |
|  | 135.0  |  |  | -39.0  |
| uralsaponin B (**17**) | 821.4  | 351.0  | √ | -180.0  | -60.0  |
| 22*β*-acetoxylglycyrrhizic acid (**18**) | 879.4  | 351.0  | √ | -180.0  | -60.0  |

Q1 = Parent ion, Q3 = Product ion, DP = Declustering Potential, CE = Collision Energy.

 Table S3. Calibration curves, LOD and LOQ data of twelve compounds in quantitative analysis with UHPLC-QqQ-MS.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Compound | Calibration curves | *r2* | Linear range (ng/mL) | LOD (ng/mL) | LOQ (ng/mL) |
| liquiritin apioside (**1**) | y=32124.8x + 127665 | 0.9981 | 5 - 800 | 1.00 | 5.00 |
| liquiritin (**2**) | y=19624.5x + 30853.7 | 0.9987 | 5 - 800 | 2.00 | 5.00 |
| liquiritigenin (**3**) | y=60791.8x + 33723.7 | 0.9991 | 5 - 800 | 0.80 | 2.00 |
| isoliquiritin apioside (**4**) | y=39978.3x + 20909.3 | 0.9974 | 5 - 800 | 2.00 | 5.00 |
| isoliquiritin (**5**) | y=883.7x + 11986.3 | 0.9988 | 10 - 1000 | 5.00 | 10.00 |
| isoliquiritigenin (**6**) | y=102525x + 55869.1 | 0.9978 | 5 - 800 | 0.80 | 2.00 |
| glycyrrhizic acid (**7**) | y=1546.2x + 19154.8 | 0.9975 | 10 - 1000 | 5.00 | 10.00 |
| glycyrrhetinic acid (**8**)  | y=6130.4x - 5593.5 | 0.9984 | 5 - 800 | 3.00 | 5.00 |
| 2,4-dihydroxyacetophenone (**9**)  | y=83722.4x + 69557.4 | 0.9969 | 5 - 800 | 0.80 | 5.00 |
| *D*-fructose (**10**) | y=27.7x + 2788.6 | 0.9957 | 40 - 2000 | 20.00 | 40.00 |
| *D*-glucose (**11**) | y=25.4x + 2690.2 | 0.9961 | 40 - 2000 | 20.00 | 40.00 |
| 5-hydroxymethylfurfural (**12**) | y=994.9x - 6025.8 | 0.9956 | 10 - 1000 | 8.00 | 10.00 |

Table S4. Precision, stability and recovery of 12 analytes in quantitative analysis with UHPLC-QqQ-MS. (*n* = 6)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Compound Concentration (ng/mL) | Intra-day Precision RSD (%) | Inter-day Precision RSD (%) | Recovery | Stability (%) |
| Average (%) | RSD(%) |
| liquiritin apioside (**1**) |  |  |  |  |  |
| 600 | 1.75  | 1.54  | 103.52  | 2.92  | 1.54  |
| 200 | 2.09  | 5.77  | 102.63  | 6.94  | 4.49  |
| 100 | 1.50  | 2.01  | 105.10  | 2.33  | 2.09  |
| liquiritin (**2**) |  |  |  |  |  |
| 600 | 1.63  | 3.62  | 107.48  | 3.57  | 1.80  |
| 200 | 3.31  | 2.56  | 100.74  | 3.01  | 2.56  |
| 100 | 2.59  | 7.24  | 102.71  | 4.69  | 3.62  |
| liquiritigenin (**3**) |  |  |  |  |  |
| 600 | 1.07  | 5.97  | 100.47  | 1.03  | 2.14  |
| 200 | 1.43  | 1.60  | 102.19  | 3.25  | 5.97  |
| 100 | 2.17  | 4.17  | 106.74  | 2.60  | 4.75  |
| isoliquiritin apioside (**4**) |  |  |  |  |  |
| 600 | 2.06  | 2.37  | 106.15  | 3.14  | 3.50  |
| 200 | 1.43  | 3.15  | 105.35  | 5.02  | 3.74  |
| 100 | 2.59  | 3.50  | 106.53  | 4.06  | 6.73  |
| isoliquiritin (**5**) |  |  |  |  |  |
| 600 | 2.26  | 4.42  | 104.68  | 2.74  | 4.42  |
| 200 | 2.20  | 6.78  | 99.08  | 4.23  | 6.78  |
| 100 | 2.00  | 5.13  | 103.23  | 4.31  | 5.13  |
| isoliquiritigenin (**6**) |  |  |  |  |  |
| 600 | 1.22  | 1.42  | 103.63  | 3.91  | 2.00  |
| 200 | 1.28  | 2.70  | 101.59  | 0.90  | 3.33  |
| 100 | 2.41  | 6.92  | 106.96  | 2.07  | 2.70  |
| glycyrrhizic acid (**7**) |  |  |  |  |  |
| 600 | 5.81  | 3.98  | 102.00  | 6.01  | 5.33  |
| 200 | 4.89  | 3.13  | 104.69  | 6.76  | 6.64  |
| 100 | 3.40  | 5.51  | 103.68  | 8.72  | 8.97  |
| glycyrrhetinic acid (**8**)  |  |  |  |  |  |
| 600 | 2.51  | 2.76  | 101.60  | 5.86  | 3.56  |
| 200 | 2.48  | 3.56  | 105.51  | 3.11  | 6.52  |
| 100 | 3.02  | 6.52  | 102.00  | 5.50  | 6.23  |
| 2,4-dihydroxyacetophenone (**9**)  |  |  |  |  |
| 600 | 1.12  | 1.56  | 102.83  | 5.69  | 2.20  |
| 200 | 1.76  | 4.66  | 101.48  | 1.49  | 2.15  |
| 100 | 5.12  | 2.20  | 102.82  | 2.71  | 4.66  |
| *D*-fructose (**10**) |  |  |  |  |  |
| 600 | 2.83  | 3.25  | 101.77  | 5.10  | 5.55  |
| 200 | 6.64  | 3.86  | 104.61  | 5.96  | 6.64  |
| 100 | 5.60  | 5.55  | 99.42  | 3.36  | 5.60  |
| *D*-glucose (**11**) |  |  |  |  |  |
| 600 | 2.33  | 1.80  | 98.29  | 4.45  | 4.55  |
| 200 | 8.24  | 5.05  | 102.58  | 8.29  | 8.24  |
| 100 | 4.55  | 5.36  | 100.91  | 8.79  | 5.05  |
| 5-hydroxymethylfurfural (**12**) |  |  |  |  |
| 600 | 1.44  | 4.76  | 101.38  | 3.50  | 5.77  |
| 200 | 4.38  | 2.37  | 102.87  | 2.52  | 6.80  |
| 100 | 5.52  | 5.77  | 103.71  | 6.71  | 7.81  |

Table S5. Calibration curves, linear range, LOD and LOQ of four compounds in rat plasma analysis with UHPLC-QqQ-MS.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Compound | Calibration curves | *r*2 | Linear range (ng/mL) | LOD (ng/mL) | LOQ (ng/mL) |
| liquiritin apioside (**1**) | y=32.9x + 462.8 | 0.9975 | 4 - 600 | 1.00 | 4.00 |
| liquiritin (**2**) | y=70.2x + 1218.0 | 0.9918 | 4 - 600 | 2.00 | 4.00 |
| liquiritigenin (**3**) | y=4136.4x - 980.4 | 0.9960 | 2 - 400 | 0.80 | 2.00 |
| glycyrrhetinic acid (**8**)  | y=7.2x + 57.6 | 0.9932 | 8 - 800 | 4.00 | 8.00 |

Table S6. Precision, extraction recovery, matrix effect and stability of four compounds in rat plasma analysis with UHPLC-QqQ-MS. (*n* = 6)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Compound Concentration (ng/mL) | Intra-day Precision RSD (%) | Inter-day Precision RSD (%) | Stability (%) | Extraction recovery (%) | Matrix Effect (%) |
| liquiritin apioside(**1**) |  |  |  |  |  |
| 400 | 8.16  | 6.50  | 8.30  | 104.09 ± 11.16 | 97.39 ± 14.36 |
| 40 | 8.40  | 7.04  | 7.34  | 97.27 ± 10.42 | 95.06 ± 9.10 |
| 8 | 10.16  | 8.00  | 7.45  | 99.81 ± 5.08 | 96.78 ± 9.33 |
| liquiritin(**2**) |  |  |  |  |  |
| 400 | 7.48  | 8.18  | 7.57  | 98.35 ± 3.41 | 104.99 ± 5.31 |
| 40 | 9.45  | 9.21  | 7.20  | 97.24 ± 7.98 | 97.55 ± 7.49 |
| 8 | 9.83  | 10.39  | 9.51  | 96.60 ± 6.73 | 96.46 ± 12.12 |
| liquiritigenin(**3**) |  |  |  |  |  |
| 200 | 8.66  | 5.51  | 9.21  | 97.64 ± 6.62 | 97.69 ± 5.08 |
| 20 | 7.46  | 7.79  | 7.02  | 95.08 ± 6.39 | 97.91 ± 3.25 |
| 4 | 9.87  | 8.39  | 10.45  | 98.71 ± 8.37 | 98.82 ± 5.17 |
| glycyrrhetinic acid (**8**)  |  |  |  |  |
| 600 | 4.82  | 5.20  | 9.73  | 97.69 ± 5.76 | 102.51 ± 7.26 |
| 100 | 5.11  | 5.94  | 8.08  | 104.77 ± 6.99 | 96.76 ± 3.97 |
| 20 | 8.85  | 6.41  | 10.32  | 97.85 ± 10.49 | 97.80 ± 11.58 |

Table S7. Pharmacokinetic parameters for liquiritin apioside (**1**), liquiritin (**2**), liquiritigenin (**3**), and glycyrrhetinic acid (**8**) in rat plasma after single oral administration of raw licorice decoction (8 g/kg (raw licorice)), fried licorice decoction, honey-fried licorice decoction, honey analogue (natural deep eutectic solvents, NADES)-fried licorice decoction. (*n* = 6)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Analytes | Group | *C*max (ng/mL) | *T*max (h) | AUC0-12/24h (h·ng/mL) |
| liquiritin apioside (**1**) | R | 34.02 ± 10.13 | 0.29 ± 0.10 | 100.69 ± 16.09 |
|  | F | 35.42 ± 4.46 | 0.38 ± 0.31 | 99.42 ± 6.61 |
|  | N | 44.14 ± 8.00\* | 0.42 ± 0.13 | 146.40 ± 25.51\*# |
|  | H | 42.59 ± 9.70 | 0.54 ± 0.25 | 149.20 ± 3.55\*# |
| liquiritin (**2**) | R | 114.45 ± 13.92 | 0.54 ± 0.37 | 314.05 ± 25.59 |
|  | F | 121.49 ± 24.67 | 0.38 ± 0.31 | 310.86 ± 25.25 |
|  | N | 166.26 ± 23.90\*# | 0.37± 0.31 | 331.93 ± 34.77 |
|  | H | 169.36 ± 28.45\*# | 0.25 ± 0.00 | 316.93 ± 47.53 |
| liquiritigenin (**3**) | R | 15.22 ± 2.02 | 0.86 ± 1.53 | 71.44 ± 13.06 |
|  | F | 17.52 ± 3.35 | 0.25 ± 0.00 | 82.33 ± 9.55 |
|  | N | 22.24 ± 5.21\*# | 0.86 ± 1.53 | 101.59 ± 12.66\*# |
|  | H | 23.65 ± 4.04\*# | 0.86 ± 1.53 | 103.06 ± 4.06\*# |
| glycyrrhetinic acid (**8**) | R | 423.33 ± 54.92 | 8.67 ± 1.63 | 4840.39 ± 310.59 |
|  | F | 452.40 ± 60.53 | 8.67 ± 1.63 | 4864.55 ± 506.31 |
|  | N | 542.01 ± 72.88\*# | 8.67 ± 1.63 | 6292.81 ± 827.17\*# |
|  | H | 561.01 ± 64.57\*# | 9.33 ± 2.07 | 6463.73 ± 641.21\*# |

\* P < 0.05 vs raw licorice decoction group; # P < 0.05 vs fried licorice decoction group.

Figure S1



**Figure S1** Bar plots of the levels of some quality difference markers (R, raw licorice; F, fried licorice; H, honey-fried licorice; N, NADES-fried licorice; *n* = 6, \* P < 0.05 vs R group; # P < 0.05 vs F group. 6''-*O*-acetylliquiritin apioside (**13**), 6''-*O*-acetylisoliquiritin apioside (**14**), 6''-*O*-acetylliquiritin (**15**), 6''-*O*-acetylisoliquiritin (**16**), uralsaponin B (**17**), 22*β*-acetoxylglycyrrhizic (**18**))