Chemical influence of *Scutellaria baicalensis–Coptis chinensis* pair on the extraction efficiencies of flavonoids and alkaloids at different extraction times and temperatures

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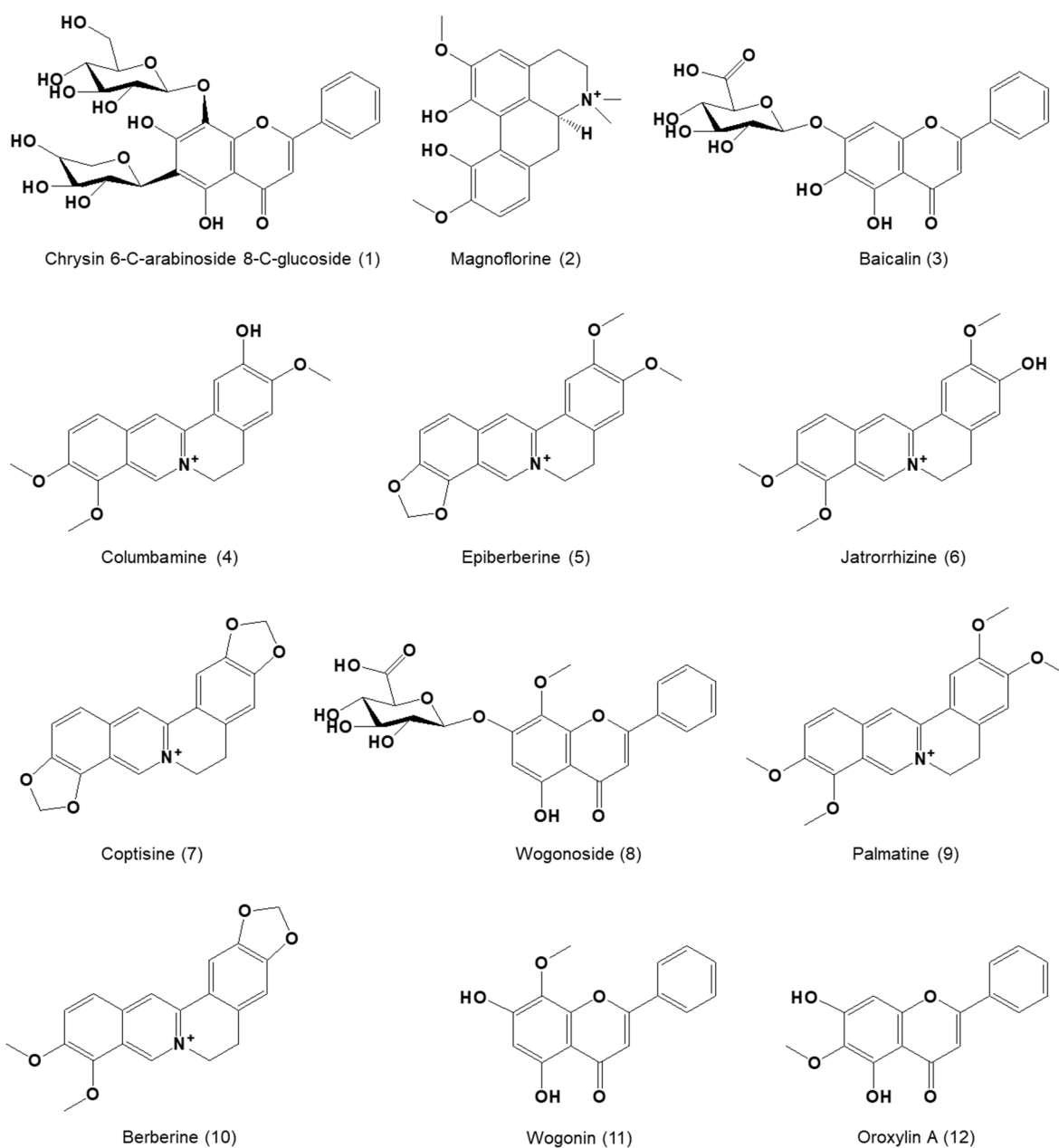* Author to whom correspondence should be addressed.

Figure S1. Chemical structures of the twelve marker compounds found in a water extract of the roots of *Scutellaria baicalensis* and the rhizomes of *Coptis chinensis*.

Optimization of chromatographic conditions and method validation

The type of column and the mobile phase modifier, as well as the composition of mobile phases in gradient elution were mainly considered as crucial factors for optimisation of analytical conditions. In our previous study, we used an ADME column, which has an adamantly functional group as packing material instead of C_{18}–coated silica, to successfully separate three flavonoids and four alkaloids from the decoctions of *S. baicalensis* and *C. chinensis* [1]. As a result, an ADME column was used in this study to better separate the five flavonoids and seven alkaloids found in the extracts.

Acid-added mobile phase aids in preserving the acidic compounds' original structure during analysis and can help sharpen peak shapes by reducing tailing or fronting [2]. Under an aqueous mobile phase containing 0.1% TFA (*v/v*) and optimally adjusted gradient composition of mobile phases at their maximum absorption wavelengths, the 12 marker compounds of interest, particularly baicalin and wogonoside, which have glucuronic acid by glycosidic bond, showed improved peak shapes and separation (all flavonoids at 275 nm and alkaloids at 345 nm, except for epiberberine and coptisine at 355 nm). Figure S2 shows the chromatograms of optimally separated peaks.
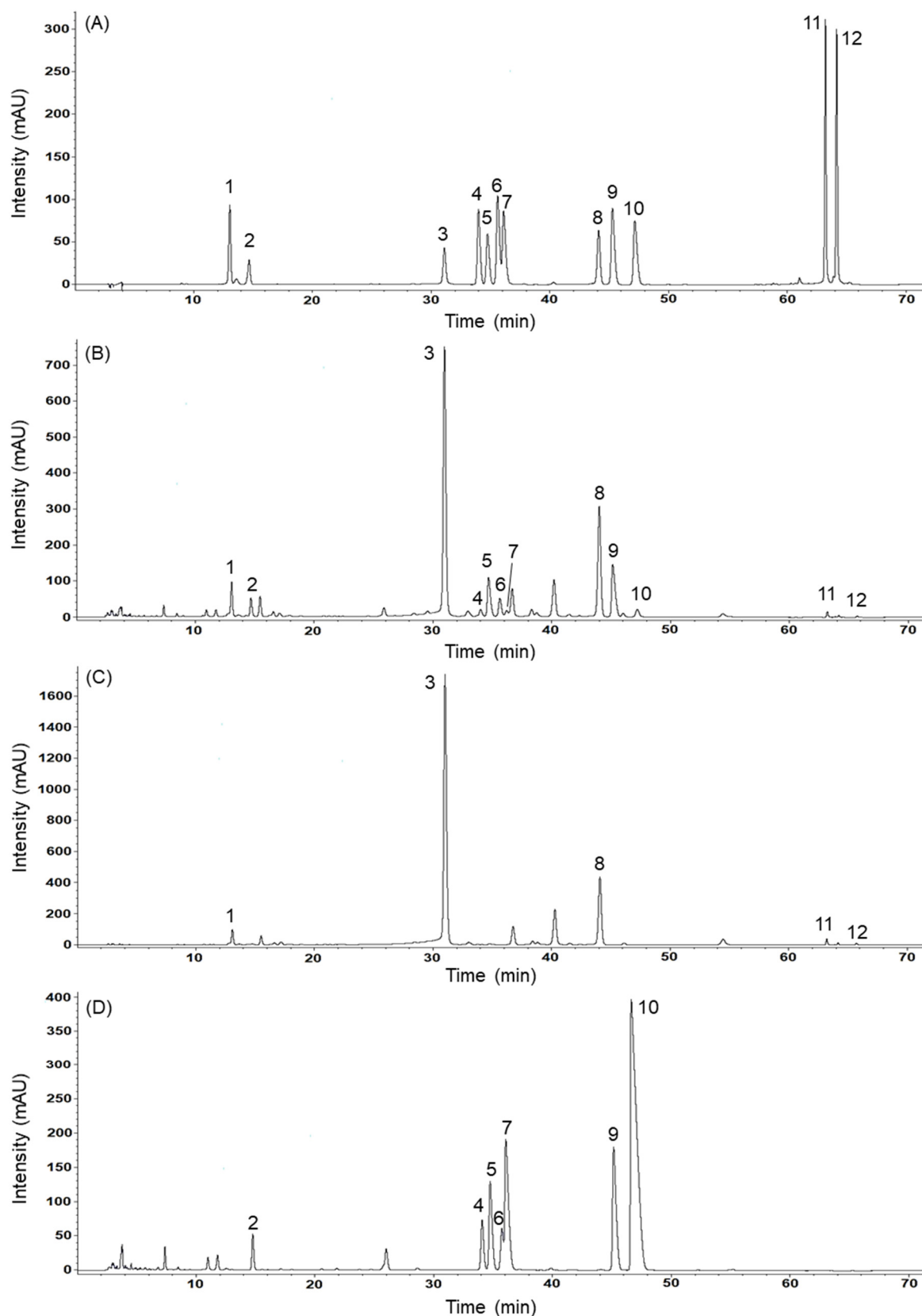
Figure S2. Chromatograms of the twelve marker compounds **(A)**, the paired extract of *S. baicalensis* and *C. chinensis* **(B)**, the single extract of *S. baicalensis* **(C)**, and the single extract of *C. chinensis* **(D)** at a detection wavelength of 275 nm. 1, Chrysin 6-C-arabinoside 8-C-glucoside; 2, magnoflorine; 3, baicalin; 4, columbamine; 5, epiberberine; 6, jatrorrhizine; 7, coptisine; 8, wogonoside; 9, palmatine; 10, berberine; 11, wogonin; 12, oroxylin A.

Within the linear range, the correlation coefficients of the marker compounds, which indicate linearity, ranged from 0.9991 to 0.9999. LODs and LOQs had ranges of 0.01–0.12 μg/mL and 0.07–0.40 μg/mL, respectively. Within a single day, the precision values as RSD were < 2.0% with accuracies ranging from 95.27% to 110.01% and < 4.0% with accuracies ranging from 97.50% to 107.02% over three consecutive days. The spiked concentrations in the extracts were recovered in 96.39%–105.71% with RSD values < 7.0% (Table S1). The developed analytical methods were validated in terms of precision and accuracy, and they were successfully applied to the analysis of the twelve marker compounds in the *S. baicalensis* and *C. coptsis* extracts.

Table S1. Linear equations, correlation coefficients (r^2), LOD, LOQ, and the values of analytical method validation of the marker compounds.

Compound	UV	Regression equation	r^2	Linear range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	Precision Intraday (n = 3) RSD (%)	Intraday Accuracy (%)	Interday (n = 3) RSD (%)	Interday Accuracy (%)	Recovery Average (n = 3, %)	RSD (%)
Chrysin 6A8G	275nm	y = 21.452x + 9.3833	0.9999	1.88–120.00	0.04	0.12	0.37	105.72	1.79	104.99	96.93	4.80
Magnoflorine	275nm	y = 9.8582x + 1.4167	0.9999	1.88–120.00	0.12	0.40	1.00	110.01	3.83	107.02	105.71	4.25
Baicalin	275nm	y = 24.819x − 152.82	0.9991	9.38–600.00	0.12	0.39	0.80	99.29	1.64	99.51	96.39	3.09
Columbamine	345nm	y = 30.219x + 6.3563	0.9999	0.94–60.00	0.06	0.20	0.31	104.84	1.28	103.99	100.63	5.76
Epiberberine	355nm	y = 23.791x + 18.406	0.9997	4.06–260.00	0.08	0.25	0.38	99.38	0.31	100.07	97.75	6.34
Jatrorrhizine	345nm	y = 39.356x + 13.134	0.9999	1.25–80.00	0.03	0.09	0.29	105.33	1.30	104.40	100.30	6.51
Coptisine	355nm	y = 40.411x − 0.7563	0.9999	0.31–20.00	0.02	0.07	0.10	102.92	1.08	102.16	101.42	6.22
Wogonoside	275nm	y = 30.622x + 53.866	0.9998	6.25–400.00	0.03	0.10	0.83	99.62	0.48	100.73	99.61	4.74
Palmatine	345nm	y = 36.543x + 45.079	0.9996	4.06–130.00	0.02	0.08	0.38	100.50	0.20	101.00	99.65	4.26
Berberine	345nm	y = 33.928x + 4.7384	0.9997	0.63–40.00	0.04	0.13	0.25	99.99	0.28	100.55	99.88	5.61
Wogonin	275nm	y = 45.553x − 2.1063	0.9999	0.16–10.00	0.01	0.03	0.64	99.14	1.74	99.17	104.42	3.71
Oroxylin A	275nm	y = 45.955x − 3.1184	0.9998	0.16–10.00	0.01	0.03	1.90	95.27	1.08	97.50	104.74	0.69

LOD, limit of detection; LOQ, limit of quantification; y, peak area (mAU); x, concentration of the compound (µg/mL).

RSD, relative standard deviation (%) = (SD/mean) × 100.

Chrysin 6A8G, Chrysin 6-C-arabinoside 8-C-glucoside.

References

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