Chemistry of Natural Compounds, Vol. 46, No. 1, 2010

HPLC ANALYSIS OF GLYCYRRHIZIN AND LICOCHALCONE A IN *Glycyrrhiza inflata* FROM XINJIANG (CHINA)

Junbo Xie,[1,2] Yanqing Zhang,[2] and Wenquan Wang[1*]

UDC 547.92

Glycyrrhiza inflata is one of the main botanical sources of licorice. It is distributed throughout central Asia: Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, and Uzbekistan, Mongolia, and west China [1]. In Chinese Pharmacopoeia, as *Glycyrrhiza glabra* and *G. uralensis*, it is listed as one of the sources of licorice.

Extensive chemical studies revealed that *G. inflata* contained the same bioactive constituents as *Glycyrrhiza glabra* and *G. uralensis*, which possess a wide array of biological properties. Triterpenoids such as glycyrrhizin, glycyrrhetinic acid [2], apioglycyrrhizin, araboglycyrrhizin [3], and inflasaponins II, VI [4] and flavonoids such as liquiritin and isoliquiritigenin are the major compounds [5, 6]. However, *G. inflata* also contains characteristic retrochalcones, such as licochalcone A–D, which are distinguished from ordinary chalcones by the absence of the oxygen functionality at the 2-position [7].

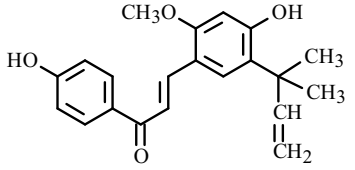
Glycyrrhizin exhibits many pharmacological effects, including antiinflammatory, antitumorigenic, and antihepatotoxic activity, and it enhances the regeneration of liver mass and function [8]. Licochalcone A, as the main retrochalcone in *G. inflata*, showed various biological properties, such as antiparasitic, estrogenic, antitumor, and so on [10–13].

Various methods have been published for the analysis of these compounds in licorice, including high-performance liquid chromatography (HPLC) [14–17], high-performance thin-layer chromatography (HPTLC) [18], and capillary electrophoresis [19, 20]. However, these published methods mainly involve *Glycyrrhiza glabra* and *G. uralensis* [21–22]. There are few special studies reported on the analysis of *Glycyrrhiza inflata.*

Based on the above-mentioned purpose, the aim of the current study was to develop a simple reversed-phase high-performance liquid chromatography (HPLC) method for simultaneous quantification of glycyrrhizin and licochalcone A. In order to validate the HPLC quantitative method, the specificity, linearity, accuracy, precision, and limits of detection and quantification of the method were investigated. The specific content of these compounds in eight samples using our technique was also examined.

Each of the six standards for glycyrrhizin, liquiritin, and licochalcone A was analyzed in three replicates. Table 1 shows the results. The calibration curve was constructed by plotting the peak area against the concentration (μg/mL) using linear regression analysis. The relative standard deviation (RSD) values of the peak areas of three replicate injections were between 1.52 and 3.29%.

Dilute solutions of the reference compounds were further diluted to a series of concentrations with methanol to assess the limits of detection (LOD) and quantification (LOQ). The LOD and LOQ under the present chromatographic conditions were determined at a signal-to-noise (S/N) ratio of 3 and 10, respectively.The LOD values for glycyrrhizin and licochalcone A were 4.2 ng and 3.3 ng. The LOQ values were 14.3 ng and 10.5 ng, respectively.



Licochalcone A

1) School of Chinese Pharmacy, Beijing University of Chinese Medicine, Beijing 100102, P. R. China, fax: +861084738611, e-mail: wwq57@126.com; 2) Department of Pharmaceutical Engineering, Tianjin University of Commerce, Tianjin 300134, P. R. China. Published in Khimiya Prirodnykh Soedinenii, No. 1, pp. 126–128, January–February, 2010. Original article submitted September 2, 2008.

TABLE 1. Analytical Parameters of the Calibration

Regression equation	Glycyrrhizin	Licochalcone A	Regression equation	Glycyrrhizin	Licochalcone A
Range	8.2–82.0 μg/mL	12.4–99.1 μg/mL	Intercept	22.912	24.936
Slope	13.264	834.18	r^2	0.9983	0.9994

TABLE 2. Precision and Reproducibility of Glycyrrhizin and Licochalcone A

Analyte	Precision (n = 3)				Reproducibility (n = 5)	
	Mean, mg/g[a]	R.S.D, %[a]	Mean, mg/g[b]	R.S.D, %[b]	Mean, mg/g	R.S.D, %
Glycyrrhizin	36.833	1.71	36.827	1.85	36.831	2.18
Licochalcone A	10.134	1.24	10.132	2.03	10.133	2.91

a: intra-day, b: inter-day.

TABLE 3. Accuracy Study of the Method for Determination of Glycyrrhizin and Licochalcone A (n =3)

Analyte	Added mean, mg	Recorded mean, mg	Recovery mean, %	R.S.D, %(n = 5)
Glycyrrhizin	1.285	1.279	99.53	1.78
Licochalcone A	1.057	1.010	95.55	3.27

TABLE 4. Results of Analysis of Glycyrrhizin and Licochalcone A in *Glycyrrhiza inflata*, mg/g

No.	Glycyrrhizin	Licochalcone A	No.	Glycyrrhizin	Licochalcone A
G1	36.83	10.13	G5	35.21	8.46
G2	19.92	8.67	G6	14.02	4.31
G3	23.13	10.11	G7	14.82	9.47
G4	26.39	9.18	G8	24.90	8.33

The intra- and inter-day precision was determined by analyzing calibration samples during a single day and on three different days, respectively. To confirm the reproducibility, five different working solutions prepared from the G1 sample were analyzed. The RSD was taken as a measure of precision and reproducibility (Table 2).

The recovery test was used to evaluate the accuracy of this method. Accurate amounts of reference compounds were added to the G1 sample and then extracted and analyzed as described before. The average recoveries were calculated with the formula: recovery (%)=(amount found–original amount)/amount spiked×100%, and RSD (%)= (SD/mean)×100% (n = 5) (Table 3).

The developed method was applied for the determination of glycyrrhizin and licochalcone A content in eight samples (G1–G8). Samples G1–G8 were from Xinjiang (China).The results showed that this method is suitable for the assay of glycyrrhizin and licochalcone A in *Glycyrrhiza inflata* (Table 4). The amount of different bioactive markers varied in the samples.

A specific, precise, and accurate HPLC method was developed and validated for the analysis of glycyrrhizin and licochalcone A in *Glycyrrhiza inflata.* It was successfully used for the analysis of glycyrrhizin and licochalcone A in samples from Xinjiang (China), and it can be applied for quality control of raw materials and extracts.

Eight plant samples of *Glycyrrhiza inflata* were obtained from different areas in Xinjiang (China). They were all identified by Prof. Wenquan Wang. The voucher specimens were deposited in the School of Chinese Pharmacy, Beijing University of Chinese Medicine, Beijing, China.

The HPLC equipment used was an HP-1100 system (Agilent, USA) including an HP-1100 quaternary pump, a degasser, a photodiode array detector, and HP ChemStation for LC 3D software. The column was a Pinnacle II C18 (i.d., 5 μm, Restek, USA). The balance was BP211D (Sartorius, Germany). An SK3200LH ultrasonic cleaning instrument (Shanghai Kudos Ultrasonic Instrument Co., Shanghai, China) was used.

Chromatography. Separations were achieved using a Pinnacle II C18 column (i.d., 5 μm, Restek, USA). Gradient elution began with a mobile phase of the aqueous phase containing 0.1% phosphoric acid –acetonitrile at a flow-rate of 1.0 mL/min. The percentage of acetonitrile in the mobile phase was programmed as follows: 10% (0 min) – 25% (10 min) – 32% (20 min) – 70% (60 min). The injection volume was 20 μL. Then the column was re-equilibrated for another 30 min, using a mobile phase composition of 90:10 (aqueous phase-acetonitrile) before the next injection. The elution was carried out at ambient temperature (35°C). Detection was made simultaneously at two different wavelengths, i.e., 254 nm and 360 nm.

Sample Solutions. A total of 25 mL of 70% methanol (aqueous) was added accurately to 0.1 g plant powder (passed through a 60 mesh screen) in a 50 mL volumetric flask. Then it was extracted by sonication for 50 min at room temperature. Before being injected into the HPLC system, all solutions were filtered through 0.45 μm membrane filters.

Standard Solutions. Stock solutions of the reference compounds were prepared by dissolving accurately weighed standards in methanol, transferring them to a 10 mL volumetric flask separately, and then adding methanol to make up the volume. The stock solution was further diluted to different concentration ranges. The calibration curve was plotted with at least six appropriate concentrations.

ACKNOWLEDGMENT

This research was financially supported by National Facilities & Information Infrastructure for Science and Technology Foundation (No. 2005DKA21000) and National Natural Science Foundation of China (No. 30572328).

REFERENCES

1. K. Y. Yen, *An Illustrated Pharmacognosy*, SMC Publishing Inc., Taipei, 1996.
2. S. L.Yang and Y. L. Liu, *Acta Botanica Sinica*, **30**, 176 (1988).
3. I. Kitagawa, K. Hori, M. Sakagami, F. Hashiuchi, M. Yoshikawa, and J. Ren, *Chem. Pharm. Bull.*, **41**, 1350 (1993).
4. K. Zou, Y. Y. Zhao, and R. Y. Zhang, *J. Chin. Pharm. Sci.,* **4**, 113 (1995).
5. G. Yoon, Y. D. Jung, and S. H. Cheon, *Chem. Pharm. Bull.*, **53**, 694 (2005).
6. H. Haraguchi, K. Tanimoto, Y. Tamura, K. Mizutani, and T. Kinoshita, *Phytochemistry*, **48**, 125 (1998).
7. K. Kajiyama, S. Demizu, Y. Hiraga, K. Kinoshita, K. Koyama, K. Takahashi, Y. Tamura, K. Okada, and T. Kinoshita, *Phytochemistry*, **31**, 3229 (1992).
8. K. Mitsutoshi, M. Tadashi, M. Hajime, M. Hiroyuki, S. Mariko, O. Hiroshi, I. Hideo, S. Toshitsugu, and O. Masahiko, *Eur. J. Pharmacol.,* **579**, 357 (2008).
9. K. Okada, Y. Tamura, M. Yamamoto, Y. Inoue, R. Taka-gaki, K. Takahashi, S. Demizu, K. Kajiyama, and Y. Hiraga, T. Kinoshita, *Chem. Pharm. Bull*., **37**, 2528 (1989).
10. M. M. Rafi, R. T. Rosen, A. Vassil, C. T. Ho, H. Zhang, G. Ghai, G. Lambert, and R. S. DiPaola, *Anticancer. Res*., **20**, 2653 (2000).
11. S. Shibata, H. Inoue, S. Iwata, R. D.Ma, L. J. Yu, H. Ueyama, J. Takayasu, T. Hasegawa, and H. Tokuda, A. Nishino, *Planta Med.*, **57**, 221 (1991).
12. R. De Vincenzo, C. Ferlini, M. Distefano, C. Gaggini, A. Riva, E. Bombardelli, P. Morazzoni, and P. Valenti, F. Belluti, F. O. Ranelletti, S. Mancuso, and G. Scambia, *Cancer Chemother. Pharmacol.*, **46**, 305 (2000).
13. H. Haraguchi, K. Tanimoto, Y. Tamura, K. Mizutani, and T. Kinoshita, *Phytochemistry*, **48**, 125 (1998).

14. V. Andrisano, D. Bonazz, and V. Cavrini, *J. Pharm. Biomed. Anal.*, **13**, 597 (1995).
15. Y. C. Wang and Y. S. Yang, *J. Chromatogr. B*, **850**, 392 (2007).
16. J. R. Hennell, S. Lee, C. S. Khoo, M. J. Gray, and A. Bensoussan, *J. Pharm. Biomed. Anal.*, **47**, 494 (2008).
17. H. Hayashi, S. Hattori, K. Inoue, K. Sarsenbaev, M. Ito, and G. Honda, *Chem. Pharm. Bull.*, **26**, 867 (2003).
18. S. F. Cui, B. Q. Fu, F. Lee Sen-Chun, and X. R. Wang, *J. Chromatogr. B*, **828**, 33 (2005).
19. C. Sabbioni, R. Mandrioli, A. Ferranti, F. Bugamelli, M. Addolorata Saracino, G. Cantelli Forti, and S. Fanali, M. Augusta Raggi, *J. Chromatogr. A*, **1081**, 65 (2005).
20. F. Rauchensteiner, Y. Matsumura, Y. Yamamotoa, S. Yamaji, and T. Tani, *J. Pharm. Biomed. Anal.*, **38**, 867 (2005).
21. T. P. Arystanova, M. P. Irismetov, and A. O. Sopbekova, *Chem. Nat. Comp.*, **37**, 89 (2001).
22. S. B. Denisova, E. G. Galkin, and Y. I. Murinov, *Chem. Nat. Comp.*, **42**, 285 (2006).