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Studies on Chromatography Fingerprint of Hongqi by High-performance Liquid Chromatography

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Abstract: Chromatography fingerprint (CFP) of 10 samples of hongqi were studied. 23 common peaks were analyzed, their average similarity was 97.29%. CFP were positioned with main index composition such as formononetin, calycosin and then the contents of index composition were determined. The character and exclusive of CFP of 10 samples of hongqi were clear. CFP and content determination of index composition of hongqi could be used to evaluate the quality of hongqi comprehensively.

Keywords: Hongqi, chromatography fingerprint, high-performance liquid chromatography.

Under present conditions, it is almost impossible to determine all chemical composition of traditional Chinese medicine (TCM), due to its complexity. But it does not mean that we have no way to evaluate the quality of TCM comprehensively. Chemical composition and content of TCM are different for different species, places of production and harvest time. The difference can be determined by advanced analysis method -CFP. Hongqi(*Hedyssarum polybolrys Hand-Mazz*[1]) is growing in southern part of Gansu province mainly, it is used as a substitute for huangqi for a long time in Gansu province. Up to now, there are some routine identification methods to control its quality. However there is no report on the determination of content of isoflavones and their CFP for hongqi.

Experimental

Reagents and samples

Hongqi was collected from southern part of Gansu and identified by professor Ru Neng ZHAO of Lanzhou Medical College. Formononetin and calycosin were provided by Shanghai TCM institute.

Chromatographic conditions

Instrument: Waters Alliance HPLC (USA) with a diode array detector. Analysis was

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performed with Hypersil C_{18} column, using water- acetonitrile as gradient mobile phase (**Table 1**). Spectrum of chromatography collecting range was from 190 to 400 nm.

Table 1 Mobile phase for determination of CFP of hongqi

time（min）	（mL/min）	H_2O（%）	CH_3CN（%）
0	1.0	96	4
20	1.0	75	25
40	1.0	55	45
60	1.0	30	70
70	1.0	20	80
80	1.0	0	100
120	1.0	0	100

Sample preparation

Butanol extraction: Hongqi (2.5 g) was extracted with methanol first, then methanol was evaporated and the residue was dissolved in water and extracted by *n*-butanol. After evaporation of solvent, the residue was dissolved in 5 mL methanol and filtrated. 10 μL of this solution was injected into HPLC.

Methanol extraction: The methanol extract of hongqi was filtrated into 10 mL volumetric flask and 20 μL of this solution was injected into HPLC.

Results and Discussion

Chromatograms of wavelength were chosen between 190-400 nm. The peaks at 254 nm were separated clearly. The CFP of hongqi showed in great number of peaks (**Figure 1**), formononetin and calycosin had the maximum absorption, which were used as references of CFP.

Sample solutions were prepared with *n*-butanol and methanol, respectively, and analyzed. The results showed that some sediment in methanol solution affected the stability of CFP and the number of peaks were less. Butanol could remove sugars and polysaccharides, and prolong the column life. The number of peaks of *n*-butanol solution were increased and separated well.

It was found that the retention time and UV spectra of the peaks 10 and 14 in CFP of hongqi were the same as those of formononetin (RT=28.404) and calycosin (RT=37.379) (**Figure 1**).

Analysis data and similarity of CFP: 10 different samples of hongqi were determined and compared with hongqi produced locally in Wudu Micang mountain and each UV spectrum was analyzed in turn to identify 23 common peaks, the similarity was calculated with Waters Pater Mach Microsoft as follows:

$$\text{similarity}=\frac{\sum_{i=1}^{n} X_i Y_i}{\sqrt{\sum_{i=1}^{n} X_i^2}\sqrt{\sum_{i=1}^{n} Y_i^2}}$$

X_i: (n≥10). peak area of peak i of each sample, Y_i: peak

area of peak i. The calculation showed that the result similarity of the 10 samples was 97.297%, which accord with the demands of technique guide to TCM injection CFP.

Precision and repetition of similarity: Inter-day precision of similarity in average 99.888%, RSD=0.880%. Intra-day precision of similarity (5 days) in average 92.811%, RSD=3.290%. Repetition of similarity in average 93.156%, RSD=1.157%.

Determination of formononetin and calycosin from hongqi was performed with Kromasil C_{18} column, using water-methanol (40-60—50-50, 10 min; 50-50—60-40, 30 min) as gradient mobile phase at a flow rate of 1.0 mL/min. Detection wavelength was 254 nm (**Figure 2**).

Figure 1 Typical chromatograms for determination of CFP of hongqi from 1. Wudu Mi cang mountain(date acquired: 2003-4-3 2:32), 2. Minxian (date acquired: 2003-4-3 4:42), 3. Longxi Shouyang (date acquired: 2003-4-3 15:36). All samples were extracted with butanol.

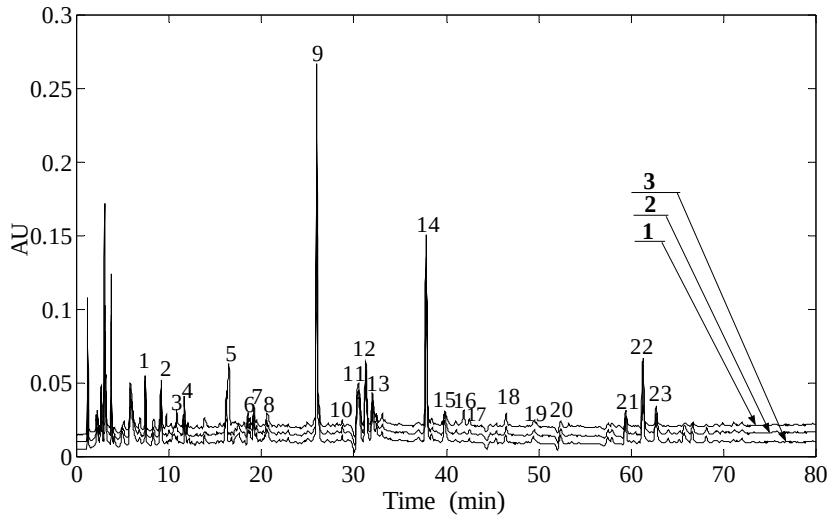
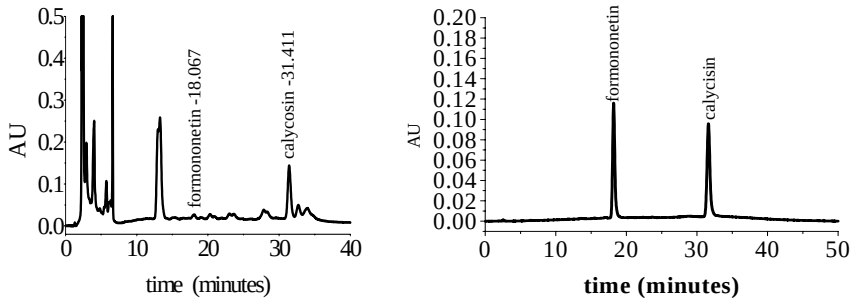


Figure 2 Chromatogram of formononetin and calycosin in hongqi



Sample of hongqi Standards

Conclusion

The similarity of CFP of 10 samples was over 90% and their characteristic and specificity were apparent. The CFP of hongqi showed no apparent difference between unripe hongqi in July and in October. The similarity was calculated by the peak areas of the CFP. CFP peaks not only can represent the content of composition,but also indicate the character of each peak. So CFP can be used for qualitative and quantitative analysis of hongqi.

It was reported[2] that the difference between hongqi and huangqi was apparent. There was no astragaloside IV in hongqi. Our study further proved this result. Formono- netin and calycosin were contained in both hongqi and huangqi, but in hongqi the content of calycosin was higher than that of formononetin, while in huangqi it was adverse. The combination of the CFP and the content determination of formononetin and calycosin in hongqi could be used to evaluate the quality of hongqi comprehensively.

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