

황기의 품질관리를 위한 isoflavonoid 동시분석방법

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Simultaneous Determination of Isoflavonoid for Quality Control in *Astragalus membranaceus*

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Objectives

- The natural resources of *A. membranaceus* is diminishing due to increasing demand for Radix Astragali and hence the bulk of the commercial supply is mainly taken from farming sources. The level of the active ingredients varies widely depending on the regions where the plants are grown and the period when they are harvested. The isoflavonoids, considered “marker compounds” for the standardization of Huangqi, can be detected by HPLC analysis. However the experimental conditions reported so far do not give well separated individual peaks.
- The purpose of the present studies is to quantify the pharmaceutical active compounds and marker compound, formononetin, calycosin, and its derivate from different tissue parts, harvested year and cultivation period of *A. membranaceus*.

Materials and Methods

- We investigated calibration, chromatographic condition, extraction recovery and stability of six isoflavonoid for determination simultaneous quantitative analysis method
- Determination of optimal cultivation periods and harvest time used to marker compound analysis using determined simultaneous quantitative analysis method

Results

- In this study a HPLC-UV method is described which permits simultaneous quantitative analysis of six major isoflavonoids, calycosin 7-O-h-d-glucoside (1), formononetin 7-O-β-d-glucoside (2), (6αR, 11αR)-3-hydroxy-9,10-dimethoxypterocarpan 3-O-β-D-glucoside (3), 7,2'-dihydroxy-3',4'-dimethoxyisoflavan-7-O-β-D-glucoside (4), calycosin (5) and formononetin (6) in Radix Astragali.
- All six HPLC calibration curves of isoflavonoids (1-6) exhibited good linearity with excellent correlation coefficient. The lowest LOD is 0.765 ng (2) and the highest is 1.343 ng (6). The precision of the results using the HPLC system was tested by six repeated injections of each standard (1-6) and the RSDs are less than 4% (RSD%: 1, 1.03 ; 2, 0.90; 3, 0.96; 4, 3.56; 5, 3.72; 6, 1.38).

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- The consistency of the comparatively low recovery of 3 (81.6–88.8%) at all levels is noted for future reference in developing standardization assay. As a whole the results clearly indicate that the level of isoflavonoids present does not affect the % recovery provided the level is within the linear range.
- The stability profile of the six isoflavonoid from root of *Astagalus membranaceus* was determined. The results show that there are no significance changes of these compounds during the 72 h storage period.
- *A. membranaceus* mainly accumulates its root isoflavonoids from May to the first frost day, in November. Thus, the optimal harvest time for isoflavonoid such as formononetin and calycosin is around the November in main and later root part of *A. membranaceus*. One-year-old plants and 2-year-old plants have similar concentration of isoflavonoids whereas the concentration of these compounds in 3-year-old plants are 50% higher than those in 1 and 2-year-old plants.
- The results strongly indicate the significance of isoflavonoid formononetin or calycosin in contributing to the biological activities of *A. membranaceus*.

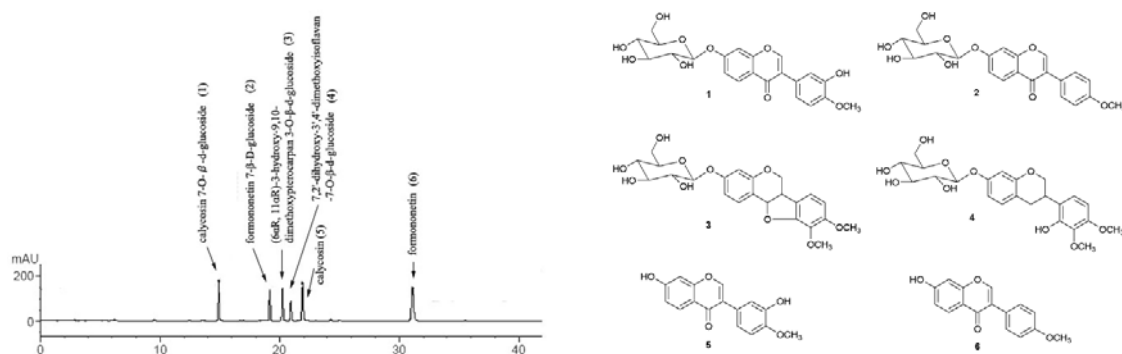


Fig. 1. Chromatograms of HPLC eluted with a gradient mobile phase on six isoflavonoid form root of *Astagalus membranaceus* (0% to 30% B in 15 min, flow rate from 1.2 ml/min to 1.0 ml/min; 30% to 40% B from 15 min to 30 min, flow rate 1.0ml/min; and 40% to 100% B from 30 min to 60 min, flow rate 1.0ml/min; column temperature: 40°C).

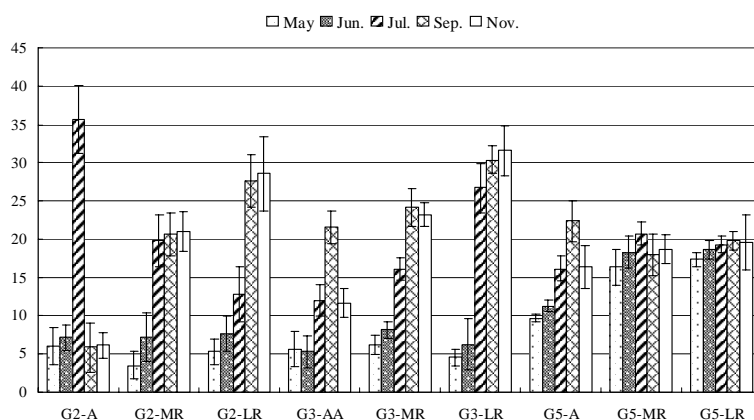


Fig. 2. Comparison of formononetin contents in *Astagalus membranaceus* by different parts and cultivation periods. G: years of perennation; A : aboveground parts; MR : main roots; LR : lateral roots