

Supplementary data 1: Investigated the concentration of 3,6'-Disin apoysucrose, tenuifoliside A, tenuifoliside B and tenuifoliside C in Radix Polygalae and its' stability of water extract solutions.

Materials

3,6'-Disin apoysucrose (98%, purity) was purchased from the Welch Materials, Inc. (Shanghai, China). Tenuifoliside A, tenuifoliside B and tenuifoliside C were purchased from the Shanghai Yilin Biology Science Technologies Co. LTD (Shanghai, China), the purity of these three compounds were greater than 95%. Acetonitrile (HPLC-grade) was purchased from Fisher Scientific. (Fair Lawn, NJ, USA). Radix Polygalae was provided by Beijing Tongrentang Co., Ltd (Beijing, China).

Methods

Chromatography conditions

The analysis of 3,6'-Disin apoysucrose, tenuifoliside A, tenuifoliside B and tenuifoliside C were performed using a Prominence LC-20A HPLC system equipped with a SPD-20A UV-Detector (SHIMADZU Corporation, Japan) on a Merck Purospher® STAR LP RP-C18 endcapped HPLC column (250 mm×4.6 mm, 5µm). The column was maintained at 30°C, the detection wavelength was 320 nm, the flow rate was 1.0 mL min⁻¹, and the injection volume was 10µL. The gradient elution employed acetonitrile as solvent A and 0.1% formic acid aqueous solution as solvent B. The gradient program was as follows: 0-10 min, 10% A; 10-25 min, 10-20% A; 25-50 min, 20-35% A; 50-55 min, 35-90% A; 50-60 min 90% A; 60-61 min, 90-10% A 61-70 min 10% A.

Sample prepared

Determination sample prepared: The powdered sample (1.00 g, through No.4 sieve) was refluxed extracted with 50 mL 70%-methanol for 90 min, cooled at room temperature and subsequently filtered through 0.22µm nylon membrane filters for HPLC analysis.

Stability sample prepared: The Radix Polygalae was decocted with 10 fold water for two times, each time for 1.5 h. The resulting filtrates were combined and determined

at 0h and 6h after heating at 100°C. The solutions were filtered through 0.22µm nylon membrane filters for HPLC analysis.

Standard samples prepared: 3,6'-Disin apoysucrose, tenuifoliside A, tenuifoliside B and tenuifoliside C were weighed accurately, dissolved in methanol and diluted to the concentrations were 0.2mg/mL.

Results

The analytical method was subsequently applied to the analysis of four compounds in this extracts. The contents of 3,6'-Disin apoysucrose, tenuifoliside A, tenuifoliside B and tenuifoliside C were 26.89, 11.73, 5.41, 5.93 mg/g, respectively, which were calculated with external standard methods. The chromatogram is shown in Fig.1. The stability studies showed that the contents of 3,6'-Disin apoysucrose, tenuifoliside A and tenuifoliside C were decreased 15.6%, 19.0% and 32.7% after heated under 100°C for 6 hours, respectively. But the contents of tenuifoliside B was increased 5.9%, this might be caused by the conversion of other ingredients under this condition.

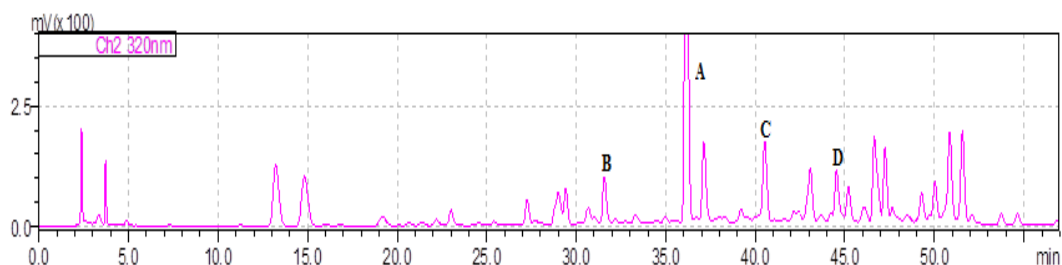


Fig.1. The HPLC chromatogram of Radix Polygalae. (A=3,6'-Disin apoysucrose, B=Tenuifoliside A, C=Tenuifoliside B, D=Tenuifoliside C)