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Determination of Arctiin and Arctigenin Contents in *Arctium Tomentosum* Mill. by HPLC Method

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Abstract: A simple, precise, rapid and accurate, binary-phase high performance liquid chromatographic method has been developed for the determination of arctiin and arctigenin contents in the *Arctium tomentosum* Mill. with short run time. Chromatographic separation was achieved by using HPLC system, consisting of a Shimadzu LC-6AD and Kromasil C_{18} column (250×4.6 mm, 5 μm, with pre-column), the mobile phase consists of methanol and water (55: 45). Detection wavelength was 280 nm. The speed of flow was 1.0 mL/min. The specimen handing quantity was 10 μL. The arctiin's linearity range was 1.575～4.725 μg (r=0.9995). The arctigenin's linearity range was 0.613, 3.063 μg (r = 0.9998) and the linear relationship was accurate. The average recovery (n=5) of arctiin and arctigenin were 101.55% (RSD=2.23%)、101.63% (RSD =1.49 %) respectively. The contents of arctiin and arctigenin in *Arctium tomentosum* Mill. were 10.69 mg/g and 0.15 mg/g, respectively. Therefore, the developed HPLC method can be applied to both *in vitro* studies of arctiin and arctigenin formulations as well as drug estimation in biological samples.

Keywords: HPLC, Arctiin, Arctigenin, *Arctium tomentosum* Mill.

Introduction

Arctium tomentosum Mill., burdock biennial herb, is dried ripe fruit[1]. Plants to 250 cm with large basal leaves and alternate leaves.[2]; Flowering summer–early fall (Jul–Oct). *Arctium tomentosum* Mill. distribute in Siberia, Middle Asia (Kazakhstan; Kyrgyzstan; Tajikistan), China (Xinjiang), Europe, Switzerland and so on[3]. The literature furnishes numerous data on their

anti-inflammatory, hepatoprotective, antitumor, antimicrobial, antifungal, anti-aging and hypoglycemic effects[4-5]. Meanwhile, it contains multiple chemical components, mainly lignans, volatile oils, phenols and sulfur polyacetylenes, *etc*.[6]. A research focus on the lignan compounds, which are mainly arctiin, arctigenin[7]. Modern pharmacology researches indicate that Fructus Arctii active components is arctiin[8]. After oral administration, under the action of bacteria in the gut, arctiin changes into demethylation of aglycone, which revert to the aglycone under the action of catechol-*O*-methyltransferase (COMT) in the liver[9]. So that control arctiin (Figure 1) and arctigenin (Figure 2) contents in arctium radix simultaneously, it can achieve the purpose of control the medicinal quality**.** The authors detect the arctiin and arctigenin contents in arctium radix by the method of high performance liquid chromatography (HPLC).

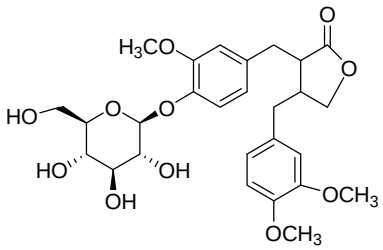


Figure 1. Chemical formula of arctiin

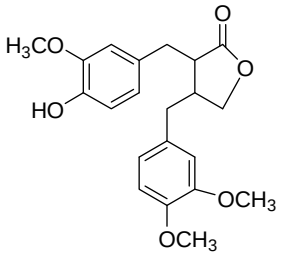


Figure 2. Chemical formula of arctigenin

Currently, under referring to relevant literatures, the researches about chemical composition and pharmacological activity of arctii are reported extensively. However, there are few reports about research on *Arctium tomentosum* Mill**.** No report about research on different parts of *Arctium tomentosum* Mill. and content of arctigenin. The research with different parts of *Arctium tomentosum* Mill., containing root, stem, leaf, child and inflorescence, *etc.,* which to better provide the basis for development and utilization of *Arctium tomentosum* Mill.

Experimental

Arctium tomentosum Mill is collected from Altai mountain of Xinjiang Province, China. Voucher specimens were deposited in Traditional Chinese Medicine College Museum of Chinese herbal samples of Xinjiang Medical University.

Arctiin and arctigenin are obtained from national institute for the control of pharmaceutical and biological products, 090516, 090712; HPLC grade Methanol supplied by USA, Fisher Scientific, 201-796-7100. Potassium phosphate monobasic of analytical grade is obtained from Tianjin, Tianxin chemical reagents company. All reagent solutions and buffers are prepared with water from Millipore Q3 ultra-pure water system (Millipore, USA).

Instrumentation and chromatographic conditions

The HPLC system consists of a Shimadzu LC-6AD, SPD-20AVP variable wavelength UV detector and Shimadzu CBM-20A station for data analysis. The analytical column is Kromasil C_{18} column (4.6×250 mm, 5 μm, with pre-column). The mobile phase consists of methanol and water (55:45). Detection wavelength is 280 nm. The speed of flow is 1.0 mL/min. The specimen handing quantity is 10 μL. All separations are performed at ambient temperature.

Sample preparation

Powder samples (0.5 g) were extracted by methanol (50 mL) for 30 min with ultrasonic extraction, filtered, dried in water bath, dissolved with methanol and transferred to a 10 mL measuring flask and finally have the volume to the calibration using methanol and shaken evenly before filtering in a 0.45 μm membrane filter.

Standard solution preparation

A standard solution, containing 0.63 mg/mL of arctiin and 0.49 mg/mL of arctigenin, was prepared by dissolving in methanol.

Results and Discussion

Optimization of the chromatographic conditions

The optimum mobile phase consists of methanol and water (55:45). The speed of flow is 1.0 mL/min and detection wavelength is 280 nm. Retention time for arctiin and arctigenin are 7.06 min and 16.96 min, respectively. The results are shown in Figure 3 and 4. An HPLC chromatograph of arctiin and arctigenin in standard solution is shown in Figure 3, an HPLC chromatograph of arctiin and arctigenin in sample solution is given in Figure 4.

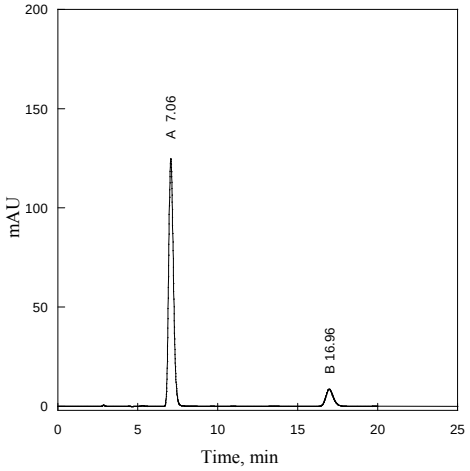


Figure 3. Standard solution chromatogram chromatogram (A: arctiin, B: arctigenin)

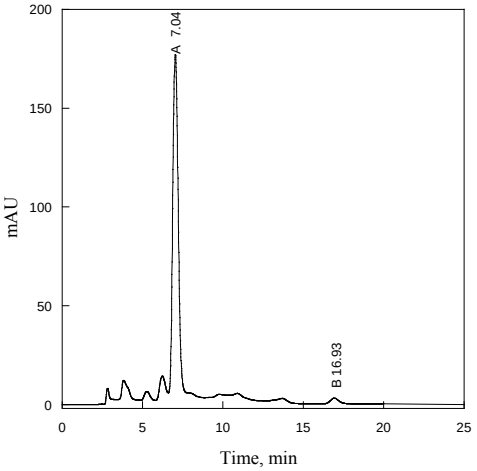


Figure 4. *Arctium tomentosum Mill.* sample solution (A: arctiin, B: arctigenin)

Specification curve

Arctiin standard curve: Y=331550X-141006 (x is the concentration of arctiin and y is the peak area integral value of arctiin). The linearity range of arctiin was 1.575～4.725 μg, with a mean correlation coefficient r = 0.9995.

Arctigenin standard curve: Y=9806.6X +4230.7 (x is the concentration of arctigenin and y is the peak area integral value of arctigenin). The linearity range of arctigenin was 0.613～3.063 μg, with a mean correlation coefficient r = 0.9998.

Precision

The contents of arctiin and the arctigenin was determined according to the above chromatographic condition, which was mixed with 10 μL standard comparison solution.

The specimen was handled for six times. The arctiin and the arctigenin peak area integral values were determined. The relative standard deviation (RSD) % is 2.9% and 3.6%, respectively. The results obtained was confirmed a good precision of the method developed.

Stability

The identical sample was taken to supply the test solution for 0, 2, 4, 8 and 24 h, respectively and determined the arctiin and the arctigenin peak area integral values. The relative standard deviation (RSD) % is 2.2 % and 0.51%, respectively. The experimental results indicate that samples are stable in 24 h.

Reproducibility

Arctium tomentosum Mill. powder 0.5 g was taken according to the method explained in the experimental section to prepare sample. The arctiin and the arctigenin peak area integral values were determined according to the above chromatograph condition. The relative standard deviation (RSD) % is 2.68% and 0.89%, respectively. The experiment results indicate that the accuracy is good.

Recovery

0.25g *Arctium tomentosum* Mill. of the known content for each group was taken and 27.4 mg arctiin standard solution and 0.38 mg arctigenin standard solution was added, then the spotting recovery rate was calculated. According to the method explained in the experimental section to prepare sample and standard curve method was used to compute the content, the average recovery (n = 6) of arctiin is 101.55% (RSD=2.23%) and of arctigenin is 102.3% (RSD = 1.49%). The results are shown in Tables 1 and 2.

Table 1. Recovery rate of arctiin

No.	Sampling quantity, g	Sample content A, mg	Addition B, mg	Determination C, mg	Recovery, %	Average, %	RSD, %
1	0.254	27.15	27.4	55.38	103.02		
2	0.257	27.47	27.4	54.37	98.16		
3	0.258	27.58	27.4	55.14	100.58	101.55	2.23
4	0.257	27.47	27.4	55.43	102.03		
5	0.253	27.05	27.4	55.53	103.96		

Recovery rate = [(C-A)/B] ×100%

Table 2. Recovery rate of arctigenin

No.	Sampling quantity, g	Sample content A, mg	Addition B, mg	Determination C, mg	Recovery, %	Average, %	RSD, %
1	0.254	0.38	0.38	0.77	102.63		
2	0.257	0.39	0.38	0.78	102.63		
3	0.258	0.39	0.38	0.78	102.63	101.63	1.49
4	0.257	0.39	0.38	0.77	99.21		
5	0.253	0.38	0.38	0.76	101.05		

Recovery rate = [(C-A)/B] ×100%

Quantification of arctiin and arctigenin in sample

The standard addition and recovery experiments were conducted to determine the accuracy of the present method for the quantification of arctiin and that of arctigenin in *Arctium tomentosum* Mill. samples. The contents of arctiin and arctigenin in *Arctium tomentosum* Mill. are 10.69 mg/g and 0.15 mg/g, respectively.

Conclusion

A simple and sensitive HPLC method is developed for the determination of arctiin and arctigenin contents in *Arctium tomentosum* Mill. This easy method provided excellent sensitivity, accuracy and precision, with relatively short retention time for both arctiin and arctigenin. This developed HPLC method can therefore be applied to both *in vitro* studies of arctiin and arctigenin formulations as well as drug estimation in biological samples. The method was validated and found to be rapid, precise, accurate and linear for the concentration detection therefore can be used to quantify arctiin and arctigenin contents in *Arctium tomentosum* Mill. Fast experiment running time, confident level in analytical results and cost-effectiveness are key objectives for today's analytical laboratories.

Acknowledgment

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