Analysis and Evaluation on the Quality of Pueraria Lobata in the Market Based on High Performance Liquid Chromatography (HPLC)

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Abstract: Objective: To evaluate the quality of Pueraria lobata sold in the market and establish a HPLC method for the analysis of Pueraria lobata. Method: Acclaim 120 C18 chromatographic column (5 μm. 4.6 * 250mm), acetonitrile-0.1% formic acid aqueous solution was used as the mobile phase for gradient elution, and the contents of 5 components, 3'-methoxy puerarin, puerarin, daidzin, daidzein, and 3'-hydroxypuerarin, were determined in 11 commercial pueraria lobata from different habitats. Results: The contents of these five components in pueraria lobata from different habitats were different. Anhui No. 003 and No. 006 have higher chemical constituents. Conclusion: The method established in this experiment is accurate, with good separation effect, and can be used to detect the content of five different substances in Pueraria lobata. The determination results can provide valuable reference information for the purchase of traditional Chinese medicine stores and provide effective scientific basis for the development of Pueraria lobata resources.
***Keywords*:** HPLC, pueraria lobata, chinese medicine market, place of origin

1. Introduction

Pueraria lobata belongs to the dried roots of Pueraria lobata (wild) Ohwi or Pueraria thomsonil Benth. It has the functions of relieving fever, producing fluid and relieving thirst, rising Yang and relieving diarrhea with curative functions on indications of fever, strong pain on the top and back, fever and thirst. Modern studies have shown that the main pharmacological components of puerarin, daidzin, daidzein, triterpenoids and other compounds have significant effects on antidrinking, reducing blood pressure and myocardial oxygen consumption, as well as reducing blood lipid and blood glucose. In addition, Daidzein contained in Pueraria lobata also has certain inhibitory effects on a variety of cancers.

Currently, Pueraria lobata is extensively used, and its processed products involve food, fishery, cosmetics and other industries. Therefore, the quality evaluation of Pueraria lobata is of paramount importance. The storage and processing methods, harvesting methods, harvesting season and varieties of Pueraria lobata from different places vary accordingly, so the manufacturers have different needs for Pueraria lobata. This experiment will evaluate the quality of Pueraria lobata from different places, establish a HPLC method for analyzing and determining the effective chemical components of Pueraria lobata,so as to provide a reliable component evaluation for Pueraria lobata for the market and a visual scale for different industries' different needs for Pueraria lobata.

2 Preparation before experiment

2.1 Materials

The 10 different kinds of Pueraria lobata samples were collected and purchased in Shaanxi, Hebei, Hubei, Anhui, Hunan and Henan provinces in May 2022 with the following sample numbers:

Table 1. Study on information table of sample collection

Sample Name	Sample No.	Batch No.	Place of origin
Pueraria lobata	001	\	Hubei
Pueraria lobata	002	2112085	Hunan
Pueraria lobata	003	2205001	Anhui
Pueraria lobata	004	21022102	Anhui
Pueraria lobata	005	\	Shaanxi
Pueraria lobata	006	\	Yuexi, Anhui
Pueraria lobata	007	21053001	Anhui
Pueraria lobata	008	22022101	Anhui
Pueraria lobata	009	\	Yingshan, Hubei
Pueraria lobata	010	21053001	Hebei
Pueraria lobata	011	20220314	Henan

2.2 Reagents and instruments

Acclaim 120 C18 column (5μm, 4.6*250mm); 3'-methoxypuerarin, Puerarin, Daidzin, daidzein, 3'-hydroxypuerarin were purchased from Chengdu Biopurify Phytochemicals Ltd. Liquid Chromatograph UltiMate 3000 HPLC (Thermo Scientific); Milli-Q pure water meter Me-rck Millipore (Merck Millipore); Numerical control ultrasonic cleaner KQ-500DE (Kunshan Ultrasonic Instrument Co., Ltd.); BJ-200 (Deqing Baijie Electric Appliance Co., Ltd.); Electronic balance BCE95PI-1CEU (Actual indexing value: 0.01mg, Sartorius); Electronic balance BCA224I-1OCN (Actual indexing value: 0.1mg Sartorius); Electronic balance BCE4202-1CCN (Actual indexing value: 0.1g Sartorius).

3. Condition optimization

3.1 Selection of chromatographic column

In view of the complexity of components in Pueraria lobata, C18 （5μm, 4.6*250mm was preferred for multi-component separation.The mobile phase was obtained from literature survey with Acetonitrile-0.1% formic acid in water.

The separation spectrum is shown in Figure 1. Multiple groups of chromatographic peaks can be separated on C18 column of the test solution. Compared with the control solution, the peak of the test solution is 3' -hydroxypuerarin at 4.9min, the peak of the test solution of Pueraria lobata stand at 7.7min, and the peak of the test solution of 3'-methoxy puerarin is at 8.7min. The peak of Daidzin at 13.8min is reached and the peak of Daidzein is reached at 15.8min. This column is therefore chosen to continue development. It was observed that there were interference peaks near 3'-hydroxypuerarin and Daidzein in the solution spectrum of the test sample, and there were multiple groups of envelope peaks. In order to study its fingerprint, the extraction solvent and chromatographic conditions should be further optimized.

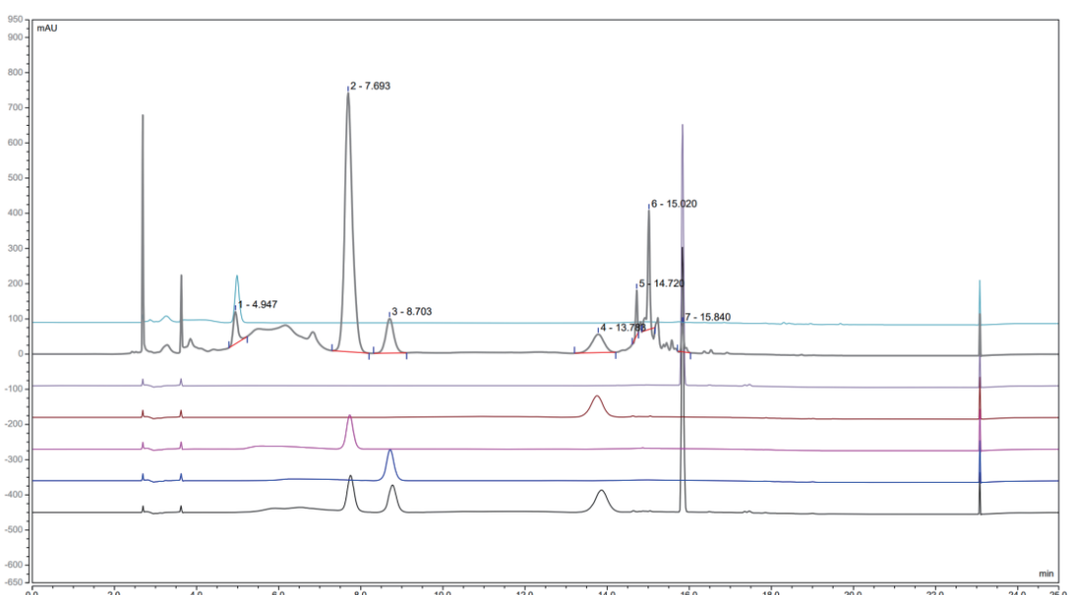

Figure 1. Reference substance separation on C18 column

(From bottom to top,the sequence is as 50ppm mixed standard solution of reference substance, 3' -methoxy puerarin reference substance solution, Puerarin reference substance solution, Daidzin reference substance solution, Daidzein reference substance solution, test substance solution, 3'-hydroxy puerarin reference substance solution)

3.2 Selection of extraction solvent

As the tested products are Chinese herbal decoction pieces, the characteristic components are mostly water-soluble or alcohol-soluble components. Methanol, 30% methanol, 50% methanol, 70% methanol; Ethanol, 30% ethanol, 50% ethanol and 70% ethanol were used as diluents to compare the extraction efficiency of different solvents for the characteristic components in Pueraria lobata decoction pieces.

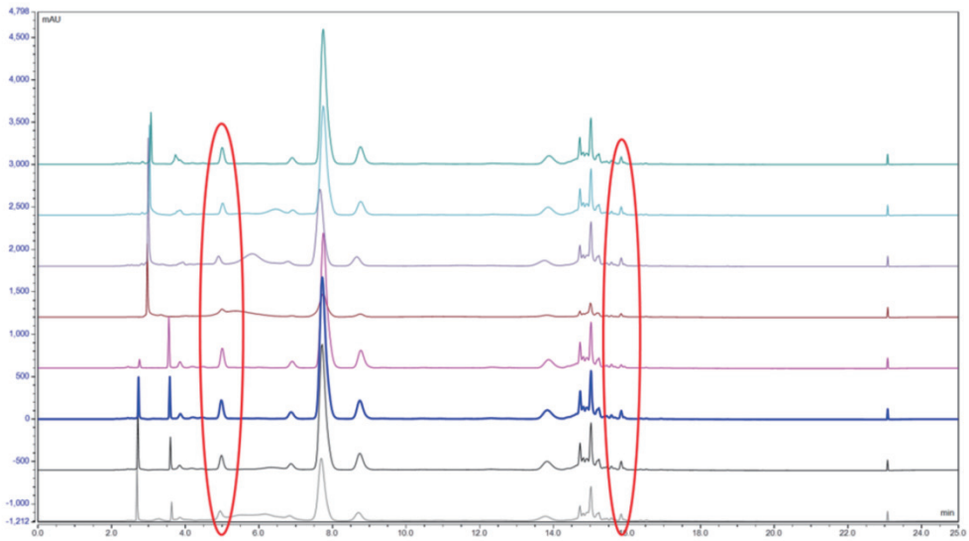

Figure 2. Separation of characteristic peaks of Pueraria lobata sample solution under different extraction solvents

(From bottom to top, methanol, 70% methanol, 50% methanol, 30% methanol, ethanol, 70% ethanol, 50% ethanol, 30% ethanol are used as the extraction solvent for the test solution)

Based on the map, when 50% methanol or 30% ethanol was used as the extraction solvent, the 3'-hydroxypuerarin in the test solution was not easily disturbed by the solvent and other substances, and the extraction content of Puerarin was higher. Compared with 30% ethanol, 50% methanol as extraction solvent. The extraction content of Daidzein is higher and it is easier to be accurately quantified. Therefore, 50% methanol was selected as the extraction solvent of Pueraria lobata to study the fingerprint of Pueraria lobata. Due to the large number of unseparated chromatographic peaks between Daidzin and Daidzein, the chromatographic conditions need to be further optimized.

3.3 Optimization of chromatographic conditions

After optimized chromatographic conditions, the separation spectra are shown in Figure 3. The chromatographic peaks of the components to be tested can be completely separated from the chromatographic peaks of other components in the test solution, and in addition to the five quantitative characteristic peaks, six characteristic peaks can be selected as fingerprint peaks. Therefore selected as the test chromatographic conditions.

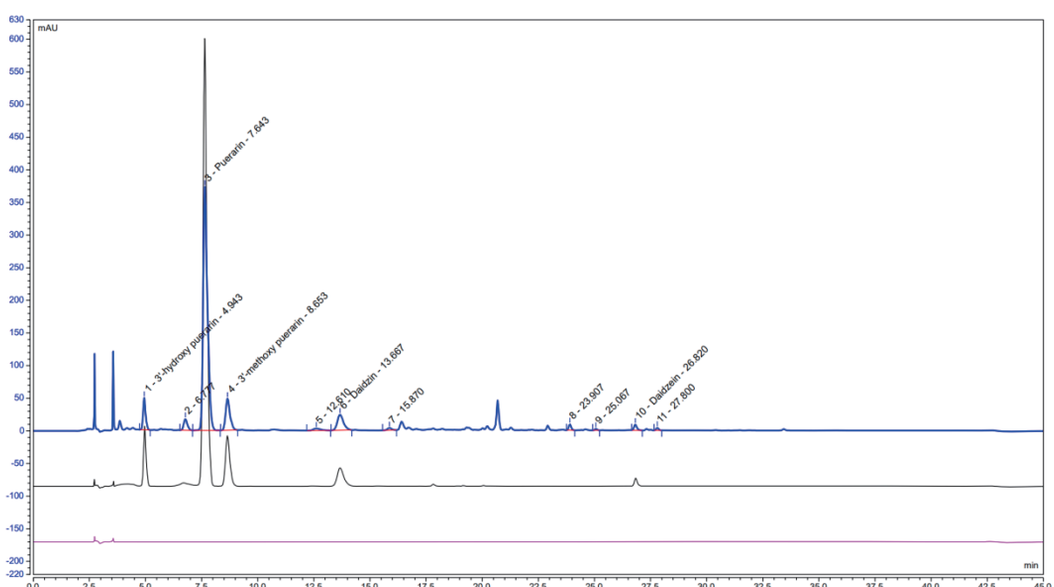

Figure 3. Separation of Pueraria lobata fingerprint on C18 column

(From bottom to top,the sequence is: blank solution, reference mixed standard solution, test solution)

4. Method pre-verification

4.1 Linear relationship

3'-hydroxy puerarin: y = 0.4288x +0.4873, r^2=0.998, linear range is 1.355~40.65μg/ml; Puerarin: y = 0.6893 x +1.4269, r^2=0.999, linear range is 10.04~200.9μg/ml; 3'-methoxypuerarin: y = 0.5713 x+0.2228, r^2=0.999, linear range is 1.581~47.43μg/ml; Daidzin: y =0.6340 x +0.0028, r^2=0.999, linear range is 0.8078~24.23μg/ml; Daidzein: y = 0.8982 x +0.0978, r^2=0.999, linear range stands 0.1082~6.493μg/ml; The linear correlation coefficients are all greater than 0.95, which proves that the linear relationship is good.

4.2 Inspection of specificity

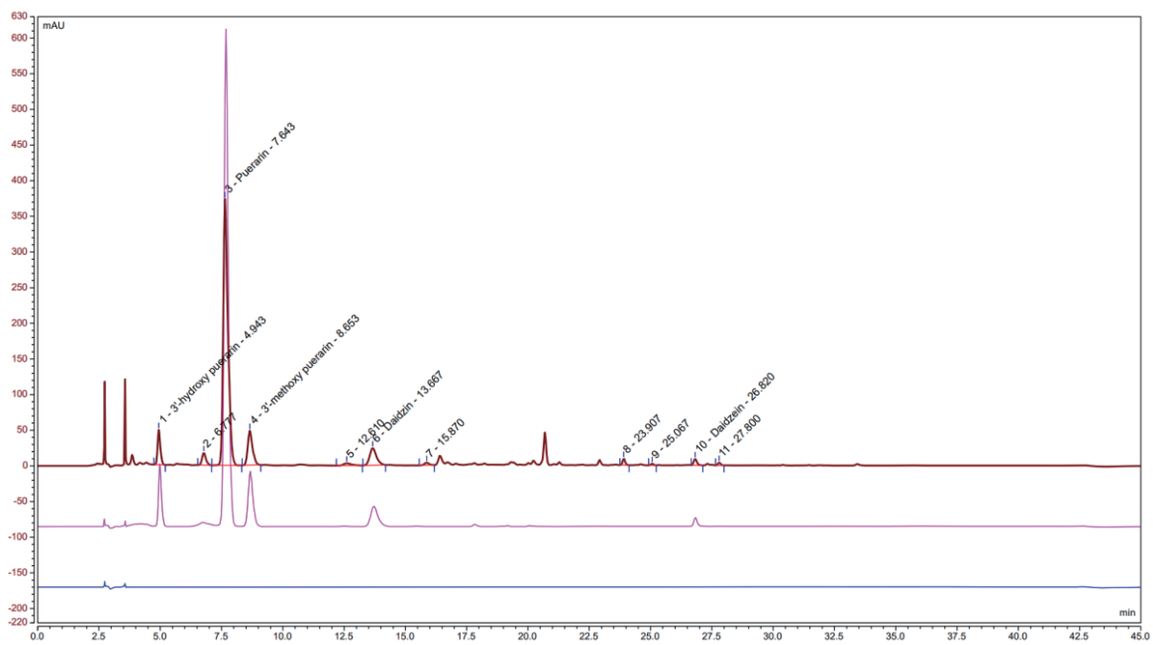

Figure 4. Specificity separation spectrum

(From bottom to top, the sequence is: blank solution, linear solution LIN100 and test solution)

Based on the investigation results, the blank solution, linear solution LIN100 and test solution had no interference at the peak of the target.

4.3 Precision test

The recoveries of six 100% concentration spiked solution of Puerarin stood at 97.0%~102.7%. The recovery rate of 3'-methoxypuerarin 6 was 95.6%~100.7%. The recoveries of 6 samples of Daidzin with 100% concentration were 94.3%~101.1%. The recoveries of six Daidzein solutions with 100% concentration were 91.3%~98.3%. The recoveries of the five substances to be tested were all in the range of 90%~108%. The accuracy of this method is good.

4.4 Repeatability test

The reproducible RSDS of 5 components in 6 test solutions were not more than 3.0%. This method shows good repeatability.

4.5 Stability test

Within 43 hours, the RSD of the peak area of the substance to be measured in the reference solution was not more than 6%. The concentration of the substance to be measured in the test solution was calculated based on the peak area of the substance to be measured in the reference solution, and the RSDS of the substance to be measured in the test solution were not more than 6% within 38 hours. The results showed that the control solution was stable within 43 hours at room temperature, and the test solution was stable within 38 hours at room temperature.

5. Sample determination

5.1 Sample testing

A single sample of 0.1g of cool Pueraria lobata fine powder was added to 50ml of 50% methanol for ultrasonic extraction for 30min, which can be evenly shaken, filtered, and the subsequent filtrate was taken as the solution of the sample.

Table 2. Test results of wood Pueraria lobata

Sample No.	Content %				
	3'-hydroxypuerarin	Puerarin	3'-methoxypuerarin	Daidzin	Isoflavoues Aglycone
001	0.707	5.720	0.906	0.559	0.075
	0.706	5.700	0.914	0.561	0.077
002	1.115	4.617	0.876	0.434	0.160
	1.123	40643	0.881	0.437	0.161
003	0.112	7.148	1.078	0.713	0.092
	1.073	70267	1.091	0.728	0.086
004	0.895	4.607	0.759	0.550	0.238
	0.811	4.503	0.738	0.531	0.224
005	1.053	5.753	1.198	0.560	0.053
	1.127	5.931	1.243	0.581	0.050
006	1.188	5.170	0.943	0.574	0.242
	1.222	5.204	0.959	0.585	0.242
007	1.029	4.792	0.833	0.622	0.210
	0.965	4.479	0.787	0.599	0.192
008	0.807	4.887	0.779	0.531	0.148
	0.825	4.887	0.778	0.528	0.145
009	0.988	4.659	0.812	0.574	0.147
	0.943	4.680	0.808	0.579	0.150
010	1.080	4.559	0.786	0.616	0.192
	1.100	4.659	0.803	0.631	0.197
011	0.975	5.081	0.836	0.534	0.157
	0.930	4.944	0.821	0.521	0.156

5.2 Fingerprint pattern

The fingerprint of 11 batches of wood Pueraria lobata is shown in Figure 5-1, with a total of 11 characteristic peaks. With Puerarin as S peak, the relative retention time and relative peak area are calculated, and the results are shown in Table 5-2 to 5-3. The relative retention time error of each peak is in the range of 5%, which can be used as fingerprint characteristic peak research.

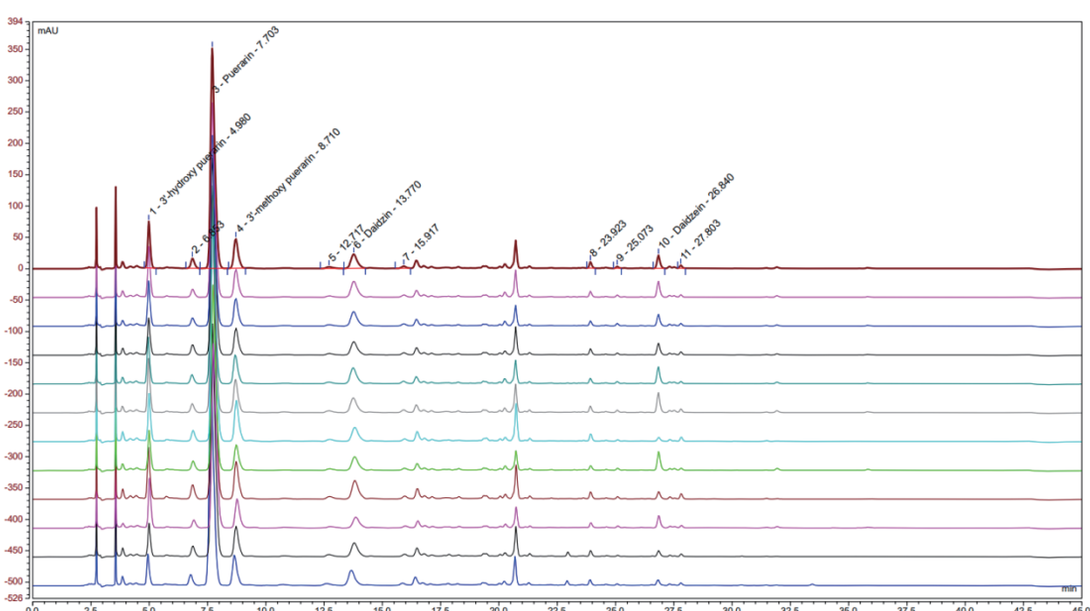

Figure 5. The fingerprint of 11 batches of wood Pueraria lobata (001-011 of test solution from bottom to top)

Table 3. Fingerprint results of 11 batches of wood Pueraria lobata - relative retention time

Sample No.	Relative retention time										
	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11
001	0.65	0.89	1.00	1.13	1.65	1.79	2.07	3.10	3.25	3.48	3.61
002	0.65	0.89	1.00	1.13	1.65	1.79	2.06	3.09	3.24	3.46	3.59
003	0.64	0.89	1.00	1.13	1.65	1.79	2.07	3.10	3.25	3.48	3.60
004	0.65	0.89	1.00	1.13	1.65	1.79	2.06	3.10	3.25	3.47	3.60
005	0.65	0.89	1.00	1.13	1.65	1.79	2.06	3.09	3.24	3.47	3.60
006	0.65	0.89	1.00	1.13	1.65	1.79	2.07	3.11	3.26	3.49	3.61
007	0.64	0.89	1.00	1.13	1.65	1.79	2.07	3.11	3.26	3.49	3.62
008	0.65	0.89	1.00	1.13	1.65	1.79	2.06	3.10	3.25	3.48	3.61
009	0.65	0.89	1.00	1.13	1.65	1.79	2.07	3.11	3.25	3.48	3.61
010	0.65	0.89	1.00	1.13	1.65	1.79	2.07	3.10	3.25	3.48	3.61
011	0.65	0.89	1.00	1.13	1.65	1.79	2.07	3.11	3.25	3.48	3.61

Table 4. Fingerprint results of 11 batches of wood Pueraria lobata

Sample No.	Relative peak area										
	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8	Peak 9	Peak 10	Peak 11
001	0.0815	0.0375	1.00	0.132	0.0148	0.0884	0.00894	0.0139	0.00271	0.0174	0.00623
002	0.154	0.0354	1.00	0.157	0.0122	0.0847	0.00914	0.0149	0.00496	0.0459	0.00660
003	0.100	0.0406	1.00	0.125	0.0133	0.0905	0.0089	0.0131	0.00426	0.0172	0.00968
004	0.126	0.0417	1.00	0.137	0.0130	0.1070	0.0098	0.0131	0.00565	0.0685	0.00535
005	0.118	0.0390	1.00	0.172	0.0139	0.0881	0.00909	0.0171	0.00357	0.0121	0.0101
006	0.147	0.0336	1.00	0.151	0.0132	0.100	0.0114	0.0159	0.00587	0.0622	0.00859
007	0.138	0.0375	1.00	0.144	0.0134	0.117	0.0118	0.0146	0.00602	0.0580	0.00587
008	0.108	0.0428	1.00	0.133	0.0118	0.098	0.0096	0.0163	0.00487	0.0402	0.00813
009	0.136	0.0355	1.00	0.145	0.0115	0.111	0.0109	0.0142	0.00763	0.0417	0.00732
010	0.152	0.0344	1.00	0.143	0.0113	0.122	0.0105	0.0159	0.00585	0.0599	0.00642
011	0.124	0.0383	1.00	0.137	0.0119	0.095	0.00933	0.0163	0.00491	0.0410	0.00758

6. Results and analysis

Under the chromatographic conditions of this experiment, the peaks of each compound in the sample achieved good baseline separation. Through methodological investigation, the accuracy of this method can be further confirmed, and the accuracy of this model in measuring and analyzing Pueraria lobata sold in the market can be greatly improved.

Pueraria lobata of different origin:

006 > 002 > 003 > 005=010 > 007>009>011>004>008>001

Comparison of isoflavone content:

003>005>001>006>011>008>009>007>002>010>004

Comparison of Puerarin content: 005>003>006>001>002>011>007=009>010>008>004

Comparison of Daidzin content: 003>010>007>001>006>009>005>004>008>011>002

Comparison of Daidzein content: 006>004>007>010>002>011>009>008>003>005>001

It can be concluded that the commercial Pueraria lobata of different origin varies in chemical composition. According to the final data, the contents of compounds in the Pueraria lobata of No. 003 and No. 006 were higher, which were all from Anhui. It can be concluded that the content of compounds in Pueraria lobata from Anhui Province is relatively high. This paper is conducive to providing reference in Chinese medicine management in the source of purchasing and further selection.

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