A SIMPLE HPLC METHOD FOR THE DETERMINATION OF FIVE BIOACTIVE COMPONENTS AND FINGERPRINT ANALYSIS OF *Pueraria lobata*

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Introduction

The root of *Pueraria lobata* used in traditional Korean medicine. According to traditional practice, it is used to relieve fever, promote salivation and relieve thirst, facilitate eruption and dispel pathogenic factors from the superficial muscles and to top diarrhea. In the present study, we developed a simple and reliable HPLC-PDA method by optimizing the extraction, separation and analytical conditions for characteristic fingerprint analysis in combination with simultaneous determination of five bioactive components, such as puerarin, genistin, genistein, daidzin and daidzein in *Galgen* and *Galgen Ex San*, and the chromatographic peaks were confirmed by LC-MS-MS analysis

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Materials and Methods

- **Plant materials** : Reference herb of *Pueraria lobata(KFDA)* , local market(60), Galgen Ex San (Hanzung Pharmaceutical Company)
- **HPLC conditions** : Column(YMC Pro C18 (4.6 x 250㎜, 5㎛)), Mobile phase(0.1% acetic acid, ACN), Detector(UV260nm), 0.5ml/min
- **Sample preparation** : Extracted with 50% ethanol by sonication for 1hr

Results

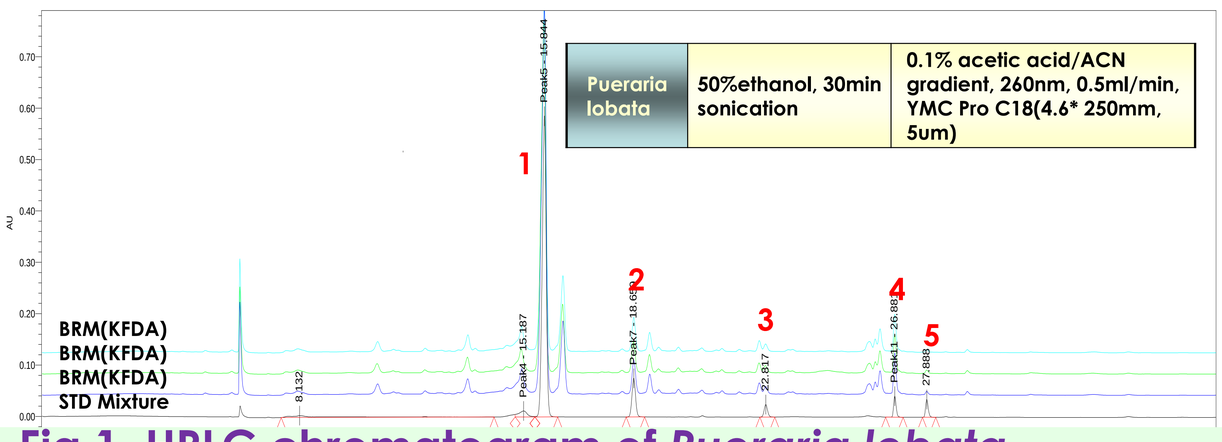


Fig 1. HPLC chromatogram of *Pueraria lobata*
1;Puerarin, 2;Daidzin, 3;Daidzein, 4;Geinstin, 5;Geinstein

Table 1. The regression data, LODs and LOQs for five bioactive compounds analyzed by HPLC

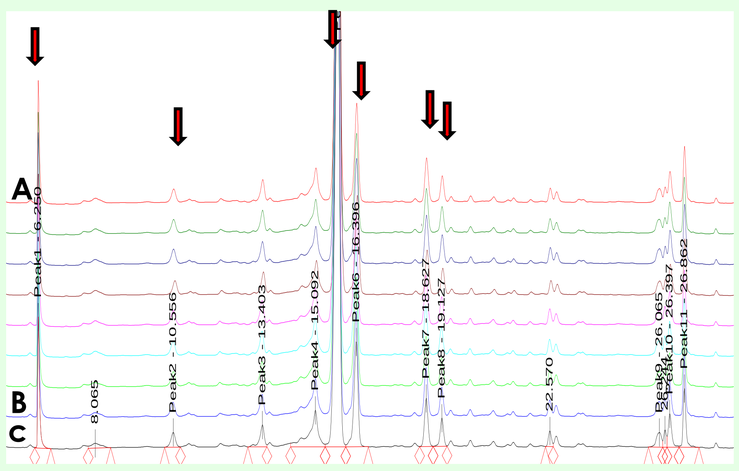
Compound	Regression equation (Y=Ax+b)	R^2	Linear range(㎍/㎖)	LOD (㎍/㎖)	LOQ (㎍/㎖)
Puerarin	Y=3.719+0.021	1	0.1936-100	0.048	0.145
Daidzin	Y=3.918+0.097	0.99999	0.1936-100	0.047	0.142
Genistin	Y=4.107+0.093	1	0.1936-100	0.054	0.164
Daidzein	Y=6.052+0.527	0.99997	0.1936-100	0.033	0.101
Genistein	Y=5.147+0.919	0.99978	0.1936-100	0.055	0.165

Table 2. Reproducibility and stability of five compounds

Compound	Reproducibility(n=6)		Stability(24h, n=3)	
	Mean(mg/g) ±SD	RSD(%)	Percentage decrease(%)*±SD	RSD(%)
Puerarin	0.2405±0.0031	1.2773	2.5041±0.0704	2.8101
Daidzin	0.0228±0.0004	1.5735	1.0003±0.0327	3.2678
Genistin	0.0023±0.00005	1.9426	2.7101±0.0813	2.9998
Daidzein	0.0043±0.0001	1.5962	1.4973±0.0426	2.8471
Genistein	0.0056±0.0002	3.0450	2.9602±0.0902	3.0465

Table 3. Intra-day and inter-day precision of five bioactive compounds

Compound	Concentration(㎍/㎖)	Intra-day precision (n=6)RSD(%)	Inter-day precision (n=5)RSD(%)
Puerarin	12.5	0.256	0.650
	25	0.349	0.701
	50	0.144	1.185
Daidzin	12.5	0.246	0.831
	25	0.026	0.849
	50	0.051	1.285
Genistin	12.5	0.079	0.748
	25	0.188	0.738
	50	0.182	1.147
Daidzein	12.5	0.069	0.780
	25	0.340	0.762
	50	0.304	1.191
Genistein	12.5	0.163	0.765
	25	0.263	0.776
	50	0.149	1.142



peak	RRT (mean)	RRT(median)	RPA (mean)	RPA(median)
1	0.381	0.381	0.574	0.573
2	0.817	0.817	0.239	0.231
3	0.964	0.964	5.633	5.412
4	1.000	1.000	1.000	1.000
5	1.136	1.136	0.396	0.398
6	1.166	1.167	0.222	0.219

Fig 2. HPLC fingerprint of *Pueraria lobata*
A; Sample 1~7, B; Galgen Ex-san, C;Standard fingerprint

Table 4. The similarities of 60 Galgen samples

	Mean	Median		Mean	Median		Mean	Median
sa1	0.9955	0.9949	sa21	0.9985	0.9984	sa41	0.9931	0.9928
sa2	0.9978	0.9975	sa22	0.9966	0.9962	sa42	0.9988	0.9986
sa3	0.9969	0.9965	sa23	0.9963	0.9959	sa43	0.9940	0.9938
sa4	0.9991	0.9990	sa24	0.9990	0.9986	sa44	0.9995	0.9994
sa5	0.9981	0.9980	sa25	0.9974	0.9975	sa45	0.9964	0.9959
sa6	0.9552	0.9537	sa26	0.9961	0.9958	sa46	0.9882	0.9878
sa7	0.9972	0.9969	sa27	0.9957	0.9956	sa47	0.9930	0.9929
sa8	0.9867	0.9862	sa28	0.9961	0.9959	sa48	0.9817	0.9813
sa9	0.9957	0.9953	sa29	0.9982	0.9978	sa49	0.9990	0.9987
sa10	0.9948	0.9950	sa30	0.9969	0.9965	sa50	0.9960	0.9963
sa11	0.9948	0.9944	sa31	0.9960	0.9955	sa51	0.9898	0.9906
sa12	0.9957	0.9953	sa32	0.9968	0.9964	sa52	0.9970	0.9971
sa13	0.9961	0.9960	sa33	0.9994	0.9994	sa53	0.9983	0.9979
sa14	0.9977	0.9974	sa34	0.9992	0.9991	sa54	0.9976	0.9975
sa15	0.9992	0.9991	sa35	0.9986	0.9983	sa55	0.9992	0.9991
sa16	0.9990	0.9988	sa36	0.9966	0.9965	sa56	0.9982	0.9980
sa17	0.9990	0.9988	sa37	0.9959	0.9955	sa57	0.9929	0.9926
sa18	0.9975	0.9971	sa38	0.9979	0.9976	sa58	0.9987	0.9984
sa19	0.9974	0.9969	sa39	0.9983	0.9979	sa59	0.9973	0.9968
sa20	0.9958	0.9959	sa40	0.9980	0.9977	sa60	0.9948	0.9943

Conclusions

we have successfully developed the HPLC fingerprint of *Galgen* 60 batches of *Galgen* from different sources were assessed and differentiated by chromatographic fingerprint analysis in combination with similarity analysis. It is demonstrated that HPLC coupled with multiple compound determination and fingerprint analysis is a powerful, practical tool for comprehensive quality control of traditional Korean medicines.

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