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HPLC 同时测定忍冬藤中 9 种活性成分含量

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摘　要:建立同时测定忍冬藤中 9 种活性成分(新绿原酸、绿原酸、隐绿原酸、马钱苷、芦丁、木犀草苷、异绿原酸 B、异绿原酸 A、异绿原酸 C)含量的 HPLC 法。HPLC 分析条件:色谱柱 ZORBAX SB-C_{18}(4.6 mm × 250 mm,5 μm),流动相 0.2% 甲酸水-乙腈梯度洗脱,流速 1 mL/min,柱温 30 ℃,检测波长 325 nm(新绿原酸、绿原酸、隐绿原酸、异绿原酸 B、异绿原酸 A、异绿原酸 C),350 nm(芦丁、木犀草苷),254 nm(马钱苷)。实验结果显示 9 种待测成分分离度良好;各成分质量浓度与峰面积在测定范围内均呈良好线性关系($R^2 \geqslant 0.9996$),新绿原酸、绿原酸、隐绿原酸、马钱苷、芦丁、木犀草苷、异绿原酸 A、异绿原酸 B、异绿原酸 C 的平均回收率(RSD)分别为 102.70%(2.54%)、100.05%(3.29%)、102.11%(1.47%)、100.04%(1.32%)、101.72%(0.51%)、100.58%(0.51%)、101.88%(1.02%)、100.55%(0.27%)、101.39%(1.29%)。HPLC 法灵敏、准确、可靠、重复性好,可用于忍冬藤药材的质量评价。

关键词:忍冬藤;同时测定;HPLC

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Simultaneous Determination of 9 Bioactive Components in *Lonicerae japonicae* Caulis by HPLC

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Abstract: The aim of this study was to establish a HPLC detection method for the simultaneous determination of 9 bioactive components (neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, loganin, rutin, cynaroside, isochlorogenic acid B, isochlorogenic acid A, isochlorogenic acid C) in *Lonicerae japonicae* Caulis. The chromatographic separation was carried out on a ZORBAX SB-C_{18} (4.6 mm × 250 mm, 5 μm) column with gradient elution of acetonitrile and 0.2% formic acid in water at a flow rate of 1 mL/min and column temperature was 30 ℃. The detection wavelength were set at 254 nm for loganin, 325 nm for neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, caffeic acid, isochlorogenic acid B, isochlorogenic acid A, isochlorogenic acid C, and 350 nm for rutin, cynaroside, respectively. Excellent chromatographic separation was achieved with good linearity ($R^2 \geqslant 0.9995$) within the studied concentration ranges. The average recovery rates (RSD) of neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, loganin, rutin, cynaroside, isochlorogenic acid B, isochlorogenic acid A, isochlorogenic acid C were 102.70% (2.54%), 100.05% (3.29%), 102.11 (1.47%), 100.04% (1.32%), 101.72% (0.51%), 100.58% (0.51%), 101.88% (1.02%), 100.55% (0.27%), 101.39% (1.29%), respectively. The established method was of high sensitivity and good reproducibility, and it can be used for quality control of *L. japonicae*.

Key words: *Lonicerae japonicae* Caulis; simultaneous determination; HPLC

Introduction

Rendongteng, derived from the dried stems and branches of *Lonicera japonica*, is popularly used as an agent for the treatment of acute fever and epidemic disease in traditional Chinese medicine (TCM) practice[1]. With 98 species, China possesses numerous reserves of *Lonicerae* genus, of which are widely distributed in Shandong and Henan provinces. According to Pharmacopoeia of the People's Republic of China (2010 edition), chlorogenic acid and cynaroside were used for

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the quality control of this medicinal plant. However, Extensive phytochemical and pharmacological studies have shown that luteolin and other flavonoids of *L. japonicae* have tremendous activities, such as anti-oxidant, anti-inflammation, anti-bacterium, anti-virus, anti-cancer, *etc*[2-8]. Thus more and more attention was also paid on such components. It has become an urgent target to develop analysis methods for multi-index evaluation of *L. japonicae*. In this study, a HPLC method was developed for the simultaneous determination of 9 bioactive components in *L. japonicae*.

Materials and Methods

Main instruments

An Agilent 1260 high performance liquid chromatography (G1311C quaternary gradient pump, G1329B automatic sampler, G1315B diode array detector, G1316A temperature box) was used for chromatographic separation; KQ-500DE ultrasonic cleaner was purchased from Kunshan Ultrasonic Instrument Co., Ltd.; Electronic balance was purchased from Shanghai Mettler Toledo Instrument Systems Co., Ltd..

HPLC conditions

The mobile phases were 0.2% formic acid in water (A) and acetonitrile (B). The gradient elution program was as follows: 0-10 min, 92%-90% A; 10-20 min, 92%-85% A; 20-30 min, 85% A; 30-40 min, 85%-75% A; 40-45 min, 75%-55% A; 45-55 min, 55%-100%; 55-60 min, 100% A. The column oven temperature was maintained at 30 ℃ and flow rate was 1 mL/min. The injection volume was 20 μL. The UV detection wavelengths were set at 254 nm for loganin, 325 nm for neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, isochlorogenic acid B, isochlorogenic acid A, isochlorogenic acid C and 350 nm for rutin, cynaroside, respectively. The chromatograms of reference substance and sample were shown in Fig. 1 under this chromatographic conditions.

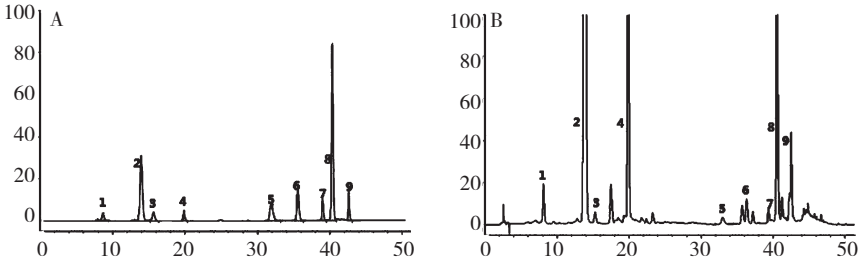

Fig. 1 HPLC chromatograms of the mixed standards (A) and sample solution (B)

Note: 1: neochlorogenic acid; 2: chlorogenic acid; 3: cryptochlorogenic acid; 4: loganin; 5: rutin; 6: cynaroside; 7: isochlorogenic acid B; 8: isochlorogenic acid A; 9: isochlorogenic acid C

Chemicals and materials

The standard samples of rutin (Batch Nos: 100080-200306) was purchased from The National Institute For The Control of Pharmaceutical and Biological Products; Neochlorogenic acid (PA0819RA13, Purity ≧ 98%), chlorogenic acid (20130415, Purity ≧ 98%), cryptochlorogenic acid (ZS0922BA13, Purity ≧ 98%), loganin (20121022, Purity ≧ 98%), cynaroside (20130521, Purity ≧ 98%), isochlorogenic acid B (20131021, Purity ≧ 98%), isochlorogenic acid A (20130816, Purity ≧ 98%), isochlorogenic acid C (20130924, Purity ≧ 98%) were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. Acetonitrile and formic acid were chromatographically pure and other reagents were analytically pure. The double distilled water was self-made.

The samples of *L. japonicae* were obtained from the planting base in Shandong, Henan, Hebei Province at June-August, 2013. All dry stems and branches of *L. japonica* samples were identified by Prof. ZHANG Yongqing from Shandon University of Traditional Chinese Medicine. A voucher specimen was deposited in the herbarium of Shandong university of TCM (SDCM).

Standard preparation

External standard method was used to analyze the contents of samples. The stock solutions were prepared according to the following steps. Neochlorogenic acid (6.70 mg), chlorogenic acid (6.51 mg), cryptochlo-

rogenic acid (11.00 mg), loganin (1.44 mg), rutin (2.67 mg), cynaroside (3.56 mg), isochlorogenic acid B (9.25 mg), isochlorogenic acid A (8.70 mg), isochlorogenic acid C (10.90 mg) were weighted accurately and transferred into 5 mL volumetric flask, topped-up to the volume with 50% methanol, respectively.

Sample preparation

Each sample (0.5 g) was accurately extracted with 25 mL of 50% methanol in an ultrasonic water bath for 30 min, and allowed to stand to cool. The sample was weighted again and the loss was complemented with 50% methanol, then it was shaken up and centrifuged at 4500 rpm, and the supernatant was filtrated through a 0.45 μm membrane filter.

Results and Discussion

Method validation

Linearity

A series of mixed solutions including above mentioned 9 standard solutions were used to determine linear range of analytes. The results were summarized in Table 1 and good correlations were found between the peak area (y) and concentration of compounds (x) within the tested ranges. In general, each component gave a wide calibration range for routine analysis.

Table 1 Linearity of each component

Component	Linear range (mg/mL)	Regression equation	Correlation coefficient (R^2)
Neochlorogenic acid	0.0269 ~ 1.3434	y = 1822.3x + 10.364	0.9999
Chlorogenic acid	0.0273 ~ 15.636	y = 1851.3x + 13.69	0.9997
Cryptochlorogenic acid	0.0451 ~ 0.4331	y = 1432.7x + 3.3910	0.9995
Loganin	1.4400 ~ 7.2461	y = 659.15x-5.8300	0.9996
Rutin	0.01926 ~ 4.4111	y = 591.67x-0.6221	0.9998
Cynaroside	0.0721 ~ 5.1511	y = 867.31x-4.3101	0.9998
Isochlorogenic acid B	0.0370 ~ 0.2773	y = 2497.9x-9.6004	0.9999
Isochlorogenic acid A	0.5220 ~ 12.571	y = 2229.8x + 27.950	0.9999
Isochlorogenic acid C	0.1308 ~ 3.033	y = 1068.4x-4.9689	0.9998

Repeatability

Repeatability of this method was obtained by analyzing 6 different samples. The RSD of neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, loganin, rutin, cynaroside, isochlorogenic acid B, isochlorogenic acid A, isochlorogenic acid C were 2.5%, 3.4%, 2.1%, 2.0%, 3.5%, 1.6%, 2.8%, 3.4%, 3.2%, respectively, which satisfied the criteria of quantitative analysis.

Stability

The peak areas of 9 compounds in sample solution were analyzed in 0, 10, 12 and 24 h. The RSD values of 9 compounds were all lower than 3.0%. The results suggested that it was feasible to analyze samples within 24 h.

Accuracy

20 μL of samples were measured 6 times by HPLC under the chromatographic condition. The relative standard deviations (RSDs) of the peak areas of neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, loganin, rutin, cynaroside, isochlorogenic acid B, isochlorogenic acid A, isochlorogenic acid C were 1.1%, 3.0%, 2.5%, 2.0%, 3.3%, 2.5%, 3.0%, 3.3%, 3.1%, respectively, suggesting that the instrument was capable of high precision in operation process.

Recovery

Recovery experiment was carried out to evaluate the accuracy of the method. Known concentration of 9 mixed standard solution were added to 6 samples solution. Each solution was injected in triplicate. The content of each component was determined by the corresponding calibration curve, while the content of each spiked component was calculated by subtracting the detected amount of the corresponding component present in the o-

riginal sample powder from the total content. Consequently, the average recoveries of the 9 compounds were all between 100.04%-102.70% (Table 2).

Table 2 Recovery of 9 ingredients for quantitative analysis ($n = 6$)

Compound	Original (mg)	Spiked (mg)	Detected (mg)	Average recovery (%)	RSD (%)
Neochlorogenic acid	0.1532	0.1340	0.2908	102.70	2.54
Chlorogenic acid	5.0212	5.0075	10.0312	100.05	3.29
Cryptochlorogenic acid	0.0539	0.0563	0.1114	102.11	1.47
Loganin	10.192	10.092	20.288	100.04	1.32
Rutin	0.2322	0.2470	0.4834	101.72	0.51
Cynaroside	0.1708	0.1869	0.3588	100.58	0.51
Isochlorogenic acid B	0.0350	0.0338	0.0693	101.88	1.02
Isochlorogenic acid A	4.0020	3.3710	7.3915	100.55	0.27
Isochlorogenic acidC	0.4244	0.4190	0.8492	101.39	1.28

Content determination

The contents of neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, loganin, rutin, cynaroside, isochlorogenic acid B, isochlorogenic acid A, isochlorogenic acid C were calculated by external standard method (Table 3).

Table 3 Determination of 9 bioactive constituents in 9 batches of *L. Japonicae* sample

Population and Batch No.		Contents (mg/g)								
		Neochlorogenic acid	Chlorogenic acid	Cryptochlorogenic acid	Loganin	Rutin	Cynaroside	Isochlorogenic acid B	Isochlorogenic acid A	Isochlorogenic acid C
Shandong	71804	0.1429	1.7279	0.0944	1.29945	0.1826	0.4706	0.0696	2.8056	1.0885
	71805	0.1921	1.5594	0.1003	1.38953	0.1269	0.6888	0.0424	1.7056	0.6938
	71806	0.0494	1.0869	0.0161	1.21929	0.1747	0.1623	0.0285	0.9765	0.3184
Hebei	72301	0.1836	5.7358	0.1057	1.06077	0.126	0.2609	0.0834	2.5536	0.6803
	72302	0.2372	6.4671	0.0984	1.36909	0.2285	0.4998	0.0666	3.5329	0.8484
	72303	0.2176	5.971	0.101	1.10112	0.1383	0.3948	0.0708	2.7715	0.7914
Henan	73001	0.2537	4.4697	0.1638	1.23515	0.1909	0.4491	0.0762	2.596	1.1786
	73002	0.2113	3.6712	0.1148	1.09701	0.1653	0.3371	0.0891	2.731	1.3261
	73003	0.2192	5.7185	0.1695	1.47105	0.1904	0.476	0.084	2.4773	0.955

Conclusions

In this article, a simultaneous HPLC method for the determination of 9 bioactive components was developed. The analysis conditions including formic acid system, phosphoric acid system, and detection wavelength were evaluated. In summary, the developed HPLC method can be applied to measure the contents of 9 components in *L. japonicae* and laid a solid foundation for the quality control of *L. japonicae* due to its simplicity, reliability and good repeatability.

The contents of chlorogenic acids showed obvious difference for samples of *L. japonicae* from different origins due to the differences of growth environment (soil, temperature and sunshine). In general, the content of chlorogenic acid in *L. japonicae* sample from Hebei was much higher than that of Henan, followed by Shandong. The flavonoids and iridoid glycosides have no significant difference.

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