Asian Journal of Chemistry; Vol. 23, No. 10 (2011), 4709-4710

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

NOTE

Determination of Chlorogenic Acid, Ferulic Acid and Flavonoids in Flos Loniceae by High Performance Liquid Chromatography

YAN QING-HUA[1,*], YANG LI[2], WANG QING[2] and MA XIAO-QIN[2]

[1]Department of Life Science and Technology, Xinxiang Medical College, Xinxiang 453003, Henan Province, P.R. China
[2]Department of Experimental Center, Henan Institute of Science and Technology, Xinxiang 453003, Henan Province, P.R. China

*Corresponding author: E-mail: yanqinghua2010@gmail.com

(*Received*: 8 January 2011; *Accepted*: 1 July 2011) AJC-10138

RP-HPLC method has been developed for the determination of chlorogenic acid, ferulic acid and flavonoids in flos loniceae simultaneously. The extracts was separated on Eclipse XDB-C_{18} (4.6 × 250 mm, 5 µm) column using acetonitrile and 0.1 % phosphoric acid with gradient elution as the mobile phase at a flow 0.8 mL min^{-1}, the column temperature was 25 ºC and the detection wavelength was 328 nm. The result indicated that the liner correlation coefficient of all subjects was between 0.9992 and 0.9999. The relative deviation is less than 3.02 % and the recovery range varies from 95.00 to 98.94 %. The method is easy, sensitive, quick, reliable, repeatable and suitable for quality test.

Key Words: HPLC, Flos lonicerae, Chlorogenic acid, Ferulic acid, Rutin, Luteoloside.

Flos lonicerae, a famous Chinese traditional medicine, is dry flower buds of Lonicera plants, also known as Yinhua, Golden or honeysuckle. Flos lonicerae contains organic acids, flavonoids, inositol, saponins and tannins and other active ingredients[1]. The main components of organic acids is chlorogenic acid which is considered to be the main active ingredient of antibacterial, detoxification and diminishing inflammation herbs and proprietary Chinese medicines, whose content has become one of the indicators to measure the quality of honeysuckle[2]. Flavonoids can reduce myocardial oxygen consumption, increase the blood flows of coronary and cerebral vascular and the soften blood vessels, especially flavonoids, which is also a natural antioxidants, can scavenge superoxide anion radicals in the body and have the physical role of anti-aging and increasing immunity[3]. This experiment established the high performance liquid chromatography method to detect flavonoid and organic acids in flos lonicerae.

SURVEYOR High performance liquid chromatography mass spectrometer (Finnigan Corporation of USA); RE-52AA Rotary Evaporator (Shanghai Yarong Biochemical Co., Ltd.).

Acetonitrile, methanol (HPLC grade); ethanol, phosphoric acid (AR); Luteolin, chlorogenic acid, rutin and ferulic acid standards were HPLC grade (Cisco Biotechnology Co., Ltd. Chengdu, China). Flos lonicerae was bought in the pharmacy of Xinxiang City, which was identified by the Chinese medicine laboratory of the College of Pharmacy of Xinxiang Medical University.

Chromatographic conditions: Chromatographic column: Eclipse XDB-C18 (4.6 mm × 250 mm, 5 µm; Tianjin Beijing Agela Technologies Co.,Ltd.); Mobile phase: 0.1 % Phosphoric acid solution: acetonitrile = 78:22; flow speed: 0.8 mL min^{-1}; Column temperature: 25 ºC; Detection wavelength: 328 nm; The injection[4] volume: 20 µL.

Preparation of sample solution: Weighed 5 g of Flos lonicerae meal accurately, setting into conical flask with stopper, adding 80 % ethanol 50 mL, soaked 24 h, transferred to the flask and then ultrasonic extraction in the hood for 0.5 h, filtration, combined filtrate, concentrated to 10 mL in rotary evaporator at 60 ºC and defatted with petroleum ether, filtered through 0.45 µm microporous membrane, the sample solution, cold storage backup for detection.

Determination of detection wavelength: Three-dimensional scan the luteoloside, chlorogenic acid, rutin and ferulic acid by a diode array detector, extract their UV spectrum in the mobile phase. The results showed that the characteristic absorption of luteolin in the 248 nm wavelength and 323 nm; absorption of chlorogenic acid wavelength 218, 242 and 329 nm; absorption of rutin and 354 nm wavelength of 256 nm, absorption of ferulic acid in a wavelength 218, 236 and 325 nm. Extraction to compare the chromatogram of these wavelengths and taking into account strong impurity peak at 300 nm, we selected 328 nm as the detection wavelength.

Mobile phase: Choose 0.1 % phosphoric acid and acetonitrile as mobile phase for analysis, the rate of 0.1 % phosphoric

acid and acetonitrile is 78:22, where the target product could be completely separated, just as Fig. 1.

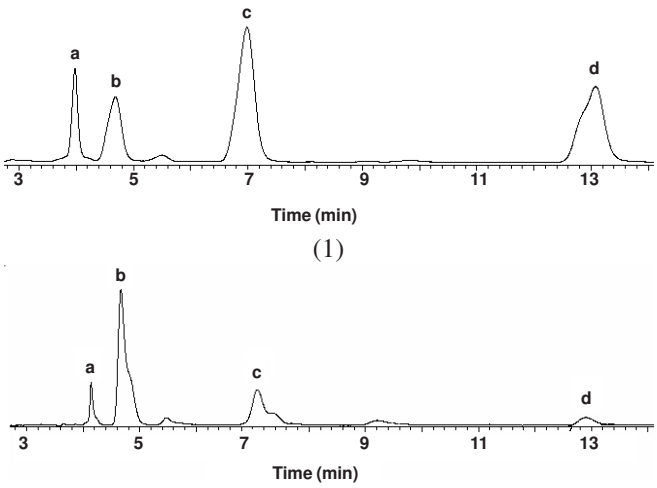

Fig. 1. Chromatograms of the standard (1) and sample (2) a, luteoloside; b, chlorogenic acid; c, rutin; d, ferulic acid

Choice of column temperature: Column temperature can influence separation of the components largely, if column temperature was too high or too low, the peak shape and analysis time are not only affected and the life of the column can be shortened. In this study 25 ºC was selected as the detecting temperature, where the peak shape is good (Fig. 1).

Standard curve: Preparation of the 1.25, 2.5, 5, 10 and 20 μg mL^{-1} solutions of luteolin, chlorogenic acid, rutin, ferulic acid standard sample storage solution, drawing standard curve in a concentration (μg mL^{-1}) for the abscissa and peak area (Y) for the longitudinal coordinates, calculating the standard curve regression equations of the above four substances (Table-1).

TABLE-1
THE REGRESSION EQUATION OF LUTEOLOSIDE, CHLOROGENIC ACID, RUTIN AND FERULIC ACID

Compounds	Regression equations	R
Luteoloside	Y = 1996.47X + 365.976	0.9996
Chlorogenic acid	Y = 2297.12X + 191.387	0.9999
Rutin	Y = 12547X - 872.899	0.9998
Ferulic acid	Y = 4036.67X + 624.139	0.9998

Precision test: Select 5 μg mL^{-1} mixed control solution, detecting by the above method, to determine the relative standard deviation of these four components by results of six successive injections, all RSDs is lower than 3.02 %, which proved that this method is precise.

Test reproducibility: Accurately weighed the same sample with 5 copies, according to the above method to prepare the samples, 20 μL injection volume, six parallel detections, the relative standard deviation of each component were all lower than 2.73 %, which proved that this method is accurate, reproducible.

Recovery test and sample determination results: The recoveries of the luteoloside, chlorogenic acid, rutin and ferulic acid, just showed in Table-2, range from 95.00 to 98.90 % and from the detecting results we can know Flos lonicerae contains the highest levels of chlorogenic acid in the above four substances.

TABLE-2
DETERMINATION RESULTS OF RECOVERY AND PRECISION (N = 5)

Compounds	Base value (μg g^{-1})	Quantity added (μg g^{-1})	Quantity found (μg g^{-1})	Recovery (%)	RSD (%)
Luteoloside	66.76	30. 00	95.46	98.66	0.69
Chlorogenic aid	345.91	50.00	391.71	98.94	0.53
Rtin	17.65	15.00	32.13	98.42	1.02
Ferulic acid	33.65	15.00	46.22	95.00	0.89

Stability test: Considering the solution of many natural extracts is prone to fail and change in the characteristics, so stability test should be done. Take the same after treatment with a sample extracting solution of Flos lonicerae, detecting a group every 2, 4, 8, 16 and 24 h, successive injecting five times for detecting Luteolin, chlorogenic acid, rutin, ferulic acid whose RSDs were 1.32, 1.27, 1.08 and 1.15 %, respectively, which proved that sample solution is stable.

Conclusion

This study established the new method of ethanol combined with ultrasonic to extract the active ingredients in Flos lonicerae and established the new method to determine the four substances of luteolin, chlorogenic acid and rutin, ferulic acid content in flos lonicerae simultaneously. This method has high precision and accuracy, small analytical error, which provide an experimental basis for determining organic acids, flavonoids and other active ingredients in Chinese herbal medicine.

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