DETERMINATION OF FLAVONOIDS IN *HYPERICUM PERFORATUM* BY HPLC ANALYSIS

WU Ying[1] , ZHOU Su-di[2] , LI Ping[1*]

(1. *Department of Pharmacognosy* , 2. *Department of Traditional Chinese Medicine' s Analysis* , *China Pharmaceutical University* , *Nanjing 210038* , *China*)

ABSTRACT: **AIM** To establish an HPLC method for simultaneous determination of four flavonoids in *Hypericum perforatum* L. , ruitn , hyperin , avicularin and quercetin . **METHODS** C_{18} column was used , the chromatography was carried out with a linear gradient program . The mobile phase was A: H_2O (pH 3.0 ~ 3.5 adjusted with phosphoric acid) and B: acetonitrile , at flow rate of 1.0 mL• min^{-1} , peaks were detected at 254 nm . **RESULTS** The linear range of rutin was 1.376 ~ 8.256 μg• mL^{-1} (γ = 0.9999) , hyperin 3.160 ~ 18.960 μg• mL^{-1} (γ = 0.9996) , avicularin 0.968 ~ 5.808 μg• mL^{-1} (γ = 0.9998) and quercetin 0.776 ~ 4.656 μg• mL^{-1} (γ = 0.9993) . The average recovery of rutin was 97.8 % , RSD 4.8 % (n = 3) , hyperin 100.7 % , RSD 3.7 % (n = 3) , avicularin 97.3 % , RSD 0.8 % (n = 3) and quercetin 100.5 % , RSD 4.4 % (n = 3) . All of RSDs of precision were less than 2 % (n = 5) , reproducibilities less than 3 % . **CONCLUSION** The method is simple , effective and feasible . It can be used to determine flavonodis in *Hypericum perforatum* .

KEY WORDS: *Hypericum perforatum* ; flavonoids ; HPLC

CLC number: R917 **Document code**: A **Article ID**: 0513 - 4870(2002)04 - 0280 - 03

Hypericum perforicum L. (Chinese name Guanyejinsitao) is a plant of genus . *Guttiferae* , which is distributed in Shandong , Shanxi , Jiangsu , Jiangxi and Sichuan provinces in China and in many areas of other countries[1] . In Europe , *H. perforatum* L. has always been used as antidepressant[2,3] . In China , the plant has been used as a folk herbal drug for 2 400 years . It is recorded in the *Dictionary of Chinese Crude Drugs* as an effectual herbal drug for dispelling heat , detoxification , stanching bleeding , detumescence , modulating inordinate menstruation and curing unknown carbuncles[4] .

There are many reported constituents in this plant , among them naphthodianthrones are regarded as the most active antidepressant compounds . Some researchers have found that flavonoids in the plant also possess antidepressant activity[5] . It is essential to establish an analytical method to control the quality by monitoring the active compound , but in general , the existing HPLC analytical methods concentrated on hyperin and pseudohyperin , there is as yet no analytic method for the control of flavonoids in the plant[6~8] . Based on previous researches , we have established a simple and effective analytical method for the simultaneous determination of four flavonoids , rutin , hyperin , avicularin and quercetin in the plant and avicularin was isolated from the plant for the first time[9] (Figure 1) .

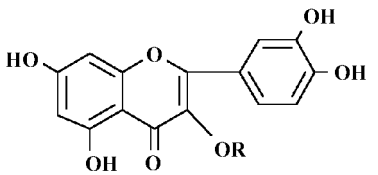

Figure 1 Structures of four flavonoids
Quercetin (R = rutinlosy) ; Hyperin (R = galactosyl) ; Avicularin (R = arabinosyl) ; Rutin (R = H)

MATERIAL AND METHODS

Chemicals and materials Acetonitrile was purchased from Tedia Co. Inc. , USA. Redistilled water was made in our own lab . Phosphoric acid (⩾85 %) was purchased from Shanghai Chemical Co. The *Hypericum perforatum* L. samples were collected in July , 1999 in Nanjing , Jiangsu , China and were identified by Prof. Zhou Su-di . Rutin , hyperin , avicularin and quercetin were isolated and purified as reference materials in our lab at a purity over 98 % (HPLC , UV detection) .

Apparatus and conditions The HPLC system consisted of a SCL-10A System Controller , LC-10AD Pump A and Pump B , DGU-12A Degasser and SPD-10A UV-VIS Detector (Shimadzu , Tokyo , Japan) . Separations were achieved with a reversed phase column (Shim-pack , CLC-ODS , 5 μm , 150 mm × 6.0 mm , Shimadzu , Tokyo , Japan) provided with a C_{18} guard column . The

Received date : 2001-07-02 .

Biographies : WU Ying (1976 -) , female , master ;
LI Ping(1960 -) , female , Professor , tutor for Ph . D .
* Corresponding author Tel : (025) 5322256 ,
E-mail : lipingli@public1 .ptt .js .cn

chromatographic data were recorded and processed by HS Chromatographic Work Station software (Yingpu Technical Development Co. Ltd, Hangzhou) .

Chromatography was carried out with a linear gradient program. Solvents were A: water (pH 3.1 ~ 3.5 adjusted with phosphoric acid) and B: acetonitrile. The gradient of A: B was 0 ~ 28 min, isocratic at 82: 18; 28 ~ 35 min, gradient up to 70: 30; 35 ~ 40 min, gradient up to 60: 40; 40 ~ 50 min, isocratic at 60: 40. The flow rate of mobile phase was kept constant at 1.0 mL• min^{-1}, the peaks were detected at 254 nm. Injection volumn was 20 μL.

RESULTS

1 Calibration Curves

A mixed stock solution consisting of rutin (1.72 mg• mL^{-1}) , hyperin (3.95 mg• mL^{-1}) , avicularin (1.21 mg• mL^{-1}) and quercetin (0.97 mg• mL^{-1}) was prepared. 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mL of the stock solution each was put into a 5 mL volumetric flask and diluted to volume with methanol. 20 μL of the above solutions were accurately taken for analysis. The coefficients of correlation of reference standards of rutin, hyperin, avicularin and quercetin were 0.9999, 0.9996, 0.9998 and 0.9993, respectively (Table 1) .

Table 1 Calibration curves of the four compounds

Flavonoid	t_R/ min	Calibration curve	r	Linear range/ μg• mL^{-1}
Rutin	16.04	Y = 13535 X - 1138.1	0.9999	1.376 ~ 8.256
Hyperin	18.85	Y = 19909 X - 4199.9	0.9996	3.160 ~ 18.960
Avicularin	29.39	Y = 23348 X - 570.14	0.9998	0.968 ~ 5.808
Quercetin	44.21	Y = 22736 X - 9044.5	0.9993	0.776 ~ 4.656

X: concentrations of injected solution; Y: peak areas

2 Precision, reproducibility and recovery

The precision was determined with the same sample solution consisting of rutin (4.128 μg• mL^{-1}) , hyperin (9.480 μg• mL^{-1}) , avicularin (2.904 μg• mL^{-1}) and quercetin (2.328 μg• mL^{-1}) . The relative standard deviation (RSD) values (n = 5) of rutin, hyperin, avicularin and quercetin were 1.5 %, 1.8 %, 1.4 % and 1.1 %, respectively.

In order to test the reproducibility, five sample solutions of *H. perforatum* were prepared. Each solution was injected twice. The contents of rutin, hyperin, aviculrin and quercetin were calculated and the RSD were as follows: rutin 2.2 %, hyperin 1.0 %, avicularin 2.2 % and quercetin 1.7 %.

The recovery experiment was carried out to evaluate the accuracy of the method. Three sample solutions of the plant materials were prepared with different contents of the four compounds, also those with a certain amount of the four standards added. Each solution was injected twice. The content of each compound was calculated and compared to the expected content. The recoveries of the four compounds were all between 95 % and 105 %, and the RSD of rutin was 4.8 %, hyperin 4.1 %, avicularin 0.7 % and quercetin 4.4 % (Table 2) .

Table 2 Recoveries of four flavonoids (n = 3)

Flavonoid	Added/ μg	Actual/ μg	Found/ μg	Recovery/ %*	Average/ %	RSD/ %
Rutin	120.00	119.46	243.40	103.2	97.8	4.2
		70.86	185.13	95.2		
		100.91	215.00	95.0		
Hyperin	276.50	209.31	493.93	102.9	100.7	4.1
		124.15	389.40	95.9		
		176.80	462.38	103.3		
Avicularin	84.00	83.72	165.46	97.3	97.3	0.7
		49.66	130.78	96.6		
		70.72	153.06	98.0		
Quercetin	67.90	69.87	135.10	96.1	100.5	4.4
		41.45	112.73	105.0		
		59.02	127.22	100.4		

* Recovery = [(found - actual) / added] × 100 %

3 Analysis of the four flavonoids in the crude drug materials

A 50 mg crushed sample of *H. perforatum* was extracted in Soxhlet' s extractor with CH_2Cl_2 for 3 h, discarded the CH_2Cl_2 solution. The dried residue was exhaustively extracted in Soxhlet' s extractor with methanol for 4 h. The methanol was concentrated to dryness, dissolved in a few mL of methanol and transferred to a 10 mL volumetric flask and diluted to volume with methanol. 1 mL of the solution was diluted in a 5 mL volumetric flask with methanol. The solution was filtered through a filter (0.45 μm) and 20 μL of the filtrate was injected for analysis. The content of each compound was calculated from the corresponding calibration curve. The retention time of rutin was 16.04 min, hyperin 18.85 min, avicularin 29.39 min and quercetin 44.21 min (Figure 2) .

For more effective application of the plant, the four compounds, rutin, hyperin, avicularin and quercetin were determined in the flower, leave, stem and the whole plant (Table 3) . The data showed that there were more flavonoids in flowers and in leaves than in the stems. Rutin existed mostly in leave and in stem, hyperin in flower and in the whole plant. The quantities of the four compounds, rutin, hyperin, avicularin and quercetin contained in the flower, leave, stems and the whole plant showed following ratios: 1.5: 5: 1: 2.5, 8: 3: 3: 1, 4: 2.5: 2: 1 and 1.5: 3: 1: 1, respectively.

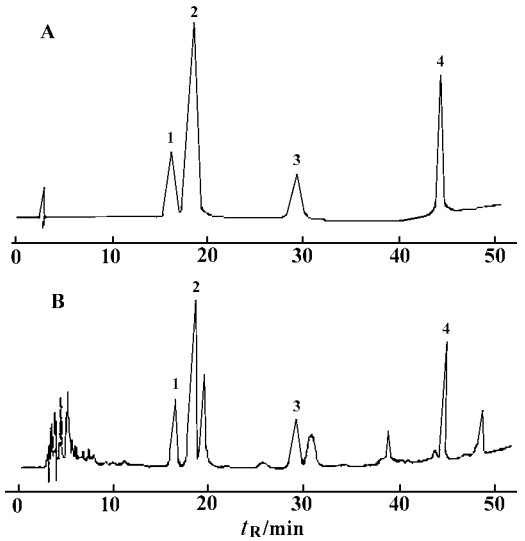

Figure 2 HPLC chromatograph of the standard (A) and samples (B)
1 . Rutin ; 2 . Hyperin ; 3 . Avicularin ; 4 . Quercetin

Table 3 Contents of the four compounds in the different part of samples

Flavonoid	Content / %			
	Flower	Leave	Stem	Whole plant
Rutin	0 .45	1 .45	0 .35	0 .47
Hyperin	1 .03	0 .52	0 .24	0 .65
Avicularin	0 .21	0 .52	0 .17	0 .26
Quercetin	0 .53	0 .19	0 .09	0 .22

DISCUSSION

To establish an HPLC method for simultaneous determination of the flavonoids , we chosed four typical flavonoids , namely rutin , a disaccharide glycoside ; hyperin , a hexose glycoside ; avicularin , a pentose glycoside and quercetin , an aglycone .

The four flavonoids were scanned from 200 to 500 nm by UV-2501 PC (Shimadzu , Tokyo , Japan) with the maxinum absorption at (254 ±1) nm , so the peaks were detected at 254 nm .

The results showed that the HPLC method established is a feasible simple , effective and suitable analytical method for flavonoids in *Hyperium perforatum* L .

REFERENCES:

[1] Liang QY . Research advances in *Hypericum* [J]. *Guihaia* (广西植物) , 1998 ,**18**(3) :256 - 262 .

[2] Ramment KF . St . John's Wort as an antidepressant [J]. *Dtsch Apoth Ztg* , 1996 ,**136**(46) :4131 - 4132 .

[3] Butterweck V, Wall A, Lieflaender-Wulf U, *et al* . Effects of the total extract and fractions of *Hypericum perforatum* in animal assays for antidepressant activity [J]. *Pharamcopsychiarty* , 1997 ,**30**(Suppl) :117 - 124 .

[4] Jiangsu New Medical College . *Dictionary of Chinese Crude Drugs* (中药大辞典) [M]. Shanghai : Shanghai Science and Technology Publishing House , 1997 .1484 - 1485 .

[5] Veronika B, Guido J, Adof N, *et al* . Flavonoids from *Hypericum perforatum* show antidepressant activity in the forced swimming test [J]. *Planta Med* , 2000 ,**66**(1) :3 - 6 .

[6] Walter K, Rainer W. Determination of naphthodianthrones (total hypericin) in St . John-Wort (*Hypericum perforatum* L .) [J]. *Pharm Ztg Wiss* , 1992 ,**5**(5) :202 - 207 .

[7] Brolis M, Gabetta B, Fuzzati N, *et al* . Identification by high-performance liquid chromatography-dioide array detection-mass spectrometry and quantification by high-performance liquid chromatography-UV absorbable detection of activity constituents of *H . perforatum* [J]. *J Chromatogr A* , 1998 ,**825**(1) :9 - 16 .

[8] Mulinacci N, Bardazzi C, Romani A . *et al* . HPLC-DAD and TLC-densitometry for quantification of hypericin in *Hypericum perforatum* L . extracts [J]. *Chromatogrphia* , 1999 ,**49**(3/4) :197 - 205 .

[9] Wu Y, Zhou SD, Li P . Studies on flavonol constituents of *Hypericum perforatum* L [J]. *Chin Tradit Herb Drugs* (中草药) , 2001 ,**32**(3) :206 .

HPLC 测定贯叶金丝桃中黄酮的含量

吴 嗤英[1] , 周素娣[2] , 李 萍[1]

(中国药科大学 1 .生药学教研室 , 2 .中药分析教研室 , 江苏 南京 , 210038)

摘要 : 目的 建立同时测定贯叶金丝桃中 4 种黄酮——芦丁、金丝桃苷、扁蓄苷和槲皮素的 HPLC 分析方法。方法 C_{18} 柱 ;流动相 A :水(磷酸调 pH 3.1 ~ 3.5) , B :乙腈 ,梯度洗脱 ;流速 1.0 mL·min^{-1} ;检测波长 254 nm。结果 线性范围为芦丁 1.376 ~ 8.256 μg·mL^{-1} (γ = 0.9999) ,金丝桃苷 3.160 ~ 18.960 μg·mL^{-1} (γ = 0.9996) ,扁蓄苷 0.968 ~ 5.808 μg·mL^{-1} (γ = 0.9998) ,槲皮素 0.776 ~ 4.656 μg·mL^{-1} (γ = 0.9993) 。平均加样回收率为芦丁 97.8 % , RSD(n = 3) 4.8 % ;金丝桃苷 100.7 % , RSD(n = 3) 4.1 % ;扁蓄苷 97.3 % , RSD(n = 3) 0.7 % ;槲皮素 100.5 % , RSD(n = 3) 4.4 % 。4 个化合物的精密度 RSD(n = 5) 均 < 2 % ,重现性 RSD(n = 5) 均 < 3 % 。结论 本方法简单、有效、可行 ,可用于贯叶金丝桃黄酮的含量测定 。

关键词 : 贯叶金丝桃 ; 黄酮 ; 高效液相色谱法