Determination of icariin in Xiaozeng granules

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ABSTRACT: OBJECTIVE To establish an HPLC method for analysis of icariin in XIAO ZENG granules. **METHODS** The HPLC system consisted of G1314A variable wavelength uv-visible detector, Hypersil ODS (4.6mm × 250mm, 5μm), HP chemstation, Hp1100 series quaternary pump and vacuum degasser (G1322A). The mobile phase was acetonitrile: water (30:70, v/v). The flow-rate was 1.0mL min^{-1}, the injection volume was 20ul, and the detector wavelength was 270nm. **RESULTS** The method was linear over the icariin range of 0.16μg to 4.0μg. the recovery of method was 98.6% ($n = 5$), the average RSD was 1.7% for precision of within-day, the average RSD was 2.1% for precision of between-day. **CONCLUSION** The method is simple, accurate and selective, can be used for determination of icariin.

KEY WORDS: Xiaozeng granules; icariin; HPLC

消增颗粒淫羊藿苷的测定

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摘要: 目的 建立高效液相色谱法测定消增颗粒中淫羊藿苷的含量方法。**方法** 采用 HP1100 高效液相色谱仪(包括 G1314A 可变波长紫外检测器, HP 化学工作站, G1322A 真空脱气机, HP1100 四元泵), Hypersil ODS 柱 (4.6mm × 250mm, 5μm)。流动相: 乙腈-水(30:70), 流速: 1.0mL · min^{-1}, 检测波长: 270 nm, 进样量: 20μl。**结果** 淫羊藿苷在 0.16 ~ 4.0μg 范围内呈现良好的线性关系, 回收率为 98.6% ($n = 5$), 日内平均 RSD 为 1.7%, 日间平均 RSD 为 2.1%。**结论** 该法简便、准确、具有专属性, 可用于测定消增颗粒淫羊藿苷的含量。

关键词: 消增颗粒; 淫羊藿苷; 高效液相色谱法

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Xiaozeng granules is a preparation of our hospital. It consisted of twelve herbs, including pericarpium citri reticulatae, herba Epimedii, Radix Glycyrrhizae ect. it can course the liver and rectify qi, transform stasis dissipate bind, using in breast gland with liver depression and qi stagnation . In order to establish the standard for quality of Xiaozeng granules, we select Herba Epimedii, using HPLC to determine icariin in Xiaozeng granules.

1 Apparatus and Reagents

1.1 Apparatus

Agilent 1100 chromatographic system (Agilent Technolo-

gies, USA) including Hp1100 Series Quaternary Pump, a variable wavelength uv-visible detector (G1314A), a vacuum degasser(G1322A) and a HP chemstation. Hypersil ODS (4.6mm × 250mm, 5μm) was slurry packed with a monolayer of octadecyl silane (Dupont company, USA). An ultrasonic degasser KQ 3200 (KUNSHAN detection instrument factory, Shanghai, China), a thermostat water bath (The fifth medical instrument corporation, Shanghai, China).

1.2 Reagents

HPLC grade acetonitrile, methanol (Si You chemical reagent factory, Tianjin, China). Distilled water made by ourselves. Analytical purity ethanol and ethyl acetate(Su Zhou chemical reagent factory, ANHUI, China). Icariin (batch: 0737-200111) supplier was National institute for the control of pharmaceutical and Biological products. The polyamide (80-100mu, Taizhou LuQiao chemicobiology plastic factory, ZHEJIANG, China). HPLC grade acetonitrile, methanol and water were filtered through a 2μm filter and degassed under vacuum prior to use. Xiaozeng granules made by ourselves.

2. Experimental methods

2.1 Chromatographic conditions

The number of theoretical plates of icariin was more than 1500, a RPLC column Hypersil ODS(4.6mm × 250mm, 5μm), the mobile phase was acetonitrile: water (30∶70, v/v). The flowrate was 1.0mL · min^{-1}, the injection volume was 20μL, and the detector wavelength was 270nm.

2.2 Established the standard curve

The standard solution were prepared by accurately weighing (icariin need dry) and dissolving in the appropriate amounts of methanol. Prepared a series solutions of icariin standard sample at 0.2, 0.1, 0.04, 0.02, 0.008 mg · mL^{-1}, then injected 20μL to measure the peak area under the above-mentioned conditions. Analyzed linear regression with area integration as abscissa (X) and injection amount(μg) as ordinate (Y). Obtained the regression equation: $Y = 0.1056 + 0.0004448X$, the linear range of icariin was 0.16μg ~ 4.0μg and the correlation coefficient: $r = 0.9999$($n = 5$).

2.3 The precision test of method

Prepare solutions of icariin standard samples that concentration were 0.2 mg · mL^{-1}, 0.1 mg · mL^{-1}, 0.04 mg · mL^{-1}, according 2.1 chromatographic conditions, measure each sample peak area five times each day, for following 5days, according to the regression equation, calculated the within-day precision and between-day precision, results were shown in table 1.

2.4 The reproduction test

The sample were prepared accurately (batch: 20021019), according to 2.7 sample extract method, measure seven times in same way. According to the regression equation, calculated the content of icariin. The RSD = 1.5% ($n = 7$).

Tab 1 The results of precision test

表 1 精密度试验结果

Concentration	Method recovery	Precision	
mg · mL^{-1}	± SD/%	Within-day RSD	Between-day RSD
0.2	97.2 ± 2.4	1.6	1.8
0.1	99.1 ± 1.8	1.4	1.9
0.04	98.7 ± 0.7	2.1	2.7

Note: the average RSD was 1.7% for precision of within-day, the average RSD was 2.1% for precision of between-day.

2.5 The recovery test

Prepare known concentration sample(batch: 20021019, content were 0.468mg/10g) 10g, mixed respectively with standard sample(concentration were 0.04mg · mL^{-1}) 10mL, according to 2.7 sample extract method, then injected 20ul to measure the peak area under the above-mentioned conditions. According to the regression equation, results of recovery were shown in table 2.

Tab 2 The results of recovery test

表 2 回收率试验结果

Sample amount (mg)	Added amount (mg)	Determinated amount (mg)	Recovery (%)	Average recovery (%)	RSD (%)
0.468	0.4	0.861	99.19		
0.468	0.4	0.879	101.27		
0.468	0.4	0.845	97.35	99.3	1.47
0.468	0.4	0.857	98.73		
0.468	0.4	0.869	100.11		

2.6 HPLC chromatographic figure

According to prescription ratio produce the negative reference sample without Herba Epimedii . Measure the standard sample, the sample and the negative reference sample under the above-mentioned conditions, seeing figure 1.

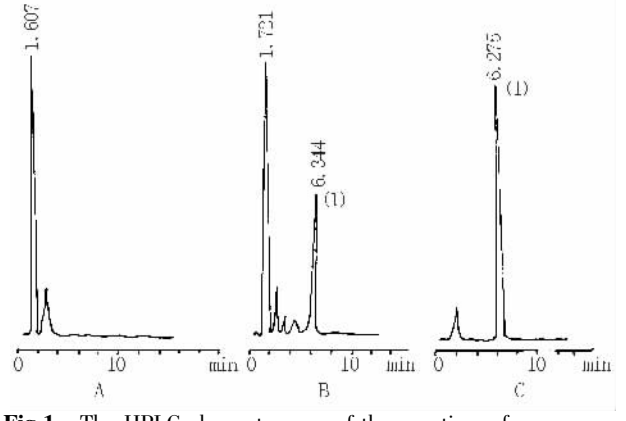

Fig 1 The HPLC chromatograms of the negative reference sample (A), the sample (B), the standard sample (c), (1): icariin

图 1 阴性样品(A), 样品(B)及对照 HPLC 色谱图

2.7 Determination of sample

Prepared Xiaozeng granules 10g (passed through 40mu) dissolving in 30mL ethyl acetate, refluxing 0.5hour, colding and filter. The filter extract two times with 1 mol · L^{-1} HCl, each time 20mL, the ethyl acetate extraction evaporate to dryness and the residue was redissolved in 2mL ethanol. The ethanol sample was passed through the polyamide column (80-100mu, 5g, dp 10mm). The polyamide column was eluted with 100mL water and the water eluate were thrown away. Then the column was eluted with 80mL ethanol and the ethanol eluate was dried using a water stream and the residue was redissolved in 10mL ethanol, then injected 20μL to measure the peak area under the above-mentioned conditions, measuring five batch Xiaozeng granules, each sample with three times. The results were shown in table 3.

Tab 3 The results of Icariin in Xiaozeng granules($n=3$)

表 3 含量测定结果

Batch	Average cotent of icariin(mg/10g)	RSD(%)
20020811	0.587	2.6
20021019	0.468	2.2
20021228	0.501	1.5
20030306	0.612	2.0
20030528	0.439	2.1

3 Discussion

3.1 There are many complex components in Xiaozeng granules. Icariin belong to flavonoid. The polyamide column was exclusive for flavonoid, it can remain flavonoid and get rid of other component, so we selected the polyamide column to purify icariin.

3.2 The polyamide column was eluted with 100mL water, one sample was collected every 10mL, ten samples were collected totally, then injected 20μL to measure the peak area of every sample separately under the above-mentioned conditions, the results suggest that there were no icariin in water sample. Then column was eluted with 80mL ethanol and collecting one sample every 10mL, Eigth samples were collected totally, then injected 20ul to measure the peak area of eight sample separately under the above-mentioned conditions, the results suggest that there were almost no icariin in eighth sample, icariin in 70mL ethanol eluate, so we selected the polyamide column was eluted with 100mL water and the water eluate were thrown away. Then column was eluted with 80mL ethanol and 80mL of eluate were collected.

The method is simple, accurate and exclusive.

RFERENCES

[1] Chinese pharmacopoeia[M] 2000, vol. 1:267.

[2] Chai YF, Li XL. Re-HPLC determination of Icariin in Epimedium brericornum and its preparation [J], Chin J Pharm Anal, 1991, 11(3):207.

[3] Ning JS, Yang JO. Determination of Icariin in Jinfukang oral Liquid [J]. Chin J Pharm Anal, 2002, 22(4):328.

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