大孔树脂预分离—薄层光密度法测定疏肝止痛片中芍药甙含量

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摘要 本文采用大孔树脂GDX104预分离，结合TLC双波长扫描，测定疏肝止痛片中芍药甙的含量。经对样品测定及稳定性、加样回收率考查，该方法准确，稳定，结果可靠。

关键词 疏肝止痛片 大孔树脂 薄层光密度法 芍药甙

采用扫描法测定芍药甙含量一般均为单味生药及小复方制剂〔张庆生，等．中国中药杂志，1991，16（9）：542〕。本品处方由柴胡、白芍等多味中药组成，成分复杂。样品经甲醇提取浓缩后的残渣用少量甲醇难于完全溶解，即使已经溶解制成样品溶液也由于粘稠度较大，对点样的精确度有一定的影响。而薄层光密度法要求被测物质在溶液中的浓度较高。如果在上样前样品液不经净化处理，则杂质的浓度亦相应增大。为此我们采用大孔树脂预分离，可以除去甲醇提取液中大部分水溶性杂质及脂溶性杂质，从而使样品液净化。

1 仪器与试药：岛津CS-9000双波长扫描仪，对照品：芍药甙（中国药品生物制品检定所），定量毛细管（CAMAG），硅胶GF_{254}（青岛海洋化工厂），大孔树脂GDX104（天津市试剂二厂），疏肝止痛片（天津市中央制药二厂），其它试剂均为分析纯。

2 薄层层析条件：薄层制备：硅胶GF_{254}加0.5%羧甲基纤维素钠湿法制板，105℃活化1h，板厚0.3mm。

展开剂：氯仿-甲醇-醋酸乙酯-氨水（50∶20∶10∶2.5），展距8.5cm。

扫描条件：a）扫描波长的选择：将芍药甙配成一定浓度的溶液，点样，展开，晾干后用扫描仪对芍药甙斑点从200～370nm扫描，得一吸收曲线，芍药甙的最大吸收波长为227nm，最小吸收波长为260nm，故$\lambda s = 227$ $\lambda_R = 260$。b）扫描方式：双波长、反射法、锯齿扫描，零点方式：Start，Sx＝3，扫描宽度10mm，$\Delta Y = 0.20$。

稳定性：芍药甙展开后取出晾干，在室温下每隔0.5h测定一次，在3h内积分值未见明显变化。

精密度：同一斑点按拟定方法连续扫描5次，测得峰面积计算变异系数CV＝0.81%。在同一薄层板上点5个同样量的斑点，按拟定的方法展开后测得对照品峰面积积分值，计算变异系数CV＝1.68%

3 含量测定方法

3.1 标准曲线绘制：精密称取芍药甙对照品，用甲醇溶液（90→100）制成2.0mg/ml的溶液，分到以2、3、4、5、6μl点于同一薄层板上，展开，取出，晾干，用薄层扫描仪测定，以积分值为纵座标，点样量为横座标，求得回归方程$Y = 27928 + 37635.9X$，相关系数r＝0.999。结果表明芍药甙在4～12μg范围内与其峰面积呈线性关系

3.2 样品溶液的制备：取本品20片，剥去糖衣，研细，取1g精密称定，置索氏提取器中，加甲醇50ml，在水浴上提取至无色（约6h），提取液浓缩至近干，残渣加热水50ml分次溶解，放冷，加入一预先装填好的大孔树脂GDX104小柱（80～100目，1.2g，内径7mm30%

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乙醇溶液湿法装柱，水洗至无醇味）顶部。洗脱，水洗脱液弃去，继续加入乙醇溶液（20→100）50ml，收集洗脱液，水浴蒸干，残渣加甲醇溶液（90→100）溶解，移入5ml容量瓶中并稀释至刻度，摇匀，作为样品溶液。

3.3 对照品溶液的制备：取80℃干燥至恒重的芍药甙对照品，加甲醇溶液（90→100）制成1.2mg/ml的溶液，作为对照品溶液。

3.4 测定：取上述2种溶液各4μl，分别点于同一含羧甲基纤维素钠为粘合剂的硅胶GF_{254}薄层板上展开扫描，由测定的峰面积计算出片剂中含芍药甙的量，结果如表。

3.5 回收率试验：本品以第一批样品作回收试验，1g样品中芍药甙的含量以7.20mg计，加入芍药甙量为7.08mg，与样品液相同操作，测定，结果回收率为99.78%，CV = 3.22%（n = 5）

表　样品中芍药甙含量

批号	含量 mg			平均$\bar{x}$	CV%
一	7.51	7.49	7.04	7.20	4.14
	7.23	7.75	7.16		
	7.39	7.10	7.28		
	6.57	6.92	6.93		
	6.90	7.29	7.38		
二	8.12	8.08	7.52	7.93	3.37
	8.17	8.12	7.80		
	7.94	8.15	7.50		
三	7.93	7.64	7.50	7.61	2.48
	7.54	7.55	7.38		
	7.81	7.38	7.73		

4 讨论

4.1 本试验，小柱中的吸附剂曾选用硅胶、氧化铝等，前者除去杂质不够理想，后者虽能除去杂质，但对芍药甙吸附力较强，至使过柱后的样品溶液中芍药甙的含量下降。故最后选定大孔树脂GDX104为吸附剂。

4.2 含芍药甙的样品水溶液上柱后，水洗脱液经浓缩加大点样量，展开后测定不含有芍药甙。

4.3 本品经采用10%，20%，30%，50%，95%浓度的乙醇梯度洗脱，结合薄层扫描检测，发现10%、20%乙醇洗脱液中均含有芍药甙，30%以上浓度的乙醇洗脱液中未检出芍药甙，故选用20%乙醇50ml洗脱，即可将柱上的芍药甙全部洗脱。实验表明，在可以将芍药甙全部洗脱的前提下，洗脱液中乙醇的含量越低洗脱后的样品溶液中杂质的含量就越少。

4.4 洗脱芍药甙后的大孔树脂小柱经用95%乙醇50ml洗后再用水100ml冲洗，即可以上第2个样品，不用重新装柱，可以反复使用多次。

4.5 展开剂中含有少量氨水，展层时可使样品中酸性杂质停留在薄层板的原点处，而且可以不用双槽层析缸。

4.6 本品经展开后测定，芍药甙吸收峰后面有一个较大的未知吸收峰，在相对湿度35%左右时，经点样展开，两峰可以达到基线分离。相对湿度过大，则影响分离。故样品点样后，如果相对湿度较大，可将薄层板放在烘箱中80℃烘烤5min，取出后放入干燥器内放冷后再展开，可以达到理想的分离效果。

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MS光谱数据与胡萝卜甙一致。晶V结构待定。

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ABSTRACTS OF ORIGINAL ARTICLES

Studies on the Chemical Constituents of Aertaihuanqi (*Astragalus altaicus*)

Cheng Jiefei, Wu Jianfei, Azi Guli, et al

Three cycloaiteosides were isolated for the first time from the n-BuOH extract of *Astragalus altaicus*. They were identified respectively as cyclosieversioside B, C and A, by FAB-MS, IR, ^1H, $^{13}CNMR$, DEPT, ^1H-^1HCOSY, ^1H-^{13}C COSY and hydrolysis.

(Original article on page 563)

Studies on the Chemical Constituents of Montane Spicebush (*Lindera reflexa*)

Zhang Junzeng, Fang Qicheng

Seven Compounds were isolated from dry Radix of *Lindera reflexa*. They were identified as launobine (Ⅰ), lindcarpine (Ⅱ), laurolitsine (Ⅲ), ±pinostrobin (Ⅳ), ±pinocembrin (Ⅴ), octacosanoic acid (Ⅵ) and β-sitosterol (Ⅶ) on the basis of chemical and spectral analyses.

(Original article on page 565)

Chemical Components of Mongolian Spirala (*Spiraca mongolica*)

Xie Haihui, Wei Xiaoyi, Wei Biyu

Six compounds were isolated for the fitst time from *Spiraca mongolica* Maxim. (*Rosaceae*) by means of chemical and spectral analysis and comparison with data of literature or authentic samples, 5 of them were identified as β-sitosterol (Ⅰ), betulin (Ⅱ), betulinic acid (Ⅲ), betulinic acid 3, 5-dihydroxyl-cinnamate (Ⅳ) and daucosterol (Ⅵ).

(Original article on page 569)

Simultaneous Quantitative Determination of Ephedrine and Strychnine in Traditional Chinese Medicine "Joufensan" by HPLC

Chen Fakui, Yoshihiro Kano, et al

A rapid, simple and sensitive method was establised for the evaluation of Chinese traditional medicine "Joufensan" by HPLC. Sample of "Joufensan" was extracted by solvent, and the extraction analysed by HPLC using an inertsil ODS column with a solvent system, acetonitrile-water-phosphoric acid-sodium dodecyl sulfate (38 : 62 : 0.1 : 0.5) at 40°C and UV detector. The new method was reliable for the evaluation of ephedrine and strychnine in "Joufensan".

(Original article on page 571)

Quantitative Determination of paeonin in "Shuganzhitong Pian" by Macroporous Resin Preseparation–TLC Densitometry Method

Shou Guoxiang, Lu Guibao

A preliminary separation procedure of paeonin in "shuganzhitong pian" by macro-porous resin GDX-104 and its subsequent quantitative determination by double wavelenth TLC scanning was developed. Through sample scanning, stability investigation and recovery tests, it reveals that the suggested method was precise and accurate, the results of determination

were reliable.

(Original article on page 573)

On the Optimization of Extraction Conditions of Tetrandrine and Demethyltetrandrine from Fourstamen Stephania (*Stephania tetrandra*)

Zhen Pan, Chen Peirong, et al

Several factors influencing the extraction of tetrandrine and demethyltetrandrine from Chinese medicinal herbs, *Stephania tetrandra* S. Moore were studied by means of Ortogonal method. The optimum Condition was eight hours steeping, in 33.3% ammonia in absolute alcohol for four hours under ultrasonic agitation. Tetrandrine and demethyltetrandrine were detected by HPLC. The contents were 0.678% and 0.616%, the detection limits were 3.87×10^{-8} and 0.48×10^{-8} mmol/L respectively.

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The Hormone Ingredient in the Egg-Oil of Chinese Woodfrog (*Rana temporaria chensinensis*) and the effect of Egg-Oil on Platelet Aggregation and blood Lipid

Li Chengyi, Chen Guangrong, Sun Wenwei

Hormone ingredient in the egg-oil, egg and Oviductus Ranae of *Rana temporaria chensinensl* is David were studied as well as the effect of egg-oil on platelet aggregation and blood lipid. Results proved that the estradiol content in the egg-oil is very high. The egg-oil can obviously inhibit blood platelet aggregation and showed an hypolipemia activity. It is expected that the egg-oil of *R. temporaria chensinensis* can be developed as an efficient medicine for the prevention and cure of atherosclerosis and hyperlipemia in elderly.

(Original article on page 584)

Protective Effects of Polysaccharide of Membranous Milkvetch (*Astragalus membranaceus*) on Acute Myocardially Infarcted Heart in Dog and Its Machanism of Action

Lu Wenwei, Lei Chunli, Chen Yu, et al

Seventeen physiological, biochemical and morphological indexes consistently showed that Astragalus polysaccharide was able to enhance the myocardia contractility, attenuate the area of myocardial infarction, and reduce the degree of myocardial damage of myocardially infarcted dog heart in vivo. The mechanism might be related to the inhibition of Na^+-Kr^+ATPase activity & the anti-free-radical-damage action.

(Original article on page 586)