HPLC 测定健心片中咖啡酸的含量

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健心片由毛冬青, 三七, 红花, 丹参, 冰片, 降香, 莶草组成。具有活血, 止痛功能。用于心肌劳损, 心绞痛、动脉硬化等症。毛冬青为方中主药, 主要含有毛冬青甲素, 原儿茶酸, 咖啡酸等[1-3]。本研究用 HPLC 测定咖啡酸的含量, 控制其质量, 取得满意的效果。

1 仪器与试药

日本岛津 LC-6A 液相色谱仪, CR-3A 记录仪, SPD-6AV 检测器, SIL-6A 自动进样器。

甲醇为色谱纯, 其他试剂为分析纯。咖啡酸对照品(中国药品生物制品检定所, 885-200001), 样品健心片及阴性空白(沈阳康田药物研究所)。

2 方法与结果

2.1 色谱条件 岛津 Shim-pack CLC-ODS 色谱柱 (6.0 mm × 150 mm, 5 μm), 柱温 40 ℃。流动相甲醇-0.025 mol·L^{-1}磷酸(16∶84), 流速 1.0 mL·min^{-1}, 检测波长 323 nm。

供试品、对照品及阴性空白样品溶液, 各进样 10 μL, 同时采集 200~400 nm 光谱数据, 经检查供试品色谱中咖啡酸蜂为单一成分。理论塔板数按咖啡酸峰计算应不低于 5 000。阴性空白溶液色谱没有干扰。

2.2 对照品溶液的制备 精密称取在 110 ℃干燥至恒重的咖啡酸对照品 12.5 mg, 置 100 mL 棕色量瓶中, 加流动相溶解并稀释至刻度, 摇匀, 精密吸取 1 mL, 置 25 mL 棕色量瓶中, 加流动相稀释至刻度, 摇匀, 即得(每 1 mL 含咖啡酸 5 μg)。

2.3 样品溶液的制备 取本品 20 片, 研细, 取粉末 0.5 g, 精密称定, 加甲醇 40 mL, 超声处理(功率 250 W, 频率 33 kHz) 30 min 放冷, 滤过, 残渣加甲醇 3~5 mL 洗涤, 合并洗液与滤液, 水浴蒸干, 残渣加流动相溶解, 转移至 50 mL 棕色量瓶中, 加流动相稀释至刻度, 摇匀, 溶液经微孔滤膜(0.5 μm) 滤过, 滤液至棕色量瓶中, 作为供试品溶液。

2.4 线性关系考察 精密称取在 110 ℃干燥至恒重的咖啡酸 12.75 mg, 置 100 mL 量瓶中, 加流动相溶解并稀释至刻度, 摇匀, 精密吸取 5 mL, 置 25 mL 量瓶中, 加流动相稀释至刻度, 制成每 1 mL 含 0.0255 mg 的溶液。

分别精密吸取上述对照品溶液各 0.5, 1, 2, 3, 4, 5 mL, 分别置 10 mL 量瓶中, 加流动相稀释至刻度, 摇匀。各进样 10 μL, 按上述色谱条件测定色谱峰面积积分值, 以进样量(μg)为横座标, 峰面积积分值为纵坐标, 绘制标准曲线, 经线性回归, 回归方程 Y = 4 574 709X − 5 451, r = 0.999 9, 线性范围为 0.006~0.127 μg。

2.5 稳定性试验 取供试品溶液在 0, 2, 4, 6, 8 h, 分别进样 10 μL, 记录峰面积积分值, 结果 RSD 2.0%, 可见供试液在 8 h 内稳定。

2.6 精密度试验 取 **2.2** 项下对照品溶液进样 5 μL, 重复进样 5 次, 峰面积积分值的 RSD 1.68%。

2.7 重复性试验 取同一批号健心片, 平行制备 5 份样品, 按样品测定法测定, 结果每片含咖啡酸平均值为 0.156 mg(RSD 2.4%, n = 5)。

2.8 回收率试验 取本品 10 片(批号 20040820, 含量 0.156 mg/粒, 0.3 g/片), 研细, 取粉末 0.5 g, 精密称定, 精密加入咖啡酸对照品溶液(0.028 4 mg·mL^{-1}) 10 mL, 按样品含量测定项下方法操作, 测定结果见表 1。

表 1 咖啡酸加样回收率试验

取样量 / g	样品中含量 / mg	测得量 / mg	回收率 / %	平均值 / %	RSD / %
0.548 8	0.285 4	0.566 8	99.1		
0.518 3	0.269 5	0.545 5	97.2		
0.501 7	0.260 9	0.531 1	95.1	97.5	1.6
0.549 9	0.285 9	0.563 0	97.6		
0.505 5	0.262 9	0.542 5	98.4		

注: 加入量均为 0.284 mg

2.9 样品测定 分别精密吸取对照品溶液与供试

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品溶液各 5 μL, 注入液相色谱仪中, 按上述色谱条件测定, 记录色谱图, 以外标法计算样品中咖啡酸的含量, 见表 2。

表 2 样品含量测定(n= 3)

批号	含量/mg/片	RSD/%
20040912	0.080	0.6
20040923	0.125	0.9
20040928	0.078	0.6

3 讨论

根据毛冬青中咖啡酸的性质, 选择了甲醇, 乙醇进行超声及回流提取比较试验, 结果表明, 样品用甲醇超声提取 30 min 效果最佳。

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[Abstract] **Objective:** To explore the influence of Kingsbrain(GETO) on the learning memory impairment of rats with cerebral ischemia. **Method:** Rats with cerebral ischemia were administered GETO orally once a day for one month. The ability of spatial-learning memory of rats was evaluated by Morris Water Maze(MWM). Duxil was used as a positive control. **Result:** the results of place navigation of MWM showed that at the 3rd time of swimming training, the escape latency of rats of the GETO group, Duxil group and Sham group were shorter than that of model group. The escape latency were (54.1±43.94), (55.9±43.49), (50.4±34.99) and (85.4±42.8) s, respectively; but there was no significantly difference. After the 6th time of swimming training, the escape latency of rats of the GETO group(37.8±38.69) s, the Duxil group(37.4±38.03) s and the sham group (26.9±21.63) s were significantly shorter than that of model rats(77.5±47.59) s, P< 0.05, respectively. Comparison of the swimming distance among groups were similar to the escape latency among groups. In the test of spatial probe, results of the ratio of the swimming time of platform quadrant(tP) vs the total swimming time(tT) and the ratio of the swimming distance of platform quadrant(dP) vs the total swimming distance(dT) indicated that the ratios of the GETO group(0.347±0.066 2, 0.344±0.055 1), the Duxil group (0.345±0.098 4, 0.34±0.093 4) and the sham group (0.35±0.066 2, 0.349±0.058 9) were significantly higher than those of the Model group(0.261±0.068 9, 0.274±0.054 4), P< 0.05, respectively. **Conclusion:** GETO can significantly improve the spatial learning and memory ability of rats with cerebral ischemia, which provides the pharmacodynamics evidence for its clinical application of improveing the learning and memory ability in poststroke patients.

[Key words] Kingsbrain(GETO); cerebral ischemia; learning and memory

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Protective effect of dl-tetrahydropalmatine on liver injury induced by carbon tetrachloride in mice

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[Abstract] **Objective:** To study the protective effect of dl-tetrahydropalmatine(dl-THP) on liver injury induced by carbon tetrachloride (CCl_4) in mice. **Method:** Mice were administracted with dl-tetrahydropalmatine ip 20, 40 mg·kg^{-1} daily for 9 d respectively, and then actue liver injury model was induced by 0.1% carbon tetrachloride ip 20 mL·kg^{-1}. The mice were killed 17 h after injection ip of CCl_4, serum alanine and aspartate aminotransferase (ALT and AST) activity were measured, and maleic dialdehyde (MDA) and superoxide dismutase(SOD) activity in liver were detected. **Result:** dl-THP significantly reduced the level of serum ALT and AST, inhibited lipoperxidation in liver, while increased SOD activity in liver tissue. Degeneration of hepatocytes was obviously prevented in mice treated with dl-THP, and the liver histological structure was well maintained. **Conclusion:** dl-THP has inhibitory effects on liver injury induced by CCl_4 in mice. The mechanisms may be related with its effects of reducing lipid peroxidation product.

[Key words] dl-tetrahydropalmatine; liver injury; carbon tetrachloride (CCl_4)

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