

丹参及复方丹参注射液质量分析研究

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摘要 对丹参及复方丹参注射液中有效成分进行紫外吸收度测定、薄层扫描测定,并建立了儿茶酚类物质总量测定法,发现市售产品有效成分含量差异很大。注射液中鞣质对红细胞及血浆蛋白的相关性研究证实,市售产品均含大量鞣质,能使人红细胞及血浆蛋白变性,会导致毒副作用。

关键词 丹参 复方丹参注射液 含量 杂质 毒副作用

丹参及复方丹参注射液是目前临床应用量较大的静脉给药中草药注射剂。但至今仍缺乏可靠的含量测定方法,其它质控项目亦有待进一步完善。本文建立了丹参有效成分含量的可见分光光度测定法,进行了化学成分的薄层扫描、注射液紫外吸收值测定、鞣质检查、对人红细胞和血浆蛋白影响研究。

1 实验材料

丹参及复方丹参注射液样品:上海××厂,批号920117,简称SJ;江苏××厂,批号891218-2,简称JD;江苏××厂,批号900215-2,简称JC;江苏××厂,批号841217,简称JW;去鞣质丹参注射液试制品,本实验室,批号930104,简称SZ。

高效硅胶薄层层析板,中国军事医学科学院;定量点样管,USA,DSCo;CS-920快速薄层扫描仪,岛津;SP-8100分光光度计,PYE UNICAM;高倍显微镜,OLYMPUS,日本。

原儿茶醛,上海试剂公司;明胶,上海试剂公司;明胶试液:将1g明胶溶于100ml蒸馏水中;其它所用试剂均为分析纯。

2 方法及结果

2.1 有效成分的含量测定:丹参水溶性成分均为邻苯二羟基类化合物^[1,2],所以采用邻苯二羟基化合物显色法进行可见分光光度法测定^[2]。

2.1.1 标准曲线的制备:称取3,4-二羟基苯甲醛适量,用水溶解,配成12.85~51.4μg/ml的系列浓度液,然后分别加入0.3ml NaNO₂(1:20)试液,放置5min后加0.3ml Al(NO₃)₃试液(1:10),5min后,再加1mol/L NaOH试液5.0ml,放置15min后,于λ_{max}525nm处测A值。以蒸馏水加显色剂为空白,测A值(n=6)得标准曲线回归方程为: $C = 157.14A - 0.916$, $r = 0.9999$, $CV\% = 0.33\% \sim 0.79\%$ 。

2.1.2 标品测定:取SJ(为100%样)、JD、JC、JW各2ml,用蒸馏水稀释125倍。精取稀释液2ml,按标准曲线法于λ_{max} = 525nm处测A值(n=3)。根据回归方程计算出各样品中有效成分含量,见表1。

2.2 样品的紫外吸收^[3,4]:精取SJ、JD、JC、JW各1.0ml。稀释至500ml,用SP-8100分光光度计于350~200nm范围内进行扫描,得λ_{max}282±1nm,于282nm处测得A值,见表2。

2.3 薄层扫描:精取SJ、JD、JC、JW各0.5ml,分别加入0.5ml无水乙醇,摇匀,为点样液,精取标准曲线项下3,4-二羟基苯甲醛液0.5ml,加0.5ml无水乙醇,摇匀,为对照液,简称S。分别将上述样品液2ml,点于同一块高效薄层硅胶板上,用二甲苯-乙酸乙酯-冰醋酸(50:50:10)展开,结果见图1。

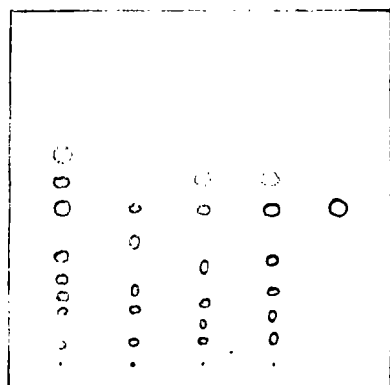
对照品斑点的R_f = 0.47,展开剂挥干后于CS-920快速薄层扫描仪上,根据对照品R_f值,于λ = 281nm处锯齿形多通道扫描,得出薄层扫描图,见图2。

各样品中相当3,4-二羟基苯甲醛色斑的面积,见表3。

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表1 各样品中有效成分浓度比较

厂家简称	浓度 (mg/ml)	相对 (%)
SJ	6.71	100
JD	1.56	23.2
JC	2.03	30.2
JW	3.08	56.6



SJ JD JC JW S

图1 薄层层析图

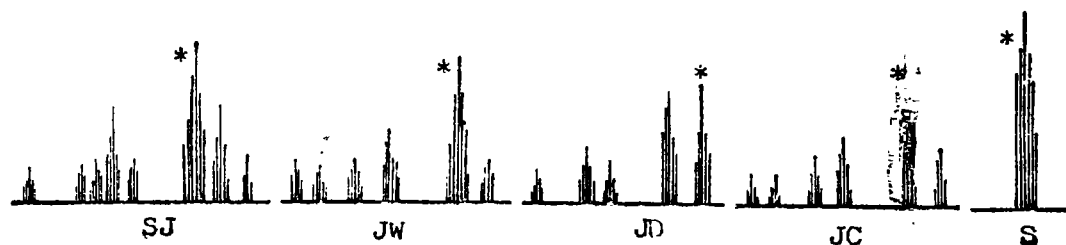


图2 薄层扫描谱(*为3, 4-二羟基苯甲醛)

表2 各样品的紫外吸收度比较

样品	SJ	JD	JC	JW
Aλ _{max}	0.707	0.323	0.270	0.713
相对 %	100	45.7	38.2	100.8

表3 各样品有效成分峰面积比较

样品	峰面积	相对 (%)
SJ	14223	100
JD	4755	33.4
JC	5192	36.5
JW	13248	93.1

2.4 鞣质检查: 取SJ、JD、JC、JW、SZ各1ml, 逐滴加入明胶试液, 如有沉淀的记为(+), 以倍倍稀释法稀释样品至无沉淀记为(-), 测得鞣质检查阳性结果的最大稀释倍数见表4。

市售注射液中的含大量鞣质, 而经精制的注射液试制品未检查出鞣质。

2.5 注射液对血浆蛋白及红细胞的影响

2.5.1 血浆蛋白及2%红细胞生理盐水试液制备:

取健康人新鲜血液, 每10ml血液加入1250U的肝素钠0.1ml, 离心, 3000r/min, 30min。倾取上清液, 即为血浆蛋白试液。取下层红细胞, 用生理盐水离心冲洗2~3次, 至上清液不呈红色为止。然后按红细胞的量用生理盐水配成2%的悬浮液。

2.5.2 对红细胞的影响: 取2%的红细胞悬浮液2.5ml于干燥试管中, 依次加入不同稀释度的样品液, 轻轻旋摇均匀, 置37℃孵箱中, 孵化3h。置显微镜下观察细胞形态。如细胞无变形, 边缘整齐, 光滑, 结果为(-); 如细胞表面不光滑, 但细胞量不少, 结果为(+); 如细胞表面不光滑且边缘稍有不整齐, 少量细胞破裂, 致细胞间液微呈混浊, 结果为(++); 如细胞表面不光滑, 不整齐, 有变形, 细胞呈絮凝沉淀, 细胞数减少, 结果为(+++)。实验结果表明, 市售注射液在一定浓度下均能使细胞变性, 但不含鞣质的注射液试制品对红

表4 各样品的最大稀释倍数

样品	原液	1/2	1/4	1/8	1/16	1/32
SJ	+	+	+	+	-	-
JD	+	+	+	+	-	-
JC	+	+	+	+	-	-
JW	+	+	+	+	+	-
SZ	-	-	-	-	-	-

细胞无影响。见表5。

2.5.3 对血浆蛋白的影响: 取1ml血浆蛋白试液于试管中, 分别加入用蒸馏水稀释成不同浓度的样品, 若生成絮状沉淀或产生沉淀或产生浑浊者为阳性, 记为(+); 若实验液澄明, 无反应者为阴性, 记为(-); 市售丹参或复方丹参注射液在一定浓度下均能与血浆蛋白产生沉淀, 而无鞣质的丹参注射液试制品则对血浆蛋白无影响, , 见表6。

表5 样品对红细胞的影响

试管号	1	2	3	4	5
2%红细胞(ml)	2.5	2.5	2.5	2.5	2.5
供试药液(ml)	0.1	0.2	0.3	0.4	0.5
生理盐水(ml)	2.4	2.3	2.2	2.1	2.0
SJ	-	-	+	++	+++
JD	-	-	+	++	+++
JC	-	-	+	++	+++
JW	-	+	+	++	+++
SZ	-	-	-	-	-

表6 样品对血浆蛋白的影响

试管号	1	2	3	4	5
血浆蛋白(ml)	1	1	1	1	1
供试药液(ml)	0.1	0.2	0.3	0.4	0.5
蒸馏水(ml)	0.9	0.8	0.7	0.6	0.5
SJ	-	-	+	+	+
JD	-	-	-	+	+
JC	-	-	-	+	+
JW	-	+	+	+	+
SZ	-	-	-	-	-

3 讨论

丹参和复方丹参注射液的有效成分主要是丹参的水溶性物质, 这些物质是原儿茶醛、原儿茶酸、丹参素、丹参酸丙(二分子丹参素缩合物)、丹参酸乙(三分子丹参素和一分子咖啡酸的缩合物)、丹参酸乙的同系物、丹参迷迭香酸、丹参酸 A、3, 4-二羟基桂皮酸等9种^[1], 均为邻苯二羟基类化合物。本文采用了可见分光光度法直接测定邻苯二羟基类有效成分的含量, 用紫外分光光度计测定 $281 \pm 3\text{nm}$ 处的吸收度, 并且用薄层扫描法来比较注射液中3, 4-二羟基类甲醛单个有效成分的峰面积。表1、2、3为3组实验数据, 均证实市售四厂家注射液质量相差甚大。有效成分含量相差4.3倍, 紫外吸收度相差2.64倍, 3, 4-二羟基苯甲醛的峰面积相差2.99倍。这势必影响临床疗效。从3组数据比较, 可以看出, 采用可见分光光度法能直接测定注射液中有有效成分的含量, 测定操作的干扰因素少, 方法简单, 精密度较高。建议丹参及复方丹参注射液质量标准应增设含量测定项目。

近年来, 丹参及复方丹参注射液临床广为应用, 但不良反应也时有发生。静脉滴注时, 可引起室性心律失常^[5]、过敏性休克^[6]、肢体剧痛^[7]、静脉炎^[8]、肝脏损害^[9]等毒副反应。本文的实验数据, 证实4种市售注射液均含大量鞣质, 能使血浆蛋白沉淀; 能使红细胞变形, 破裂, 细胞量减少。而本实验室制得不含鞣质的丹参注射液则不使血浆蛋白发生沉淀, 也不使红细胞变性。由此可知, 丹参及复方丹参注射液的不良反应主要是由鞣质引起的, 鞣质为多羟基芳香酸组成的化学性质活泼的大分子物质, 进入机体后, 可作为半抗原与血浆蛋白的氨基缩合成更大分子的复合物, 引起机体变态反应, 导致过敏性休克; 进入静脉, 粘附于血管壁, 可致静脉炎, 小血管栓塞, 引起肢体剧痛; 进入静脉的药液回流入心脏, 一旦和心肌细胞结合就有可能引起心律失常; 进入肝脏, 大量鞣质与肝细胞作用的结果势必引起炎症甚至坏死。故建议丹参注射液的质量标准应增加鞣质检查项目。

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ABSTRACTS OF ORIGINAL ARTICLES

Studies on the Chemical Constituents of Libanjisheng

(*Helixanthera parasitica*)

Li Liangqiong, Li Meirong , Feng Wentao

Four compounds were isolated from the leaves of *Helixanthera parasitica*. They were identified as ethyl gallate (I), gallic acid (II), quercitrin (III) and 5,7,3',4'-tetrahydroxyflavan (IV) on the basis of chemical properties and spectral data. Compound IV is a new flavan.

(Original article on page 283)

Studies on the Chemical Constituents of Wangzaozi

(*Rabdosia amethystoides*)

Wang Xianrong, Wang Hongping , Li Youwen

Three compounds were isolated from the leaves of *Rabdosia amethystoides* (Benth) C.Y. Wu et Hsuan. They were identified as rabdosinaiol (I), oleanolic acid (II), β -sitosterol (III) on the basis of chemical reactions and spectral data. Compounds I and II were isolated for the first time from this plant.

(Original article on page 285)

Determination of Berberine in Processed Amur Corktree

(*Phellodendron amurense*) by HPLC

Wang Jingzhu, Chen Dingyi , Su Yingying

A HPLC method for the determination of berberine⁺ in *Phellodendron amurense* processed by four different procedures has been established. The method is simple, specific and accurate. The recovery is 102.7% and coefficient of variation is 0.69%.

(Original article on page 293)

UV Second Derivative Spectrophotometric Determination of Synthetic Decanoyl Acetaldehyde in Compounded

Chinese Medicinal Prescriptions

Jiang Xinmin, Yan Zhengyu , Yan Xueqin

UV second derivative spectrophotometry was used for the analysis of compounded Chinese medicinal prescription in order to eliminate interference from prescription base. Amplitude D and ΔA were taken as the quantitative informations. Synthetic decanoyl acetaldehyde in compounded Chinese medicinal prescription were determined by two methods. The Correlation coefficients of the standard curve were found to be 0.9994 and 0.9996, the mean recovery to be 98.10% and 96.31%.

(Original article on page 296)

On the Quality of Danshen Injection and Compound Danshen Injection

Yuan Lu, Su Guilan , Hu Guanshi

Active constituents in Danshen and Compound Danshen injections were determined by UV

spectrometry and TLC scanning. Results showed that the total amount of catechol like constituents in injections obtained from the market varies differently. It was proved that all the injections contained a large amount of tannins can lead to degeneration of red blood cells and agglutination of plasma protein and cause clinical toxic actions.

(Original article on page 299)

On the Effect of Total Saponins from Common Selfheal (*Prunella vulgaris*) On Experimental Myocardial Infarction and Hypertension of Anesthetized Rats

Wang Haiho, Zhang Zhiyu, Su Zhongwu, et al

Effects of total saponins from *Prunella vulgaris* (PVS) on acute myocardial infarction and hypertension of anesthetized rats were assessed. Pretreatment with PVS at a dose of 40mg/kg ip lowered Lg PVC, Lg (VT+VF) and ischemic arrhythmic scores; at a dose of 20mg/kg ip, resulted in a significant reduction of infarct size as compared with the controls; 2.5mg/kg iv showed an hypotensive action to both systolic and diastolic blood pressure.

(Original article on page 302)

Antiinflammatory Effects of Alcohol Extract of Daoguanliou (*Caesalpinia sepiaria* var. *pubescens*)

Li Le, Zhuang Feier, Li Zengli, et al

Antiinflammatory effects of alcohol extract of *Caesalpinia sepiaria* Roxb. var. *pubescens* Tang et wang were shown by ip injection. The extract significantly decreased xylene induced swelling of mice ear; carrageenin produced edema of hind paw in rats, and decreased volume of pleural exudate on carrageenin-induced acute pleurisy of rats. It also inhibited egg white induced arthritis in rats. LD₅₀ value is 303.7mg/kg by ip in mice, confidence limit of 95% is 296.5±189.4mg/kg.

(Original article on page 304)

Experimental Study on Anti-Lipid-Peroxidation of Japanese Raisin Tree (*Hovenia dulcis*)

Wang Yanlin, Han Yu, Qian Jingping

Anti-Lipid-Peroxidation of *Hovenia dulcis* Thunb. in mice was studied. Results showed that *H. dulcis* Thunb. had the following functions: 1. decrease MAD contents of serum and tissues of liver and brain. 2. increase SOD activity in tissues of liver, kidney and brain. 3. impart tolerance toward cold and heat, and prolong swimming time of mice. The results indicate that *H. dulcis* Thunb. has function of Anti-Lipid-Peroxidation and may be valuable for the retardation of senility.

(Original article on page 306)

Preliminary Study on Germinating Conditions of Ural Licorice (*Glycyrrhiza uralensis*)

Yu Linqing, He Maotai

Germinating conditions of *Glycyrrhiza uralensis* Fisch. including temperature, water, pH