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石甘散散剂挥发油的提取工艺研究*

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摘要 目的:探讨石甘散散剂挥发油提取工艺,优选石甘散散剂挥发油提取方法,确定提取的最佳工艺条件。**方法:**利用正交试验法,以水蒸气蒸馏法提取石甘散挥发油,计算挥发油得率;并用高效液相色谱法,以面积归一化法计算石甘散挥发油中 β -细辛醚的含量为指标,研究石甘散挥发油提取工艺中的影响因素;对提取时间、浸泡时间和加水量三个影响因素的试验结果进行分析,计算不同因素影响下的石甘散散剂挥发油得率及 β -细辛醚的含量,从而优选出石甘散挥发油最佳提取工艺。**结果:**影响石甘散挥发油得率及 β -细辛醚含量最主要的因素为提取时间,其次为加水量,再者为浸泡时间。**结论:**通过这三个因素影响下的正交试验结果,筛选出的石甘散散剂挥发油最佳提取工艺为加水量10倍,浸泡时间1小时,提取时间8小时。该提取工艺简单易行、合理稳定,为石甘散散剂挥发油的最佳提取工艺条件。

关键词:提取工艺;正交试验;石甘散散剂;挥发油; β -细辛醚

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Extraction Technology Research on Volatile Oil of Shi Gan Powder*

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ABSTRACT Objective: Discussing the extraction process of volatile oil from Shi Gan powder, optimizing the extraction method of it, to determine the optimal extraction process. **Methods:** Using orthogonal test method, with volatile oil extracted by steam distillation, to calculate the rate of volatile oil. Using high performance liquid chromatography to calculate the content of β -asarone from volatile oil of Shi Gan powder, with Area normalization as calculating method. To study the influence factors in the extraction of Shi Gan powder and analyse the results of the experiment from the aspects of extraction time, soaking time and water amount. Calculating the rate of volatile oil and the content of β -asarone from volatile oil of Shi Gan powder under the influence of the different factors, and thereby to optimize the extraction process of volatile oil from Shi Gan powder. **Results:** Extraction time was the first influence factor of the rate of extraction and the content of β -asarone, and the second is the amount of water, and the third is the soaking time. **Conclusion:** Through orthogonal test under these three influence factors, screening out the optimal extraction process of volatile oil from Shi Gan powder was 10 times the amount of water, soaking time for 1 hour and extraction time for 8 hours. The extraction process was simple, reasonable and stable, and the optimum extraction condition of Shi Gan powder was determined.

Key words: Extraction process; Orthogonal test; Shi Gan powder; Volatile oil; β -Asarone

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前言

癫痫为神经系统功能失常的常见慢性疾病之一,在临床治疗以西药治疗为主,中药治疗为辅,中药可通过逐渐减少西药药量来减少其对患者产生的毒副作用^[1-3]。目前,癫痫通过中药治疗经验丰富,并且毒、副作用相对小^[4]。中药石甘散复方制剂为石菖蒲、甘松两味中药等份为散,是治疗癫痫的临床经验方剂,在治疗癫痫的有效率、消除脑电图异常方面均有较好的治疗作用^[5]。石菖蒲、甘松是石甘散复方制剂的主要成分, β -细辛醚是石甘散散剂水蒸气蒸馏法挥发油主要成分之一,亦是治疗作用的重要药效物质^[6]。本文采取水蒸气蒸馏法^[7-9],提取石甘散

散剂中的挥发油并测定挥发油中 β -细辛醚含量^[10],以其含量为指标对石甘散挥发油提取工艺实行优选^[11-14],为石甘散散剂工业生产提供试验依据。

1 材料与方法

1.1 仪器与试药

1.1.1 仪器 高效液相色谱仪(日本岛津公司):岛津LC-20AD泵、岛津SPD-20AV紫外检测器、岛津Prominence CTO-20AC柱温箱、岛津ODS C18分析柱(150 mm 4.6 mm, 5 μ m),电子分析天平(JD-4 沈阳龙腾电子有限公司),中草药粉碎机(西厨不锈钢350克型打粉机),紫外可见分光光度计(北

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京普斯通用仪器有限公司),电子调温电热套(ZNHW 上海科升仪器有限公司),旋转蒸发仪(201D 上海易研科技有限公司)。

1.1.2 试药 石菖蒲、甘松饮片购于北京同仁堂药店(哈尔滨分店), β -细辛醚对照品购于中国药品生物制品鉴定所,甲醇(色谱纯)购于天津康科德有限公司,磷酸二氢钾(分析纯)购于天津市永大化学试剂有限公司,十二烷基磺酸钠(分析纯)购于天津市致远化学试剂有限公司。

1.2 试验方法

1.2.1 工艺路线的拟定 挥发油大多具有较强的生物活性,是植物体中一类具有挥发性,与水不混溶的油状液体。 β -细辛醚作为挥发油中主要的活性物质,且是挥发油中的主要成分之一,根据石甘散散剂中各味药有效成分的理化性质及临床应用特点,采用设备相对简单、操作容易的水蒸气蒸馏法来提取挥发油,拟定出石甘散挥发油的提取工艺路线,然后以具有较高

灵敏度、稳定性和重复性的高效液相色谱法来测定石甘散挥发油中 β -细辛醚含量,以石甘散复方制剂中 β -细辛醚含量为测定指标。所以,本文采用正交设计法考察提取时间、溶剂量和浸泡时间等三个因素在不同提取条件下的挥发油得率及 β -细辛醚含量,进而确定提取石甘散散剂挥发油的最佳提取工艺。

1.2.2 正交试验设计 把石甘散复方制剂分为等质量的若干份,采用正交设计法,以水蒸气蒸馏法提取每份石甘散样品中的挥发油,根据以下三个水平,提取时间、浸泡时间和加水量三个因素,按 L9(33)正交表进行正交试验。首先称取适量石甘散复方制剂,加入适量蒸馏水浸泡一定时间,再加入一定量的蒸馏水缓慢加热至回流,提取至挥发油体积不再增多,收取挥发油,加入无水硫酸钠吸水后得黄色油状液体,即得石甘散挥发油组分,称定其质量,与所加石甘散复方制剂总质量相比,即为挥发油得率。以石甘散挥发油得率作为评价指标,因素水平见表 1。

表 1 石甘散挥发油提取正交试验因素水平表

Table 1 Factors and levels of orthogonal test for the extraction of volatile oil from Shi Gan powder

Levels	Factors		
	Extraction time of volatile oil (A/h)	Soaking time of medicinal herbs (B/h)	Solvent multiple (C/h)
1	4	1	6
2	6	2	8
3	8	3	10

Note: The amount of volatile oil no longer increases after collecting for eight hours.

1.2.3 测定方法 对试验整体结果进行数理统计分析,测定并计算该试验方法下的挥发油得率^[15]。利用高效液相色谱法扫描 β -细辛醚最大吸收波长:精密称定 β -细辛醚对照品 10 mg,放于 25 mL 容量瓶内,加色谱甲醇溶解,定容后摇匀,作为储备液。精密量取 1 mL 上述储备液,放于 10 mL 容量瓶内,加甲醇定容后摇匀。取其适量,自动进样 10 μ L,进行全波长扫描,确定高效液相色谱条件,并在该条件下测定石甘散复方制剂挥发油含量。

1.2.4 对照品溶液的制备 精密量取上述储备液 1 mL,置于 10 mL 容量瓶内,加甲醇定容后摇匀,即得。

1.2.5 供试品溶液的制备 精密称取石甘散挥发油 10 mg,放于 25 mL 容量瓶内,加色谱甲醇溶解定容,摇匀;精密量取 1 mL,置于 10 mL 容量瓶内,加甲醇定容,摇匀,即得。

1.2.6 测定 分别吸取适量对照品溶液与供试品溶液于进样杯中,设置好色谱条件,自动进样 10 μ L,测定,即可。

2 结果

2.1 工艺路线的确定

正交试验及挥发油得率结果见表 2,方差分析结果见表 3。由表 2、3 能够得出,无显著性差别。提取时间对石甘散挥发油得率影响最多,第二为加水量,最后浸泡时间影响最小。由试验结果得知,石甘散散剂挥发油提取的最佳工艺组合为 A3B1C3,此方法下提取的挥发油得率最高。通过高效液相色谱全波长范围内吸光度扫描结果,表明 β -细辛醚在 257 nm 处有

最大吸收波长。按照 2.3 测定方法,确定色谱条件为:色谱柱为 ODS C18 分析柱 (150 mm × 4.6 mm, 5 μ m);流动相为甲醇(50%);水(50%),1000 mL 流动相中加入缓冲盐磷酸二氢钾和十二烷基磺酸钠各 1.2 g;检测波长 257 nm;流速 1.0 mL/min;进样量为 10 μ L。

2.2 验证试验

在 2.1 确定的色谱条件下,多次测得最佳工艺组合下提取挥发油中 β -细辛醚含量结果见表 4,表明试验的重现性符合试验要求。

3 结论

本研究通过正交试验,对石甘散复方制剂挥发油提取工艺的影响因素进行了分析,根据正交试验结果及 β -细辛醚含量重复验证试验,得出石甘散挥发油提取最佳工艺为:加水量 10 倍,浸泡时间 1 小时,提取时间 8 小时。试验结果显示本研究中石甘散挥发油提取工艺合理稳定、简单易行、重现性良好,此方法可以为石甘散工业生产提供试验依据。

4 讨论

石甘散具有定痫熄风、豁痰开窍止痫等功效,是博士后导师自拟验方中药补脑止痫散(首乌、石菖蒲、天麻、全蝎、远志、甘松等)中选取两位中药组成的新复方制剂,凭借传统基础中医理论,根据痫证病机的特性,组成的新散剂处方^[16-19]。为了实现中药的现代化,甚至走向国际,本文结合中药的应用经验,深

表 2 石甘散挥发油提取正交试验及测定结果

Table 2 Orthogonal test and the determination results for the extraction of volatile oil from Shi Gan powder

Serial number	Factors			Rate of volatile oil(%)
	A	B	C	
1	1	1	1	1.12
2	1	2	2	1.38
3	1	3	3	1.00
4	2	1	2	1.23
5	2	2	3	1.24
6	2	3	1	1.16
7	3	1	3	1.58
8	3	2	1	1.37
9	3	3	2	1.45
K1	1.167	1.310	1.217	T=11.53
K2	1.210	1.330	1.353	
K3	1.467	1.203	1.273	
R	0.300	0.127	0.136	

表 3 石甘散挥发油提取正交试验方差分析

Table 3 Variance analysis for the extraction of volatile oil from Shi Gan powder

Source of variance	Sum of squares of deviations	Degrees of freedom	F value	Fa	Significant
A	0.158	2	3.435	19.000	indifference
B	0.028	2	0.609	19.000	indifference
C	0.028	2	0.609	19.000	indifference
Error	0.050	2			
Sum	0.264				

表 4 石甘散挥发油 β - 细辛醚含量重复验证试验Table 4 Repeated verification test for the content of β -asarone from volatile oil of Shi Gan powder

	1	2	3	RSD(%)
β -asarone	0.515 mg/g	0.493 mg/g	0.498 mg/g	0.67

入研究其安全性、有效性、稳定性,对石甘散挥发油提取工艺进行了优化。石菖蒲的挥发油含有 α - 细辛醚、 β - 细辛醚等,中外研究表明,石菖蒲药理研究多集中在挥发性成分 α - 细辛醚与 β - 细辛醚,且都具有较强的抗惊厥作用和一定的中枢镇静作用,石菖蒲、甘松均是具有较好抗癫痫作用的药材^[20-22]。文献报道^[9],不同产地的石菖蒲挥发性成分有所不同, β - 细辛醚含量仍存在一定差异性。浙江省金华和河南省嵩县的石菖蒲中 β - 细辛醚含量较高,但石菖蒲中含量较高的挥发性成分均含有 β - 细辛醚,所以本文采用测定石甘散挥发油得率和有效成分 β - 细辛醚含量共同确定石甘散提取最佳工艺,是合理可行的。所以其复方制剂所选药材必须严格控制。

本试验初次探讨了石甘散复方制剂的挥发油提取工艺,采用水蒸汽蒸馏法提取挥发油,对提取时间、浸泡时间和加水量

三方面进行了考察。石甘散散剂中只有两味中药,适用水蒸气蒸馏法提取挥发油,其提取设备简单,所选溶剂亦环保,易于所提取挥发油的分离,所以此提取方法对分析人员的操作性、对设备的要求、以及对试验的稳定性综合考虑是比较好的选择。提取挥发油温度控制在回流温度即可,不易过高,以免破坏挥发油成分,对挥发油收率的稳定性造成影响。冷凝水的流量以及温度的控制也会对挥发油得率产生影响,温度太低、流量太大会对成分中易冷凝的油不好,温度太高、流量太小会使挥发油中易挥发的成分得不到冷凝,最终也会对挥发油得率和 β - 细辛醚含量造成较大影响。在克服这些影响因素上,本文对提取时间、浸泡时间和加水量三个影响因素通过正交试验,得出最佳提取工艺条件,并对试验结果进行多次验证,挥发油得率及 β - 细辛醚含量的稳定性可靠。

本文的研究对于确保石甘散复方制剂的质量及其今后更好的阐明其药效物质基础都是不可或缺的,对于石甘散复方制剂的研究空间还很大,还需更加深入挖掘,对石甘散的质量控制方面还需深入研究。以石甘散复方作为发挥药物疗效的基础,通过所含药效成分的挥发油得率及挥发油中 β -细辛醚含量评价其提取工艺合理、稳定,能够确定其安全性和稳定性。

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