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椒目及椒目仁油中 α - 亚麻酸的含量测定研究 *

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摘要 目的:建立同时测定椒目及椒目仁油中 α - 亚麻酸含量的 HPLC 方法。**方法:**用固定相为 Kromasil C18 柱(250 mm× 4.6 mm, 5 μ m), 流动相为乙腈-1%醋酸溶液(90:10), 检测波长为 205nm, 流速为 1.0 mL·min⁻¹, 柱温:25℃, 进样量:10 μ L, 测定椒目及椒目仁油中 α - 亚麻酸的含量;**结果:** α - 亚麻酸在(22~500) μ g·mL⁻¹ 浓度范围内线性关系良好, 5 批椒目和椒目仁油中 α - 亚麻酸的平均含量分别为 4.56%, 32.72%, 平均回收率分别为 99.87%, 98.97%。**结论:**所建方法操作简便, 准确可靠, 重现性良好, 可有效的控制椒目及椒目仁油的质量。

关键词: 椒目; 椒目仁油; HPLC 法; α - 亚麻酸

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Study on the Determination of α -linolenic Acid in the Zanthoxylum Bungeanum Maxim and the Seeds Oil from Zanthoxylum Bungeanum Maxim*

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ABSTRACT Objective: To establish the HPLC method to determine the content of α -linolenic acid in the Zanthoxylum bungeanum Maxim and the seeds oil from Zanthoxylum bungeanum Maxim. **Methods:** With the stationary phase for Kromasil C18 column (250 mm × 4.6 mm, 5 μ m), mobile phase of acetonitrile and 1% acetic acid solution (90:10), detection wavelength of 205 nm, flow rate of 1.0 mL min⁻¹, column temperature: 25 °C, sample quantity: 10 μ L to determine the content of α -linolenic acid in the Zanthoxylum bungeanum Maxim and the seeds oil from Zanthoxylum bungeanum Maxim. **Results:** Linear range of α -linolenic acid was (22~500) μ g·mL⁻¹, and the average contents of α -linolenic acid were 4.56% and 32.72%, the average recoveries of α -linolenic acid were 99.87% and 98.97% for Zanthoxylum bungeanum Maxim and the seeds oil from Zanthoxylum bungeanum Maxim respectively. **Conclusion:** The method is simple, accurate, reliable and with good reproducibility, which can effectively control the quality of Zanthoxylum bungeanum Maxim and the seeds oil from Zanthoxylum bungeanum Maxim.

Key words: Zanthoxylum bungeanum Maxim; The seeds oil from Zanthoxylum bungeanum Maxim; HPLC; α -linolenic acid

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前言

椒目为芸香科植物花椒(Zanthoxylum bungeanum Maxim.)的成熟种子。椒目又名川椒目,干燥的种子呈类球形,表面黑色有光泽,表皮已脱落者,露出黑色网状纹理,种皮质坚硬。椒目气香,味辛辣^[1];椒目仁油为采用压榨法或 CO₂ 超临界流体萃取等技术,从椒目的种仁中提取的脂肪油^[2]。目前,国内外研究发现,椒目仁油中富含不饱和脂肪酸,其中 α - 亚麻酸对人体健康^[3]和疾病治疗^[4]方面有重要作用。但《中国药典》^[5]及各地标准尚未收载椒目及椒目仁油,只是有些椒目及椒目仁油制剂相关开发的报道^[6,7]。为了进一步对药材及制剂标准的制定提供试验依据和参考,本次试验对椒目及椒目仁油中有效成分的测定方法

做了进一步的研究,使其测定结果更加精准,为建立和完善椒目及椒目仁油的质量标准提供参考。

1 材料

1.1 仪器

D200 型电子分析天平(德国 Sartorius 公司); LC-2010AHT 高效液相色谱仪(日本岛津公司); KQ-300E 型超声波清洗器(昆山市超声仪器有限公司);电热恒温水浴锅(北京科伟永兴仪器有限公司)。

1.2 试剂

α - 亚麻酸(批号 111631-200502)购自中国药品生物制品鉴定所; 5 批椒目及椒目仁油样品购自久芳(韩城)花椒有限公

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司;超纯水(H_2O);美国 Millipore 纯水器制备;乙腈(色谱纯)购自韩国德山药品工业,其它试剂均为国产分析纯。

2 方法与结果

2.1 色谱条件

色谱柱:Kromasil C18 (250 mm× 4.6 mm, 5 μm);流动相:乙腈-1%醋酸溶液(90:10);检测波长:205 nm;流速:1.0 mL·min⁻¹;柱温:25 °C;进样量:10 μL 。理论塔板数按 α -亚麻酸峰计算应分别不低于 4000^[7]。

2.2 对照品溶液的制备

取 α -亚麻酸对照品适量,精密称定,加乙醇制成每 1 mL 含 0.2 mg 的 α -亚麻酸。

2.3 椒目供试品制备

取本品粉末约 1 g,精密称定。置具塞锥形瓶中,先后加入石油醚(60-90 °C)50 mL,超声提取(功率 300 w,频率 40 kHz)2

次,每次 30 min。过滤,滤至圆底烧瓶内,合并滤液,减压回收石油醚。然后,在圆底烧瓶内加入 0.5 mol/L 的氢氧化钾乙醇溶液 10 mL,回流提取 30 min,放冷,加入酚酞试液 3 滴,加 0.5 mol/L 的盐酸溶液至红色刚好退去,溶液转移至 50 mL 量瓶中,加乙醇洗涤圆底烧瓶,洗涤液并入量瓶中,加乙醇至刻度,摇匀。精密量取 1 mL 置 10 mL 量瓶中,加乙醇至刻度,摇匀,即得^[8]。

2.4 椒目仁油供试品制备

取本品约 200 mg,精密称定。置圆底烧瓶内,加入 0.5 mol/L 的氢氧化钾乙醇溶液 10 mL,回流提取 30 min,放冷,加入酚酞试液 3 滴,加 0.5 mol/L 的盐酸溶液至红色刚好退去,溶液转移至 50 mL 量瓶中,加乙醇洗涤圆底烧瓶,洗涤液并入量瓶中,加乙醇至刻度,摇匀。精密量取 1 mL 置 10 mL 量瓶中,加乙醇至刻度,摇匀,即得^[8]。

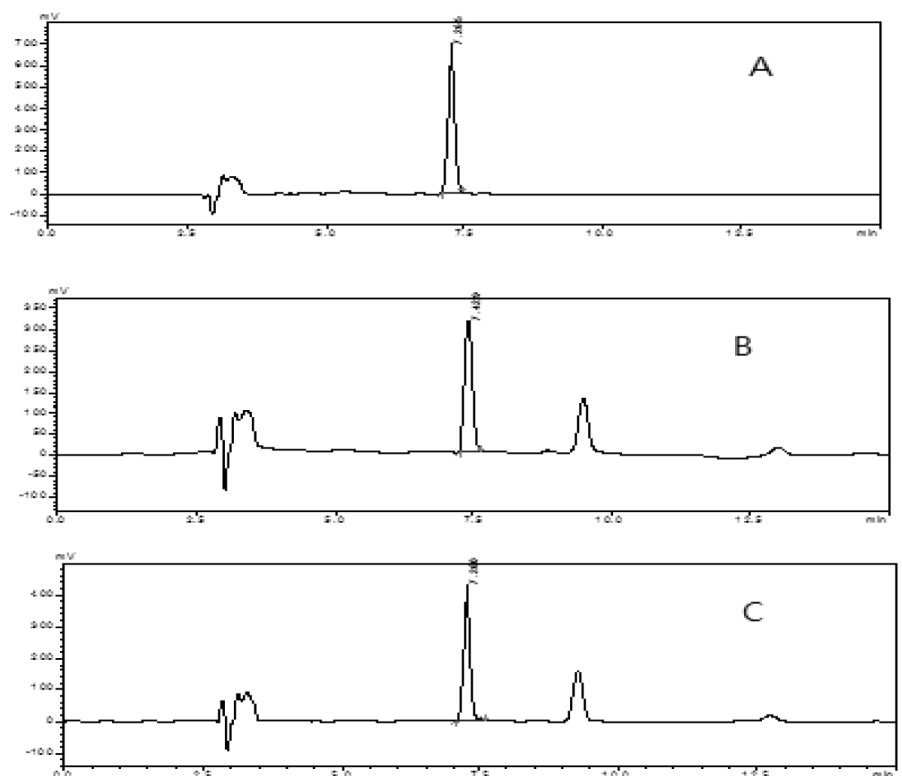


图 1 椒目及椒目仁油样品 HPLC 图

A- α -亚麻酸对照品 B-椒目供试品 C-椒目仁油供试品

Fig.1 HPLC figures of Zanthoxylum bungeanum Maxim and The seeds oil from Zanthoxylum bungeanum Maxim samples

A - a-linolenic acid B-Test samples of Zanthoxylum bungeanum Maxim C - test samples of The seeds oil from Zanthoxylum bungeanum Maxim

2.5 线性关系考察

精密量取 α -亚麻酸对照品溶液,经乙醇稀释,制成 α -亚麻酸浓度为 22,110,210,400 和 500 $\mu\text{g}\cdot\text{mL}^{-1}$ 的标准品溶液。分别精密吸取 10 μL 注入液相色谱仪按上述色谱条件测定。以浓度(X)为横坐标,峰面积积分值(Y)为纵坐标绘制标准曲线,得 α -亚麻酸的回归方程为: $Y = 33340X - 9123.5$ ($r^2 = 0.9999$)。结果表明, α -亚麻酸的浓度在 (22 ~ 500) $\mu\text{g}\cdot\text{mL}^{-1}$ 浓度范围内与各自峰面积积分值呈良好的线性关系。见图 2。

2.6 精密度试验

取同一浓度的标准品溶液,在上述色谱条件下连续进样 6 次,测定峰面积。结果, α -亚麻酸峰面积的 RSD 为 0.17%。表明仪器精密度良好。

2.7 重复性试验

取椒目和椒目仁油同一批供试品分别按 2.1.3 和 2.1.4 项下操作方法各制备 5 份样品溶液,进样测定。结果, α -亚麻酸峰面积的 RSD 值分别为 0.45%、1.64%。表明本实验所用的方法重复性良好。

2.8 稳定性试验

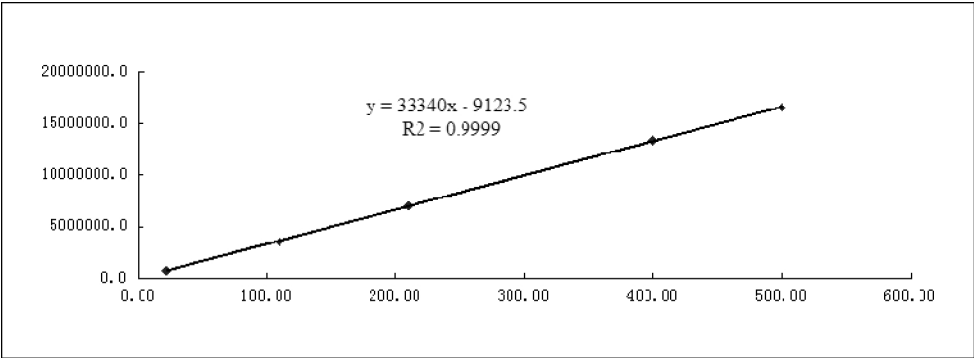


图 2 α- 亚麻酸标准曲线

Fig.2 The standard curve of a-inolenic acid

表 1 椒目加样平均回收率实验结果(n=3)

Table 1 The average recovery experiment results of Zanthoxylum bungeanum Maxim (n = 3)

Technical content (μg)	Add the scalar(μg)	Measured the amount (μg)	Recovery (%)	The average recovery(%)	RSD%
33602.64	20000	53855.81	101.27	99.87	1.24
33570.72	20000	53803.19	101.16		
30898.56	20000	50652.69	98.77		
35454.00	20000	55288.26	99.17		
32923.20	20000	52716.86	98.97		

表 2 椒目仁油加样平均回收率实验结果(n=3)

Table 2 The average recovery experiment results of The seeds oil from Zanthoxylum bungeanum Maxim (n = 3)

Technical content (μg)	Add the scalar(μg)	Measured the amount (μg)	Recovery (%)	The average recovery(%)	RSD%
43779.36	24000	67704.78	99.69	98.97	1.23
48589.20	24000	71922.80	97.22		
44531.92	24000	68314.64	99.09		
46495.12	24000	70153.08	98.57		
46102.48	24000	70172.44	100.29		

取同一浓度对照品溶液,分别于 0、1、2、4、8、12、24 h 测定峰面积,结果,对照品溶液中 α- 亚麻酸峰面积的 RSD 为 0.38%。表明 α- 亚麻酸在 24 h 内稳定。

2.1.9 加样回收率 精密称取已知含量的椒目和椒目仁油样品各 5 份,精密添加 α- 亚麻酸对照品,分别按照 2.1.3 和 2.1.4 项下的方法制备供试品溶液,进样测定,计算加样回收率,结果见

表 1、2。平均回收率分别为 99.87%、98.97%,RSD 值分别为 1.24%、1.23%。

2.1.10 样品含量测定 按上述色谱条件测定 5 批椒目及椒目仁油中 α- 亚麻酸的含量,结果见表 3、4。

3 讨论

表 3 五批椒目样品中 α- 亚麻酸含量测定结果(n=3)

Table 3 Five batches of a-linolenic acid content determination results in the Zanthoxylum bungeanum Maxim samples (n = 3)

Batch number	The average pek areaa	A-linolenic acid content (%)	Average(%)
1	3084326.0	4.59	4.48
2	3111949.0	4.67	
3	3031158.5	4.07	
4	3092718.0	4.61	
5	3090857.5	4.19	
6	3096156.5	4.65	

表4 五批椒目仁油样品中 α -亚麻酸含量测定结果(n=3)Table 4 Five batches of α -linolenic acid content determination results in the seeds oil from *Zanthoxylum bungeanum* Maxim samples (n=3)

Batch number	The average peak area	α -linolenic acid content (%)	Average(%)
1	4481353.5	33.42	31.99
2	4656817.0	33.09	
3	4632539.0	34.55	
4	4410910.0	28.63	
5	4459849.0	30.26	

椒目仁油中富含不饱和脂肪酸,如 α -亚麻酸、亚油酸等。因其中 α -亚麻酸含量最高^[6,9,10],且作用广泛^[11,12],促使对其各方面的研究^[13,14]。目前,药材和油样中 α -亚麻酸的含量测定多为 HPLC 法^[9]、气-质联用法,也有气相色谱法^[15,16],而 HPLC 法测定椒目仁油需经皂化水解、尿素包含所得的脂肪酸甲酯化产物来计算其所含 α -亚麻酸含量^[17],此法比较繁琐,不直观。我们之前用的 HPLC 法测定椒目仁油需经皂化水解、酸解等步骤,计算 α -亚麻酸含量^[7],后来发现此法不能够准确测定椒目中 α -亚麻酸的含量。鉴于此,本试验进一步探讨了其制备方法和 HPLC 法的条件,例如,本研究考察了椒目的提取溶剂、提取方法和提取时间,结果表明用石油醚(60~90℃)超声提取 30 min 提取效果较好;对供试品溶液的制备考察了提取时间和溶剂用量等,结果表明用氢氧化钾乙醇溶液 10 mL,回流提取 30 min,加酚酞试液 3 滴效果较佳。此外, α -亚麻酸分子结构中仅含非共轭双键,只有末端吸收,因此选择 205 nm 为检测波长。对于流动相,采用了乙腈-1%醋酸系统作为流动相,可在保证良好分离度的前提下,避免基线的波动干扰。结果表明:此法不仅能够准确测定其 α -亚麻酸的含量,且其含量明显提高。

综上,本研究科学、准确地测定了椒目及椒目仁油中 α -亚麻酸的含量,为椒目及椒目仁油中提取 α -亚麻酸提供了新的方法。不仅可以较好地控制椒目及椒目仁油的质量,且为其质量标准的制订提供了参考。

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