High Performance Liquid Chromatography-Electrospray Ionization Mass Spectrometry² Analysis of Acanthopanax giralddii Harms

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Abstract A high performance liquid chromatography-electrospray ionization-mass spectrometry-mass spectrometry² (HPLC-ESI-MS²) method has been developed for the qualitative analysis of Acanthopanax giralddii Harms. The separation was performed on Zorbax SB-C₁₈ column (250 mm × 4.6 mm i.d. × 5 μm) with gradient elution of acetonitrile-water. The pH was adjusted to 3.0 with acetic acid as mobile phase with a flow rate of 0.8 ml/min at 30 °C. The photodiode array detector (DAD) was set at 254 nm. The MS analysis was carried out on an Agilent 1100 series ion trap mass spectrometer with an electrospray ionization source in the positive ion mode. The ultraviolet (UV) chromatogram, total ion current chromatogram (TIC) and extracted ion chromatogram (EIC) of the sample and ESI-MS² spectra of the peaks in the chromatograms were obtained. Guanosine, adenosine and liriodendrin in the Acanthopanax giralddii Harms were identified. This method is easy to run, fast and accurate.

Key words high performance liquid chromatography-electrospray ionization-mass spectrometry², guanosine, adenosine, liriodendrin, Acanthopanax giralddii Harms, Chinese traditional medicine.
1. \[ \text{五加中化学成分进行定性分析,鉴定出红毛五加中鸟苷、腺苷和紫丁香树脂苷} \]

1.1 \[ \text{图} 1 \text{鸟苷(a)、腺苷(b)和紫丁香树脂苷(c)的分子结构式} \]

1.2 \[ \text{Agilent Agilent 1100 series Chemstation Chemstation} \]

1.2.1 \[ \text{Agilent 1100 series Chemstation} \]

1.2.2 \[ \text{Agilent 1100 series SL Chemstation} \]

2. \[ \text{实验部分} \]

2.1 \[ \text{试剂与样品} \]

乙腈为色谱纯,美国Dikma公司产;水为娃哈纯净水;其他试剂均为分析纯。红毛五加产于四川省,由沈阳药科大学孙启时教授鉴定。

2.1.1 \[ \text{仪器及条件} \]

美国Waters公司液相色谱123型多级离子阱质谱联用仪。数据处理系统为美国Waters公司化学工作站(4567)。

2.1.2 \[ \text{液相色谱部分} \]

Waters型高效液相色谱仪,包括低压四元梯度泵、二极管阵列检测器(#934),自动进样器、柱温箱、在线脱气机。

色谱柱:CEC(ABCD6),流动相:乙腈(60),水(40)溶液(用冰醋酸调),二元线性梯度洗脱,

2.1.3 \[ \text{质谱部分} \]

电喷雾离子源(J2),正离子检出模式;雾化气压力(3),干燥气(氮气)流量(93),干燥气温度(30),电喷雾电压(BLM),扫描范围(1),离子电荷控制(44)。采用自动二级质谱和自动参数设置(2670-K7076+)模式。柱后分流比(3).

2.2 \[ \text{样品的处理} \]

将红毛五加茎皮粉碎,称取(3),分别用去离子水加热回流提取(4)次(60,30 min),合并提取液,过滤,减压浓缩至约(4),加入乙醇适量,进行醇沉,使乙醇浓度达(0.45 μm),在低温(5)左右)下放置(20),后取上清液,减压浓缩后用水定容至(4),用(0.22)微孔滤膜过滤,滤液作(4)。
**Fig. 2** UV chromatogram [a] and TIC chromatogram [b] of the extract of *Acanthopanax giralddii* Harms

HPLC conditions: Zorbax SB-C18 column, 250 mm × 4.6 mm i.d., 5 μm. The mobile phase was gradient elution of acetonitrile and water, pH 3.0 with acetic acid as mobile phase with a flow rate of 0.8 mL/min at 30°C. The column effluent was monitored at UV 254 nm with diode array detector (DAD). MS conditions: electrospray ionization (ESI) positive detection mode, nebulizer gas pressure 0.24 MPa, 35 psi, drying gas flow rate 9 L/min, drying temperature 350°C.

1. guanosine
2. adenosine
3. liriodendrin.

**Fig. 3** ESI mass [a] and mass [b] spectra of guanosine

**Fig. 4** ESI mass [a] and mass [b] spectra of adenosine
色谱峰依次为鸟苷、腺苷和紫丁香树脂苷,其相对应谱图及其一级和二级质谱的碎片离子进行分析,鉴别,所以最终选用水为提取溶剂。为了防止水提液确度高、灵敏度高的特点,而且能解决因缺乏对照品谱检测。通过对的紫外色谱图中色谱峰的保留时间分别为腺苷和紫丁香树脂苷成分。以乙腈剂,对红毛五加茎皮进行加热回流提取。比较各提取液的色谱图发现,水提液的色谱图中色谱峰数最多,这样有利于对红毛五加进行

图8 9为流动相梯度洗脱,为图8 9

色谱柱,采用了水提醇沉法将这些杂质除去。多,这样有利于对红毛五加进行

五加质量控制方法的建立奠定了基础。

结论

讨论

参考文献: