Separation and Determination of Madecassic Acid in Triterpenic Genins of Centella asiatica by High Performance Liquid Chromatography Using β-Cyclodextrin as Mobile Phase Additive

PAN Jian KAI Guiqing YUAN Chuanxun ZHOU Beibei JIN Risheng YUAN Yuan

Abstract Centella asiatica L. Urban is a tropical medicinal plant with a long history of therapeutic uses. Madecassic acid and terminolic acid which are a pair of structural isomers are two constituents of Centella asiatica. A method using reversed-phase high performance liquid chromatography in which β-cyclodextrin β-CD was the additive in mobile phase has been developed for separation of the structural isomers and determination of madecassic acid. The two compounds can be isolated with high resolution on a C_{18} reversed-phase column with the addition of β-CD in the mobile phase. The separation mechanism of the isomers was discussed. It was assumed that the separation of the isomers might have been resulted from different inclusion forces of complexes with β-CD. The effects of β-CD concentration and the pH of mobile phase on resolution were investigated. It was found that the resolution of the isomers increased with the increase of β-CD concentration when the mobile phase consisted of methanol-water 65:35 v/v at pH 4. The correlation coefficient r^2 of the linear calibration curve between peak area and concentration of madecassic acid was 0.9989 in the range of 0.1–5.0 g/L. This method was successfully used to determine the madecassic acid in triterpenic genins of Centella asiatica.

Key words reversed-phase high performance liquid chromatography RP-HPLC β-cyclodextrin madecassic acid terminolic acid isomer Centella asiatica
acid. The two isomers are separated by HPLC, and the single peak is obtained when using linear gradient.
色谱第...卷...2002.10.58 \nu^2 = 0.998 \quad 0.1 \sim 5.0 \text{g/L}
\]

3. 不同浓度的...环糊精对羟基积雪草酸和...分离度的影响见图...可以看出,同分异构体的分离度随着...环糊精浓度的增加而增大。这可能是因为...环糊精浓度的增加有利于增强它与同分异构体之间的相互作用,使得同分异构体之...色谱行为差异扩大,得到不同的保留时间,从而达到分离的目的。但...环糊精浓度太高时,由于受...溶解度的限制,在流动相中不能完全溶解,使流动相变浑浊。因此通过微孔滤膜过滤后...环糊精的浓...度几乎没有增加,分离度就趋于水平了。

图...浓度对羟基积雪草酸同分异构体分离度的影响

流动性...对分离度的影响不是很...大,但对色谱峰的峰形有很大的影响。在低...值时,色谱图的基线很高;而当...值较大的时候,色...谱峰严重拖尾。实验表明,在...时,羟基积雪草酸和...的色谱峰都能达到较好的峰...形,分离度良好,因此本实验中流动相...调整为...。

2.2...10.0 \text{mg}\%
\]

\begin{align*}
\text{羟基积雪草酸的标准品的色谱图}
\end{align*}

\begin{align*}
\text{终点}
\end{align*}

\begin{align*}
\text{不同浓度的...环糊精对羟基积雪草酸和...分离度的影响见图...可以看出,同分异构体的分离度随着...环糊精浓度的增加而增大。这可能是因为...环糊精浓度的增加有利于增强它与同分异构体之间的相互作用,使得同分异构体之...色谱行为差异扩大,得到不同的保留时间,从而达到分离的目的。但...环糊精浓度太高时,由于受...溶解度的限制,在流动相中不能完全溶解,使流动相变浑浊。因此通过微孔滤膜过滤后...环糊精的浓...度几乎没有增加,分离度就趋于水平了。}

图...浓度对羟基积雪草酸同分异构体分离度的影响

流动性...对分离度的影响不是很...大,但对色谱峰的峰形有很大的影响。在低...值时,色谱图的基线很高;而当...值较大的时候,色...谱峰严重拖尾。实验表明,在...时,羟基积雪草酸和...的色谱峰都能达到较好的峰...形,分离度良好,因此本实验中流动相...调整为...。

羟基积雪草酸的定量分析

精密称取羟基积雪草酸对照品...用甲醇配制成...溶液,然后用甲醇进行倍比稀释,配成质量浓度依次为...和...的羟基积雪草酸对照品甲醇溶液。以峰面积...对质量浓度...进行线性回归,羟基积雪草酸的回归方程为...。结果表明,样品量在...范围内浓度与峰面积呈线性关系。