RP-HPLC Determination of 1,3-Dideoxygalactonojirimycin in Bombycis Faeces

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Abstract: Objective To establish a simple and rapid method for the determination of 1,3-dideoxygalactonojirimycin in Bombycis Faeces, a potent glucosidase inhibitor, by HPLC. Methods A RP-HPLC method with fluorescence detection has been developed. Results The HPLC method developed in this research has a good reliability including accuracy and precision. The detection limit was less than 72 ng. Conclusion This method is sufficiently sensitive for determining 1,3-dideoxygalactonojirimycin in Bombycis Faeces and other related products.

Key words: Bombycis Faeces; 1,3-dideoxygalactonojirimycin; extraction; glucosidase inhibitor; RP-HPLC

Introduction

Bombycis Faeces is a traditional Chinese drug. The total alkaloids from Bombycis Faeces could inhibit α-glucosidase activity cooperatively, which could successfully lower blood glucose level in the therapy of diabetes (Geng et al., 2005). 1-Deoxynojirimycin (DNJ) is a main component of the total alkaloids in Bombycis Faeces (Geng et al., 2005; 2007). While in our prior study of (2R,3R,5R)-2-(hydroxymethyl) piperidine-3,5-diol (1,3-dideoxygalactonojirimycin) (Fig. 1), a novel alkaloid obtained from Bombycis Faeces, its activity of α-glucosidase was measured by the production of glucose and determined by standard GOD-PAP enzymatic colorimetric method. We have found that 1,3-dideoxygalactonojirimycin has a promising inhibitory action on α-glucosidase activity (Zhu et al., 2011).

Fig. 1  Structure of 1,3-dideoxygalactonojirimycin

Because the total alkaloids and DNJ which are widely used in clinic for the treatment of diabetes demonstrate good inhibitory effect against α-glucosidase and 1,3-dideoxygalactonojirimycin has the similar action, it is necessary to focus on establishing a rapid and convenient method for determining 1,3-dideoxygalactonojirimycin in Bombycis Faeces.

Materials and methods

Chemicals and reagents

1,3-Dideoxygalactonojirimycin was self-restrained (purity 99%), and used as a reference standard in the experiments. Bombycis Faeces (No: 1002) was purchased from Tianjin Zhongxin Pharmaceuticals Co., Ltd. All reagents used were of analytical grade.

Chromatography conditions

The HPLC system consisted of a Shimadzu LC—6AD pump (Japan), a Shimadzu CBM—20A degasser, and a RF2000 Fluorescence Detector (Dionex) was employed in the experiment. The separation was performed on a YMC-Pack ODS-A column (250 mm × 4.6 mm, 5 μm) at room temperature. The mobile phase was 0.1% aqueous acetic acid solution-acetonitrile (35:65). The flow rate was 1 mL/min. A 20 μL sample was injected for the quantitative determination.

Extraction and quantification of 1,3-dideoxygalactonojirimycin

Powder (1.5 g, No: 1002) was added into 45 mL 0.1 mol/L HCl, vortexed for 20 min, and centrifuged at 4000 r/min for 10 min. The supernatant was removed, and the pellet was extracted again with the same method described above. The supernatants were combined and diluted to 100.0 mL with 0.1 mol/L HCl.
The diluted extract was used for subsequent derivatization.

The stock solution containing 0.018 mg/mL of 1,3-dideoxygalactonojirimycin was prepared using distilled water as solvent and stored at −4 °C until analysis.

The derivatization was carried out as following: 1.0 mL of 1,3-dideoxygalactonojirimycin standard or diluted extract was mixed with 1 mL K3BO3 buffer (pH 8.5) in a 10.0 mL volumetric flask. Three microliter of 5 mmol/L FMOC-Cl in CH3CN was added with immediate mixing and allowed to react at 20 °C for 30 min. Two microliter of 0.1 mol/L glycine was added to terminate the reaction by quenching the remaining FMOC-Cl. The mixture was diluted to 10 mL with 0.1% aqueous acetic acid.

Results and discussion

The 1,3-dideoxygalactonojirimycin has similar structure with DNJ. As compounds of this class do not have absorption in the UV-visible region, UV detection can not be used in the analysis. A fluorescence detection method was used by which 1,3-dideoxygalactonojirimycin was derivatized with FMOC-Cl according to the references (Kim et al., 2003; Ou, Chen, and Li, 2005).

Each component of the reaction mixture was separated very well by HPLC. The excitation and emission maxima were 254 and 322 nm, respectively (Fig. 2).

**Fig. 2** HPLC chromatograms of reference substances (A) and sample (B)
1: DNJ 2: (2R,3R,5R)-2-(hydroxymethyl) piperidine-3,5-diol
3: Gly-FMOC 4: FMOC-OH

Optimal conditions for the derivatization were investigated, including pH value and concentration of borate buffer, FMOC-Cl, reaction temperature, and time. The optimal range of pH value is 7.5–8.5 and optimal concentration range of FMOC for the derivatization is 3–7 mmol/L, respectively. To achieve maximum buffering capacity, the borate buffer with pH value 8.5 and 5 mmol/L for the concentration of FMOC was selected.

As a subsequent step, a time course study of the derivatization of FMOC-Cl was performed at 15, 20, 25, and 30 °C under the optimal conditions reported above. Different reaction temperatures resulted in different maximum peak areas at different times. Finally, the reaction conditions were set at 20 °C and 30 min.

Linearity and limit of detection

Various amounts of 1,3-dideoxygalactonojirimycin (72, 144, 216, 288, and 360 ng) in 20 μL were injected into HPLC. The correlation between dose and response is best described by the following equation: area units = 13701 × injected amount + 364 665; \( r^2 = 0.9998 \). The detection limit was less than 72 ng.

Method validation

In order to examine the method, we first attempted to get the accuracy of the instruments. Take an identical sample (1.5 g, No: 1002) and analyze it repeatedly for six times, then register the peak areas under the identical condition. The results showed that the RSD was 0.48%, which showed enough accuracy of the instruments.

For stability test, the prior same sample solution was analyzed every 2 h in one day at the room temperature, and the analytes were found to be rather stable within 24 h, the RSD was 1.1%.

Five powdered samples (No: 1002) were accurately weighed 1.5 g. Preparing the solution according to the prior extraction procedure, each sample was tested twice. The average content of 1,3-dideoxygalactonojirimycin in Bombycis Faeces is 0.051%, the RSD of a day is 1.8%, which shows the method has good repeatability.

The recovery test was carried out as following: the above powdered samples (No: 1002) were accurately weighed 1.4, 1.6, and 1.8 g, then added into 0.018 mg/mL of 1,3-dideoxygalactonojirimycin with concentrations of 30, 35, and 40 mL, respectively. The resultant samples were extracted and analyzed as described in Table 1. Results showed that the average recovery was 98.88%, and the RSD was 1.02%.
From the results of precision test, stability test, repeatability test, and recovery test, it can be concluded that the method manifested good precision and accuracy.

Simultaneously, we determined 1,3-dideoxygalactonojirimycin in *Bombycis Faeces* from different areas using the method mentioned in this article (Table 2).

### Table 2 1,3-Dideoxygalactonojirimycin concentration of *Bombycis Faeces* from three producing areas

<table>
<thead>
<tr>
<th>Producing areas</th>
<th>Content / %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jinhua, Zhejiang Province</td>
<td>0.055</td>
</tr>
<tr>
<td>Anguo, Hebei Province</td>
<td>0.052</td>
</tr>
<tr>
<td>Bozhou, Anhui Province</td>
<td>0.045</td>
</tr>
</tbody>
</table>

### Conclusion

We established a simple quantitative determination method for 1,3-dideoxygalactonojirimycin in *Bombycis Faeces*. This method may be applicable to the assay of 1,3-dideoxygalactonojirimycin present in various supplements and other related products.

### References


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