

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

QI Hui, ZHAO Hui, DING Zhen-ying, ZHU Yuan-yuan\*

Tianjin Key Laboratory of Molecular Drug Research, College of Pharmacy, Nankai University, Tianjin 300071, China

**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

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### Introduction

*Bombycis Faeces* is a traditional Chinese drug. The total alkaloids from *Bombycis Faeces* could inhibit  $\alpha$ -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 (2*R*,3*R*,5*R*)-2-(hydroxymethyl) piperidine-3,5-diol (1,3-dideoxygalactonojirimycin) (Fig. 1), a novel alkaloid obtained from *Bombycis Faeces*, its activity of  $\alpha$ -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  $\alpha$ -glucosidase activity (Zhu *et al*, 2011).

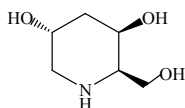


Fig. 1 Structure of 1,3-dideoxygalactonojirimycin

Because the total alkaloids and DNJ which are widely used in clinic for the treatment of diabetes demonstrate good inhibitory effect against  $\alpha$ -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-dideoxy-

galactonojirimycin 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  $\times$  4.6 mm, 5  $\mu$ m) 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  $\mu$ L 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.

\* Corresponding author: Zhu YY Address: College of Pharmacy, Nankai University, Tianjin 300071, China  
Tel: +86-139 2076 2856 Fax: +86-22-2350 3865 E-mail: zhuyy@nankai.edu.cn  
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The diluted extract was used for subsequent derivatization.

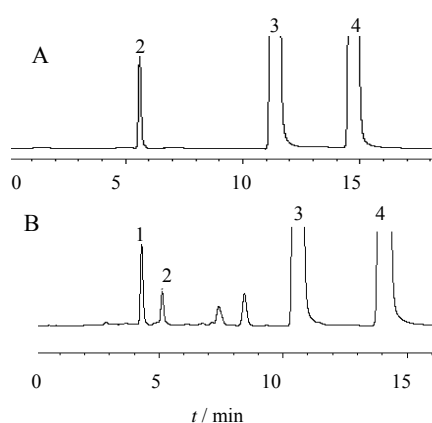
The stock solution containing 0.018 mg/mL of 1,3-dideoxygalactonojirimycin was prepared using distilled water as solvent and stored at  $-4^{\circ}\text{C}$  until analysis.

The derivatization was carried out as following: 1.0 mL of 1,3-dideoxygalactonojirimycin standard or diluted extract was mixed with 1 mL  $\text{K}_3\text{BO}_3$  buffer (pH 8.5) in a 10.0 mL volumetric flask. Three microliter of 5 mmol/L FMO-CI in  $\text{CH}_3\text{CN}$  was added with immediate mixing and allowed to react at  $20^{\circ}\text{C}$  for 30 min. Two microliter of 0.1 mol/L glycine was added to terminate the reaction by quenching the remaining FMO-CI. 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 FMO-CI 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: (2*R*,3*R*,5*R*)-2-(hydroxymethyl) piperidine-3,5-diol  
3: Gly-FMO-CI 4: FMO-CI-OH

Optimal conditions for the derivatization were investigated, including pH value and concentration of borate buffer, FMO-CI, reaction temperature, and

time. The optimal range of pH value is 7.5–8.5 and optimal concentration range of FMO-CI 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 FMO-CI was selected.

As a subsequent step, a time course study of the derivatization of FMO-CI was performed at 15, 20, 25, and  $30^{\circ}\text{C}$  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  $20^{\circ}\text{C}$  and 30 min.

### Linearity and limit of detection

Various amounts of 1,3-dideoxygalactonojirimycin (72, 144, 216, 288, and 360 ng) in 20  $\mu\text{L}$  were injected into HPLC. The correlation between dose and response is best described by the following equation: area units =  $13701 \times \text{injected amount} + 364\,665$ ;  $r^2 = 0.9998$ . 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%.

**Table 1** Recovery test of 1,3-dideoxygalactonojirimycin

Sample weight / g	Sample content / mg	Added / mg	Found / mg	Recovery / %	Average recovery / %	RSD / %
1.4098	1.0573	0.540	1.5918	98.98	98.61	0.34
1.4022	1.0516	0.540	1.5851	98.79		
1.6100	1.2075	0.630	1.8269	98.32		
1.6125	1.2116	0.630	1.8305	98.24		
1.8023	1.3517	0.720	2.0601	98.39		
1.8101	1.3575	0.720	2.0700	98.96		

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

Producing areas	Content / %
Jinhua, Zhejiang Province	0.055
Anguo, Hebei Province	0.052
Bozhou, Anhui Province	0.045

## 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.

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