Investigation of HP-β-CD Inclusion Compound Preparation Process for Pulsatilla Chinensis (Bunge) Regel Total Saponins1

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Abstract. This paper used parallel test of single factor to investigate feed ratio of inclusion compound, drying method, the volatilization temperature of ethanol for *Pulsatilla Chinensis* (Bunge) Regel total saponins-HP- β -CD inclusion compound. The results showed that the best preparation process of inclusion compound was as following: the feed ratio of *Pulsatilla* total saponin extract and HP- β -CD was 1:1; After the *Pulsatilla* total saponins extract with ethanol solution was dropwise added to the HP- β -CD aqueous solution, the mixed solution was continuously stirred for 6h at 60C and was freeze-dried for 12h. The preparation process for *Pulsatilla Chinensis* (Bunge) Regel total saponins-HP- β -CD inclusion compound was simple and it is helpful to improve the drug dissolution.

1. Introduction

Pulsatilla chinensis (Bunge) Regel can clear away heat and detoxicate, and can cool blood and arrest dysentery, which activities especially in cleaning colon heat and blood toxic heat. Pharmacological studies confirmed that *Pulsatilla chinensis* (Bunge) Regel extract had a good effect on anti-ulcer colitis [1], which can be developed as an oral colon-targeted preparation.

Pulsatilla chinensis (Bunge) Regel total saponins was extracted by ethanol from *Pulsatilla chinensis* (Bunge) Regel and separated and purified by Macroporous resin. The preliminary results showed that it had a low solubility in water [2] and was difficult to release when it was made into pellets directly. However, when it was made into hydroxypropyl-β-cyclodextrin (HP-β-CD) inclusion compound, it can significantly enhance the dissolution rate of the drug [3]. This paper had examined the prescription and process of *Pulsatilla chinensis* (Bunge) Regel total saponins-hydroxypropyl-β-cyclodextrin inclusion compound, which can lay the foundation on preparing pellets further.

2. Methods and results

2.1 Instruments and materials

The instruments used together with the suppliers were as follows: Freeze Dryer (FDU-1100, TOKYO RIKAKIKAI Co. Japan); Vacuum oven (DZF-6050, Medical Devices Ltd. Emerging Artists, Shanghai); HPLC (Agilent 1200, Agilent Technologies Ltd. USA).

The materials used together with the suppliers were as follows: Pulsatilla chinensis (Bunge) Regel total saponins (National Engineering Research Center for Manufacturing Solid Preparation of Traditional Chinese Medicine, Lot:20110804); Pulsatilla saponin D reference (National Engineering Research Center for Manufacturing Solid Preparation of Traditional Chinese Medicine, Lot:

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20111108); Hydroxypropyl- β -cyclodextrin (HP- β -CD, Biologica Chemical Ltd. Xian, Deli); Chromatography acetonitrile (Sinopharm Chemical Reagent Ltd.); Ethanol (Sinopharm Chemical Reagent Ltd.); Distilled water (Laboratory homemade). All other chemicals were of analytical-reagent and deionized double-distilled water was used throughout the study.

2.2 Establishment of the HPLC method

Hypersil ODS2 C18 column (5 μ m, 250mm × 4.6mm, Dalian Elite Ltd.), mobile phase of acetonitrile-0.1% phosphoric acid solution (39:61), flow rate 1mL·min-1, column temperature 30C, detection wavelength 203 nm, injection volume 0.02mL.

Number of theoretical plates calculated by the peak of Pulsatilla saponin D was not less than 5000. Index components from Pulsatilla saponin D had no interference on the peak position and had better separation and peak shape, and its chromatography was shown in Figure 1.



Fig.1: HPLC chromatogram

About 20mg Pulsatilla saponin D reference was accurately weighed, and was dissolved in methanol to 10mL as a stock solution. A volume of 0.25, 0.5, 1.0, 1.5, 2.5mL of the stock solution was accurately absorbed and then was respectively added methanol to 5mL. The prepared solution was injected according to the chromatographic conditions mentioned above and the results were recorded. The linear regression equation was obtained by taking concentration C (g·L-1) as the vertical axis and the peak area A (mAU·min) as the abscissa, and the results were shown as following: A = 4902.6C + 8.926, the RSD was r = 0.9995 and the linear range was 0.1009 ~ 1.009 g·L-1.

1mL sample solution, of which the content had been determined, was accurately measured, and about 10mg reference was accurately added into it. Then the methanol was added to the volume to 25ml. The same method was used to prepare three parallel solutions. The prepared solution was

determined according to the chromatographic conditions above. The results showed that the calculated recovery was 97.45% and the RSD was 1.43%.

The same sample solution was measured for three times on the same day and was continuously measured for three days according to the chromatographic conditions above. The intra-day and inter-day precision were respectively calculated, and the results were 0.13% and 0.52%, respectively.

2.3 Investigation of feeding ratio

About 3.0g *Pulsatilla chinensis* (Bunge) Regel total saponins was weighed, and the weighed sample was placed in a conical flask. 60mL ethanol was added into the conical flask. And then the sample was dissolved by ultrasound and heating. The HP- β -CD (2.0, 3.0 and 4.5g) was weighed, and was respectively placed in the 100mL beaker. Appropriate amount of distilled water was added into each beaker. The sample of each beaker was dissolved by magnetic stirring. The *Pulsatilla chinensis* (Bunge) Regel total saponins ethanol solution was dropwise added when the HP- β -CD solution was heated to 60C. After all the solution was added, the temperature was maintained at 60C for about 2h. Then the mixed solution was cooled to room temperature and was continually stirred until no alcohol taste. The final mixed solution was dried and crushed to obtain the *Pulsatilla* total saponins-HP- β -CD inclusion compound.

According to the third method of dissolution assay method in Chinese Pharmacopoeia (2010) bipartite appendix XC, the dissolution of the inclusion compound was respectively determined. Determination of the index components in the dissolution sample filtrate was according to the HPLC analysis method mentioned above. The results of the calculated dissolution were shown in Figure 2.



Fig 2. The dissolution curve of cyclodextrin inclusion compound of different feeding ratio The results showed that after the hydroxypropyl- β -cyclodextrin inclusion compound was prepared, the drug dissolution was accelerated significantly in the tested range. The higher proportion of hydroxypropyl- β -cyclodextrin, the more significantly the drug dissolution accelerated. Considering the influence of the drug content and solubilization and other factors, we can ultimately determine that the best feed ratio was *Pulsatilla chinensis* (Bunge) Regel total saponins and HP- β -CD of 1:1 for the preparation of inclusion compound.

2.4 Selection of drying methods

The inclusion compound was prepared by solution stirring method with the ratio of *Pulsatilla chinensis* (Bunge) Regel total saponins and HP- β -CD quality of 1:1. The freeze drying method and vacuum drying method were respectively used for the preparation of the inclusion compound, and the drying time was recorded. The characteristics of dried product were observed and the dissolution was determined. The investigative results were shown in Table 1. The results showed

that the prepared cyclodextrin inclusion compound from the two drying methods had no significant differences in the solubilization, but the required time of freeze-drying method was shorter and the dried product was looser, which had benefit for the preparation molding.

Table 1:	Study results of different drying methods			
Drying Method	Drying Time	Product Characters	Dissolution at 1h	
Freeze-drying	12h	loose	71.3%	
method	1211	10050	11070	
Vacuum drying	48h above	dense	70.4%	
method		actibe	, , .	

was looser, whi	ch had	benefit for	the preparati	on molding.	
Table	$1 \cdot St$	udv results	of different	drving methods	

2.5 Selection of ethanol evaporation temperature

The cyclodextrin inclusion compound was prepared by the method described above with the quality ratio of *Pulsatilla chinensis* (Bunge) Regel total saponins and HP- β -CD of 1:1. The prepared cyclodextrin inclusion compound was respectively stirred at 50, 60, 70C until no alcohol taste and then dried by freeze-drying. The required time of ethanol evaporation was recorded and the dissolution was determined. The results were shown in Table 2. The dissolution of the prepared cyclodextrin inclusion compound was accelerated with the reduction of the temperature, but the preparation time of the cyclodextrin inclusion compound was prepared at 60C and 50C had little differences in dissolution and both were significantly better than that at 70C, but the preparation time at 60C was significantly shorter than that at 50C. Considering the preparation time and solubilization effect, the volatile ethanol of 60Cwas selected for the preparation process of inclusion compound.

Temperature / C	Preparation Time / h	Dissolution at 1h			
50	12	77.2			
60	6	72.1			
70	4	60.2			

 Table 2:
 Investigation of ethanol evaporation temperature

3. Conclusion

The prepared inclusion compound by the process in this paper can significantly improve the dissolution of the drug. Meanwhile, the low temperature for the evaporation of ethanol was good for the increase of the dissolution rate of inclusion compound, which suggested that the inclusion effect for *Pulsatilla chinensis* (Bunge) Regel total saponins and HP- β -CD was reflected to be exothermic reaction.

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