Preparation and evaluation of brain-targetd thermosensitive in situ gel of geniposide

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Abstract

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Protocol

Determination of geniposide: C18 column (5 μm, 4.6 mm × 250 mm), the mobile phase acetonitrile: purified water (15: 85), 238 nm, the column temperature 25℃, the volume of each injection 20 μl.

Step 1.

Preparation of formulation: 1 Purified water was stored overnight at 4℃ in refrigerator. 2 Required amount of poloxamers were slowly added into the required volume of cold purified water with continuous stirring. 3 The upper hydrogel was kept at 4℃ until a transparent hydrogel was obtained. 4 The required amount of hydroxypropyl methylcellulose, benzalkonium chloride, sodium chloride, geniposide and borneol were dispersed into the hydrogel with continuous stirring. Volume was adjusted.

Step 2.

Clarity of formulation：Under black and white background, the clarity of formulation was observed and graded.

Step 3.

The pH value of formulation was determined by using the pH meter. The pH meter was first calibrated using solutions of pH 7.01 and pH 4.01.

Step 4.

Gelation temperature of formulation: 1 Hydrogel (0.5 ml) was transferred to small (2 ml, 12 mm × 32 mm). 2 Vial was sealed and immersed into a thermostat controlled-electric water bath at an initial temperature of 20℃. 3 Temperature of the water bath was increased in increment of 0.5℃/min. 4 Mercury bulb of a thermometer with a minimum readable scale of 0.2℃ was placed at the same level with the hydrogel. 5 The meniscus of the hydrogel didn’t move when the vial was tilted 90 degree angle. The temperature was identified as the gelation temperature.

Step 5.

The gel strength: A sample of 50 g of hydrogel was put into a 100 ml graduated cylinder and gelled in a thermostatically controlled water bath at 37±0.5℃. A weight of 35 g was placed onto the gel. The gel strength was determined by the time in second as the weight penetrated 5 cm into the gel.

Step 6.

Mucoadhesive strength: 1 A section of fresh goat nasal mucosa was prepared. 2 The goat nasal mucosa was tied to one side of the both vials (2 cm diameter). 3 Fifty milligrams of hydrogel was placed on one nasal mucosa of one vial. The two vials’ nasal mucosa were attached together for 2

min. 4 Water was poured drop by drop into one container of the balance instrument until the two vials got detached from each other. 5 The minimal water was weighed.

Step 7.

In vitro release: 1 Cold hydrogel (5 g) was transferred into a graduated test tube (1 cm diameter) and placed in water bath (34±0.5℃) and maintained 10 min. 2 A saline phosphate buffer (pH=6.4, 2.5 ml, 37±0.5℃) was layered over the surface of the gel. 3 Removed the medium at one-hour interval. 4 Test tube was cleared, weighted and layered with fresh saline phosphate buffer (2.5 ml).

Step 8.

Ex vivo drug permeation: 1 A section of fresh goat nasal mucosa was prepared. 2 The nasal mucosa was fixed on the Franz diffusion cell having effective permeation area of 2.8 cm2. 3 After 30 min of incubation time, gel 0.5 g was placed in the donor compartment. 4 The temperature of the chamber was maintained at 34±0.5℃. 5 Saline phosphate buffer (pH6.4, 6.5 ml) used as receptor medium was withdrawn from the receptor chamber at 30 min interval, and immediately replaced by the fresh. 6 The geniposide of sample was determined by the high- performance liquid chromatography method.

Step 9.