Study on novel galantamine hydrobromide sustained-release capsules and its *in vitro* releasing property

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Abstract: In present study, we prepared a novel galantamine hydro bromide sustained-release capsules with the new manufacturing technology, extrusion-spheronization method, and the optimized preparative formulation. A simple, rapid and accurate high performance liquid chromatography method (HPLC) was developed and validated for the quantification and release evaluation of galantamine hydro bromide. Experimental results showed that the method was specific, sensitive and reliable, could be effectively applied to the in vitro release study of galantamine hydro bromide sustained-release capsules. Our resulting samples had superior properties, worked better as sustained-release carriers and lasted longer hours to release drugs compared with the marketed control, Razadyne ER. The in vitro releasing characteristics of different batches of preparations are quite similar with each other, the total release proportions of galantamine hydro bromide from sustained-release capsules reached higher than 90 % within 12 h. The testing sustained-release preparation may be a promising new product for curing the related diseases.

Keywords: Galantamine hydro bromide; sustained-release; HPLC; release rate.

INTRODUCTION

By far, Alzheimer's disease (AD) has become one of the most common aging diseases of the highest risk. This chronic neurodegenerative disorder is characterized by the loss of cholinergic neurons and can cause progressive impairment of memory and cognitive function, as well as the resulting behavioral disturbances (Hebert *et al*.,2000). Although it was suggested that the pathogenesis of AD maybe involves oxidative I njury induced by free radicals (Lipton *et al*., 2007 and Moreira *et al*., 2005), cholinergic neurotransmitter system deficits (Oddo *et al*., 2006), and the elevated levels of proinflammatory cytokines (Wenk *et al*., 2002), the exact mechanism remains complicated and unknown. Nowadays, it has been indicated that many of the memory promoters, such as Brain Speed Shake, Brain Speed Smoothie, and Mocha Focus Delight etc., have chemical substances mimicking the memory promoting agents, for example, galantamine hydro bromide (Wenk, 2003).

Galantamine hydro bromide is a phenanthridine alkaloid and isolated from several members of the Amaryllidaceae family plant, such as snowdrops (de Jong *et al*., 2006). It can be used to moderate or delay the manifestation of AD symptoms as one of the selective and reversible acetyl cholinesterase (AChE) inhibitors, thus show the memory-enhancing effects (De Bruin *et al*., 2006). Further, its concentration-dependent inhibitive effect on AChE activity has antioxidative properties, involving decreased super oxide anion and NO overproduction, as well as restoring mitochondrial membrane potential (Ezoulin *et al*., 2009). Currently, among several drugs available for AD treatment, galantamine hydro bromide is the latest one recommended to improve the cognitive functions, and subsequently to treat Alzheimer's patients (U.S. National Library of Medicine, 2007). Galantamine hydro bromide has been approved most recently by the FDA for symptomatic treatment for AD and vascular dementia by oral or inject able administration. However, its pharmacological activities administrated by oral or injection would be likely to cause some severe side effects in gastrointestinal tract, and some organs or systems outside the central nervous system (CNS) (Douglas *et al*., 2008 and Valentin *et al*., 2004). Therefore, a more efficient administration route and pharmaceutical preparation to enhance delivery ability is urgently needed (Marques *et al*., 2011, Kuna *et al*., 2013 and Li *et al*., 2012).

In view of this, in the present work, we intended to develop a new galantamine hydro bromide preparation, sustained-release capsules, on this basis, establish and validate a simple, practical and accurate HPLC analysis method to determine drug contents, and further apply it to the in vitro releasing study. We also wished to confirm whether the release behavior of drug could be improved by the developed samples with that of the reference.

MATERIALS AND METHODS

Chemicals and Reagents

The reference substances of galantamine hydro bromide (purity>99.8%, fig. 1) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Potassium dihydrogen

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phosphate, sodium hydroxide and triethylamine were provided by Qinjiuhong Chemical Reagent Co., Ltd (Zhengzhou, China). Hydroxy-propyl methyl cellulose (HPMC), microcrystalline cellulose (MCC) and ethyl cellulose (EC) were provided by Beijing Huajinsheng Technology Co., Ltd. HPLC-grade methanol was purchased from Tedia Company Inc. (Fairfield, OH, USA). Other chemicals of analytical grade were provided by Nanjing Chemical Reagent Co., Ltd (Nanjing, China). Water was distilled and purified using a Milli-Q System (Millipore, MA, USA).

Preparation of galantamine hydro bromide sustained-release capsules

Sustained-release capsules were prepared by extrusion-spheronization method. In brief, an appropriate quantity of galantamine hydro bromide was weighed and mixed with MCC, after adding a solution of HPMC in water, pellets were prepared by extruding and rolling. Spray-coating technique was used to prepare immediate-release pellets with the solution of HPMC on fluidized bed bottom to envelop the sealing coat, followed by taking small samples for spraying to coat with EC water dispersion on fluidized bed bottom similarly, to obtain sustained-release pellets. Finally, encapsulated the immediate-release and sustained-release ones to capsules proportionally and packed, yielded the products.

Development of assay method for galantamine hydro bromide

Chromatography analysis condition

The chromatography separation was performed with a Diamonsil^{TM} C_{18} Column (200mm×4.6mm, 5μm) at a column temperature of 25^{o}. The mobile phase containing triethylamine phosphate buffer (PBS, pH 6.0)-methanol (75:25) was pumped at a flow rate of 1.0 ml·min^{-1}, the drug was detected at 289 nm for determining the content of galantamine hydro bromide.

Specificity

The release medium, PBS (pH 6.5), blank excipients solution including HPMC, MCC and EC (each of the excipient was weighed precisely, mixed and dissolved by release medium), and the reference solution of galantamine hydro bromide were taken and injected for HPLC analysis, respectively.

Linearity

The galantamine hydro bromide testing solutions of different concentrations were prepared. The calibration curve samples were assayed in triplicate, using concentration (C) as abscissa and peak area (A) as ordinates.

Precision and accuracy

Precision and accuracy were assessed by determining the replicate QC samples (10 μg·ml^{-1}) on the same day (intra-day precision) and three consecutive days (inter-day precision). Accuracy was described by relative error and precision was evaluated by intra- and inter-day relative standard deviation (RSD).

Recovery

Absolute recovery of analytes was evaluated by QC samples and data were determined by comparing the mean amounts obtained from the excipients solution spiked with reference solution with that of the neat standard samples. Three different concentration levels of analytes were evaluated by analyzing the five samples at each level.

Stability

The stability of galantamine hydro bromide was evaluated using the samples of 100% concentration level in recovery determination experiment. The samples were analyzed at 0, 2, 4, 8, 10 and 12 h after conditioning at room temperature, respectively.

Releasing assay method of sustained-release capsules

The oar method for dissolution test was consulted to determine release rate of galantamine hydro bromide from sustained-release capsules. 900ml release medium was taken to dissolution glass at predetermined temperature; release medium was agitated by stirring blades at the rotation speed of 50 r·min^{-1} and sampled at the scheduled time after initiating experiment. 10 ml sample was collected and filtered through a 0.45 μm membrane, filtrate was selected to determine as testing solution.

Galantamine hydro bromide content at each time point was determined by HPLC analysis. Meanwhile, the proper amounts of reference substance was dissolved and diluted quantitatively by release medium to the final concentration of 10 μg·ml^{-1} (8 mg standard) or 30 μg·ml^{-1} (24 mg standard), which was used as standard solution for the total drug amounts (W). The above solutions were analyzed by using HPLC and external standard method, accumulative release amounts and release percent were calculated according to the formula:

$$Q_x = C_x V_0 + \sum_{z-0}^{z-1} C_1 V_1 \quad \text{Accumulative release percent}(\%) = \mathrm{Q_x} / W \times 100\%$$

Noting: Qn was the accumulative release amounts at each time point, Cn was the measured concentration at each time point, V_0 was the bulk volume of release medium, V_i was the sampling volume, C_i was the measured concentration at time point *i*, W was the total drug amounts in capsules.

Releasing of galantamine hydro bromide in different medium

The in vitro releasing feature of galantamine hydro bromide was assessed using PBS (pH 6.5), hydrochloric acid solution (0.1M HCl), pH 4.5 buffer solution and purified water as release solvent, respectively. Release characteristics of galantamine hydro bromide were

Table 1: Release rate determination results of galantamine hydro bromide sustained-release capsules. (n=3). Data represent average ± standard deviation.

Time	1h (Theoretical value of 20-40%)	4h (Theoretical value of 50-70%)	12h (Theoretical value of >90%)
Tested samples	31.87%±0.61	61.48%±0.31	96.33%±0.67
Reference preparations	34.27%±1.30	66.40%±0.82	95.10%±1.81

determined at 0.5, 1, 2, 4, 6, 8, 10 and 12 h following the assay procedures described above. Filtrate was taken for HPLC analysis to determine the accumulative release amounts at each time point and draw the release curve.

Determination of release rate of galantamine hydro bromide in sustained-release capsules

The tested samples and control ones (Razadyne ER, 8 mg standard in galanthamine) were both taken for release rate assessment, drug contents as well as accumulative release rate in release medium were determined. According to the guiding principles of quality standards for sustained-release, controlled release and delayed release preparations, 1, 4, and 12 h were selected as sampling time points, the total release amounts for each capsules should attain 20-40%, 50-70% and higher than 90% corresponding to the three time points, respectively.

RESULTS

Method validation

Specificity

The HPLC chromatograms of galantamine hydro bromide were shown in fig. 2, it was indicated that the retention time (RT) was about 7.6 min, and that there was no significant interference on the determination from pharmaceutical necessities. Thus this method showed good specificity and selectivity for the following study under the selected conditions.

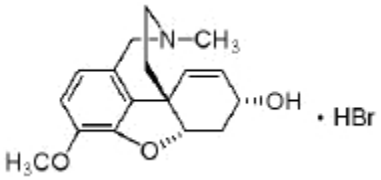

Fig. 1: The chemical structure of galantamine hydro bromide

Linearity

The calibration curves were prepared at the concentration levels of 2.5-50μg·ml^{-1} and constructed with a weight of $1/x^2$, the typical curve equation obtained was A=32986C-17970 with the correlation coefficient (r) 0.9999.

Precision and accuracy

The results of precision and accuracy assessed at low, median and high level were. The RSD values of intra-and inter-day precision were within 2%, and accuracy results extended from 95% to 105%.

Recovery

Absolute recovery of galantamine hydro bromide was determined by comparing the contents of three-level QC samples incorporated with excipients to that of the standard solutions, which were directly diluted by mobile phase. The recovery was 99.77±0.34%, 99.57±0.30% and 99.95±0.28% at low, middle and high QC concentrations, respectively, showing that the absolute recovery was high enough for the analysis of in preparation.

Stability

The room temperature stability results of galantamine hydro bromide showed that the testing samples were stable under storage conditions and routine analysis for release study.

Release characteristic of galantamine hydro bromide in different medium

The release curve of galantamine hydro bromide in four medium were shown in fig.3. It can be drawn that galantamine hydro bromide was dissolved from sustained-release capsules in the same way and at the similar speed within 12 h in different medium; all the accumulative release percents reached over 90 % at the last time point, suggesting that our sustained-release preparation had favorable release characteristic and the drugs can be fully release independent of release solvent.

Release results of galantamine hydro bromide in sustained-release capsules

The release curve of the tested samples and control ones were shown in fig. 4, release rates results contrast were summarized in table 1. We can conclude that galantamine hydro bromide in different batch of samples was released with nearly the same property, and release rates increased continuously along with the time. Although the total release amounts were almost the same, somewhat differently, the drugs in control samples were released more rapidly within 2 h and reached the highest value at about 8 h. Therefore, the results indicated that our tested capsules were of better sustained-release property comparing with the commercial preparation.

DISCUSSION

Nowadays, the most frequently used preparative methods for sustained-release pellets formation include rolling method, centrifugalization-fluidization method, spraying-congelation method, spray drying and liquid medium

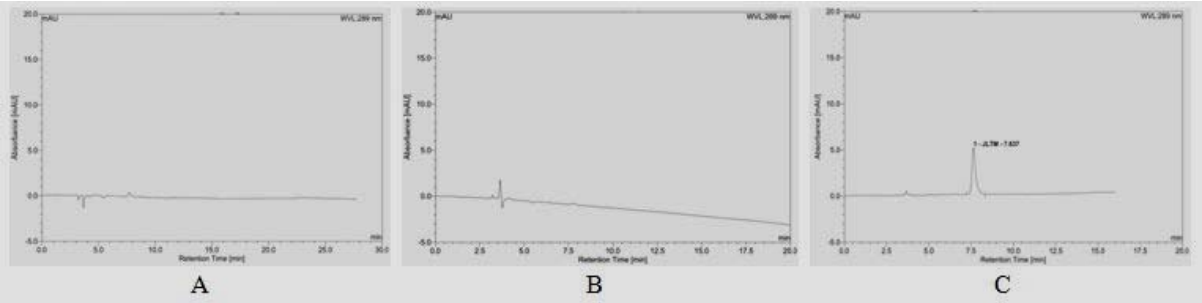

Fig. 2: Chromatograms of solvents (A), blank excipients (B) and galantamine hydro bromide standard (C).

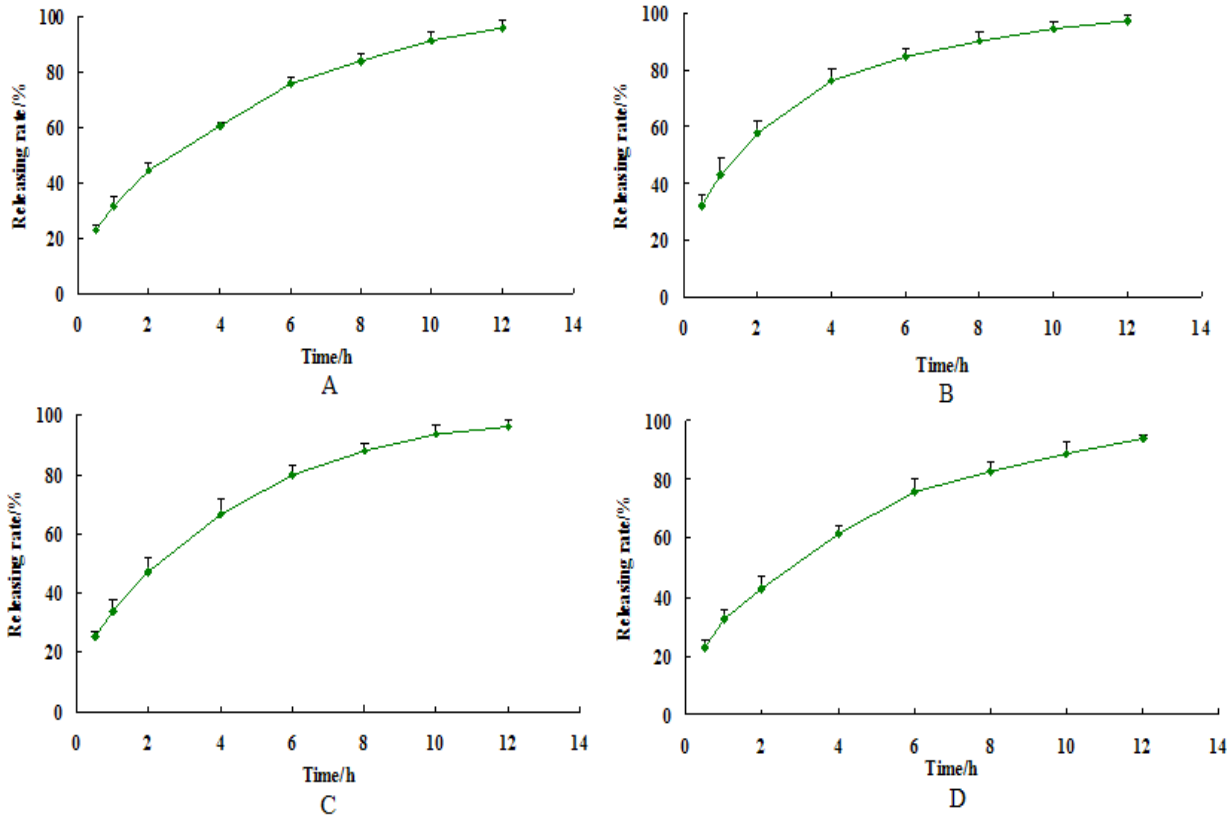

Fig. 3: Effects of mediums, PBS (A), 0.1 M HCl (B), pH 4.5 buffer solution (C), and purified water (D) on releasing of galantamine hydro bromide in sustained-release capsule (n=3).

method, and so on. However, extrusion-spheronization method is the most best with the advantage of convenient operation, having the rounded, smooth and complete resulting samples of comparable size and proper rigidity (Su *et al*., 2012, Wang *et al*., 2010 and Dong *et al*., 2013). For the post-marketing drug sample in USA, which used as control in this study, it was prepared by coating impermeable liner after fluidization dressing into the blank pellets with agent. Considering the longer dressing time is required for this method and the large drug loss result from spraying process, and the high cost of raw material drugs galantamine hydro bromide, to further save the drugs, the extrusion-spheronization method was adopted by comprehensive considering the relative merits of all the methods.

In our previous study, we has screened preparative process of pellets to optimize with the shape, friability, content and release rate as assessment index, on the basis of these results, prepared the trial-manufacture products in three successive batches to validate its feasibility and stability (Shibata *et al*., 2010 and Ishida *et al*., 2008). The results showed that the pharmaceutical preparation technique was reasonable, convenient and controllable, thus final formulation and preparation process was selected to manufacture galantamine hydro bromide immediate-release and sustained-release pellets. It should be noting that the fillings of marketed control sample

consisted of immediate-release and sustained-release ones of the equal amounts for both the 8 mg and 24 mg standards, the drug loading proportion for each immediate-release to sustained-release pellet was 1:3. By contrast, we produced with immediate-release and sustained-release ones in the proportions of 1:3, which enveloped the same amounts of drugs in each small pellet, to pack and obtain our capsules within different specifications. The testing results proved that the new formulation design would result in improved sustained property.

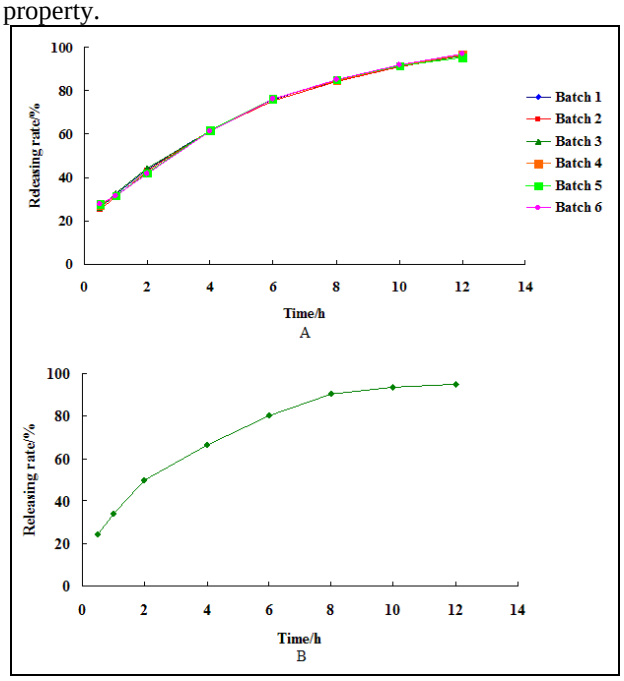

Fig. 4: The release curves of the tested (A) and control (B) sustained-release samples, with PBS (pH 6.5) as the release medium at the rotation speed of 50 r·min^{-1}.

It was suggesting that the release medium had little effect on the drug release from preparation. According to the sustained-release capsules assay provided by Dissolution Methods for Drug Products and the characteristic of product, phosphate buffer (PBS, pH 6.5) was selected for release evaluation and the dissolution methodology was studied. The release rates limit values were defined based on release curve of the control samples and the guidelines for sustained and controlled release preparation study. The experimental results indicated that 216.2 mg galantamine hydro bromide could well be resolved in 900 ml release medium, completely matching the sink condition for release rate determination (3-7 folds of raw material drug amounts equivalent to standard amount be resolved in the same volume of release medium). The maximum specification of our test samples was 30.7 mg calculating by galantamine hydro bromide, therefore, pH 6.5 PBS can entirely meet the release determination requirement of galantamine hydro bromide in sustained-release capsules.

In conclusion, we prepared galantamine hydro bromide sustained-release capsules using the new extrusion-spheronization preparative method, as well as adjusting and optimizing the immediate-release and sustained-release pellets formulation to constitute the preparation samples. The results indicated that the produced capsules had better sustained-release performance than control, which released their contents sustaining over a longer term. On the other hand, drugs could be fully released from sustained-release carriers within the specified time limit, and there was no difference in release property between batches. Furthermore, a rapid, simple and accurate HPLC method was established and validated. This method showed highly convenience and reliability for the rapid quantitative determination of galantamine hydro bromide contents in high-throughput release characteristic studies. This research offered the in vitro releasing details and may provide helps in the further pharmaceutical studied.

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