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ORIGINAL ARTICLE

Release property study on the novel divalproex sodium enteric-coated capsules

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Abstract In the present study, a novel divalproex sodium (DS) enteric-coated capsule was prepared, and high performance liquid chromatography (HPLC) assay method for DS was developed. Their uniformity, release curve and release characteristics in different solvents were examined. The release studies were performed using marketed sample as a reference and data were analyzed in terms of cumulative release amounts as a function of time. It was demonstrated by the results that assay developed was specific, rapid and reliable, which can be used to determine DS *in vitro* accurately, and our developed samples were similar to reference preparation in *in vitro* release characteristics. The release characteristics of different batches of samples were quite similar to each other, and the total release percents of DS from enteric-coated capsule were within 0–10% in HCl, and reached close to 100% in phosphate buffer. Similarity factors (f_2) of three batches between two preparations were all higher than 50. The developed enteric-coated capsule may be a promising alternative dosage form for treatment of related diseases.

1. Introduction

Bipolar disorder (BD) is a severe psychiatric disorder with worldwide lifetime prevalence estimated between 0.3% and 1.5%. It was reported that BD affects over 2% of the population (Suppes et al., 2005). The natural course of BD is characterized by acute severe affective episodes followed by high rates of relapse and recurrence. Depressions occur with greater frequency and last longer in BD, and even in treated samples, patients with BDs spend one-third to one-half of their lives with depressive symptoms. Furthermore, depression is associated with impairment in functioning that is at least equal to that observed in mania (Dutta et al., 2002; Kakkar et al., 2009). Thus, effective treatments for BD depression are urgently needed to reduce suffering and improve quality of life in patients, and the primary goal of treatment was to treat the acute episode as well as to prevent recurrences. Although numerous effective treatment options are available for acute mania, few medications have consistently been demonstrated

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to be effective in the depressed phase of BD (Bond et al., 2010). The treatment of BD remains a challenge for physicians.

To improve clinical outcomes for significant number of patients with BD, anticonvulsants and atypical antipsychotics are often used as alternatives or augmentation strategies to lithium. Divalproex is the most commonly prescribed anticonvulsant medication for patients with BD. Divalproex has demonstrated efficacy in preventing depressive episodes during maintenance treatment, but it is not routinely prescribed for the treatment of acute BD depression, and there is a perception among clinicians that there is little evidence to support its use (Muzyk et al., 2010). Divalproex has proven efficacy in treating acute mania and remains the principally accepted anticonvulsants for effective antimanic therapy. Approved for the treatment of mania in 1995, divalproex has surpassed lithium as the primary mood stabilizer employed in the treatment of bipolar disorders whether as monotherapy use or as a component of combination therapy regimens (Taher et al., 2009).

As a formulation combining valproic acid and sodium valproate, divalproex sodium (DS) is indicated for use as sole and adjunctive therapy in the treatment of patients with complex partial seizures that occur either in isolation or in association with other types of seizures. DS is also indicated for use as sole and adjunctive therapy in the treatment of simple and complex absence seizures, and adjunctively in patients with multiple seizure types that include absence seizures. However, there are several limitations of traditional routes of DS delivery (Pavuluri et al., 2004; Reed et al., 2009). These limitations include low oral bioavailability due to degradation in the stomach, inactivation and digestion by proteolytic enzymes in the luminal cavity, poor permeability across intestinal epithelium because of its high molecular weight and lack of lipophilicity.

Currently, the formulations of DS on the market in China are mainly normal tablet or capsule. However, these types of general formulations have a common drawback. Therefore, a large number of attempts have been made to deliver divalproex sodium orally using enteric-coated capsules. The enteric-coated capsules, which have a special outer covering designed to dissolve and absorb in the small intestine, offer the advantage of once daily dosing which may improve patient compliance and provide the opportunity to titrate to maximum effective dose while possibly reducing the risk of peak-concentration related side effects (Hosny et al., 2002). This feature of enteric-coated preparation not only moderates the stomach irritation or neutralizes gastric acid aroused by the long-term use of DS, but also improves the interrelated food absorption and avoids some adverse reactions (Pierre-Louis et al., 2009).

The aim of this study was to develop a novel DS enteric-coated dosage. *In vitro* dissolution testing is frequently used to determine the release characteristics of the pharmaceutical products over time. On this basis, a dissolution assay method was established and validated to the *in vitro* release study for enteric-coated capsules by high performance liquid chromatography (HPLC). The dissolution profiles of the commercial and self-made ones are compared by similar factors method, to evaluate the drug release performance of the developed formulation.

2. Materials and methods

2.1. Chemicals and reagents

The reference substances of DS (purity > 99.5%) were obtained from the Sigma–Aldrich (Shanghai, China). Potassium dihydrogen phosphate and sodium hydroxide 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. The commercial product (Razadyne ER) was purchased from the market. 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).

2.2. Development of assay method for DS

HPLC assay method was developed to determine DS (Li and Yang, 2014).

2.2.1. Chromatographic analytical procedures

The chromatography separation was performed with a Diamonsil™C_{18} Column (200 mm × 4.6 mm, 5 μm), the flow rate was 1.2 ml min^{-1}, with column temperature of 25 °C, the mobile phase was citrate buffer–phosphate buffer–acetonitrile (35:35:30, with the pH of 3.0 ± 0.1, adjusted by phosphoric acid), and the drug was detected at 210 nm for determining the content of DS.

2.2.2. Linearity

The developed capsules were 125 mg standard in DS, and the total volume of release medium was 500 ml. Its release percent should be within 0–10% in acid, and the maximum release should reach 100% in buffer solution.

The appropriate amounts of DS was weighed precisely and dissolved in phosphate buffer solution, to prepare the testing solutions of different concentrations. The testing solutions of different concentrations were taken for HPLC analysis. The calibration curve samples were assayed in triplicate, using concentration (C) as abscissa (X) and peak area as ordinates (Y).

2.2.3. Precision and accuracy

Precision was investigated by determining the replicate QC samples of 100% concentration level in recovery determination experiment on one day and three consecutive days, described as intra-day and inter-day precision, respectively. Accuracy was assessed by relative error and precision was investigated by intra- and inter-day relative standard deviation (RSD).

2.2.4. Recovery

Absolute recovery of DS was investigated by QC samples, and results were evaluated by comparing the means from the excipients solution spiked with reference solution with those of the standard samples. Three concentration levels of analytes were estimated by analyzing the samples at each level.

2.2.5. Stability

The stability of DS was investigated using the phosphate buffer solution at the concentration of 250 μg ml^{-1}. The samples were analyzed at 0, 2, 4, 6 and 8 h after conditioning at room temperature, respectively.

2.3. Dissolution assay method for enteric-coated capsules

The oar method for dissolution test was applied to determine dissolution of DS from enteric-coated capsules (Yang and Li, 2014). The quantity of 500 ml HCl solution was firstly used as release medium to take to dissolution glass at predetermined temperature; then, it was agitated by stirring blades at the rotation speed of 50 r min^{-1} and sampled at the scheduled time after initiating experiment. After 2 h the solution was filtered and the subsequent filtrate was used as test solution in acid; 500 ml of phosphate buffer solution was then added and used as release medium. After 45 min the solution was filtered and the subsequent filtrate was used as test solution in buffer.

The concentration of DS at each time point was determined by HPLC assay. Meanwhile the proper amounts of valproic acid standard reference were taken and dissolved in acetonitrile, and then diluted by 0.1 mol L^{-1} HCl solution or phosphate buffer solution (pH 7.5) to the final concentration of 250 μg ml^{-1}, which was used as control solution, which was used as standard solution for the total drug amounts (W). The above solutions were analyzed by external standard method, and accumulative release amounts and release percent were calculated according to the formula:

$$Q_n = C_n V_0 + \sum_{i=0}^{n-1} C_i V_i$$

$$\text{Accumulative release percent } (\%) = Q_n / W \times 100\%$$

Noting: Q_n was the accumulative release amounts at each time point, C_n 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, and W was the total drug amounts in capsules.

2.4. Release uniformity of DS in different medium

The *in vitro* release feature of DS was assessed in 0.1 M HCl, phosphate buffer (PBS, pH 7.5), respectively. The solution samples were taken for dissolution determination at 2 h in HCl, and at 10, 20, 30, 45 and 60 min following the assay procedures described above in PBS. Filtrate was taken to determine the accumulative release amounts at each time point and draw the release curve.

2.5. Drug release study and statistical analysis for release data

The developed formulation and marketed product were both taken for dissolution determination with the above two solutions used as release medium, respectively. Accumulative release percents were calculated for both the preparations and release performance in each medium was contrastively evaluated.

According to the guideline for bioavailability and bioequiavailability of orally administered solid drugs, similarity (f_2) measuring was applied to evaluate the closeness between the two dissolution profiles. The f_2 was calculated according to the equations given below:

$$f_2 = 50 \times \lg[(1 + Q/n)^{-1/2} \times 100] \quad Q = \sum_{t=1}^{n} (R_t - T_t)^2$$

where n is the number of time points, and R_t and T_t are the percentages of the reference and testing drug release at each time point t, respectively. In order to consider the release profiles similar, the f_2 values should be close to 100. In general, f_2 value of the two drug release profiles is between 50 and 100, and then these two drug release characteristics are similar, whereas value below 50 indicates differences between the release profiles.

3. Results

3.1. Method validation

3.1.1. Specificity

It was indicated by the HPLC chromatograms of valproic acid that its retention time (RT) was about 14.082 min, and enteric film coating was eluted at 4.3 and 6.9 min, without interfering the determination.

3.1.2. Linearity

The calibration curves were prepared at the concentration levels of 25.2–252 μg ml^{-1} in phosphate buffer. The typical curve equations were constructed with a weight of $1/x^2$ and described as $A = 7.8368C + 0.1319$, with the correlation coefficients (r) of 0.9999.

3.1.3. Precision and accuracy

The results of precision and accuracy were assessed at low, median and high levels. The RSD values of intra- and inter-day precision were within 1%, and accuracy results extended from 95% to 105%.

3.1.4. Recovery

Absolute recovery of DS was determined by comparing the contents of three-level QC samples incorporated with excipients to those of the standard solutions which were directly diluted by release medium. The recovery at low, middle and high QC concentrations was shown in Table 1. All these results

Table 1 Determination result of recovery.

Concentration (%)	Addition (mg)	Mass yield (mg)	Recovery (%)	Mean (%)	RSD (%)
50	12.9	12.70	98.47	99.31	0.94
	13.1	13.04	99.58		
	13.3	13.43	100.97		
80	21.3	20.97	98.44		
	21.0	20.88	99.45		
	21.6	21.31	98.66		
100	25.9	25.90	100.02		
	26.0	26.01	100.04		
	25.8	25.33	98.18		

showed that the absolute recoveries were high enough for the analysis of in preparation.

3.1.5. Stability

The room temperature stability results of DS with the RSD values lower than 2% showed that the testing samples were stable under storage conditions and routine analysis for release study.

3.2. Release uniformity of DS in different medium

The release results of DS in two medium are shown in Table 2 and Fig. 1, respectively. It can be drawn that the accumulative release percents calculated in different medium were different. In 0.1 M HCl, the drug release amounts were below 10% at 2 h. In phosphate buffer, the drugs can be fully released and the release percents all reached above 95%. Furthermore, the release rates were all consistent with each other both in the two medium, suggesting that DS was released with nearly the same property, and dissolution increased continuously along with the time. Thus it was indicated that there was little effect from release medium on the release performance of enteric-coated capsules.

3.3. Release rate determination for enteric-coated capsules

The release results of DS from enteric-coated capsules are summarized in Table 3. It showed that the total release percent was within 0–10% in HCl, and reached close to 100% in phosphate buffer, which met the requirement for release rate determination of oral enteric-coated preparation.

3.4. Drug release study

The dissolution curve of developed formulation and marketed product in phosphate buffer is shown in Fig. 2. The similarity factors (f_2) were calculated for the formulations using the release profile with the marketed product as the reference. The f_2 values of three batches were all higher than 50 (87.1, 76.4, and 70.5, respectively), suggesting that their release profiles were quite similar to those of the reference.

4. Discussion

In the *in vitro* release determination, in total two solutions, 0.1 M HCl solution and phosphate buffer (pH 7.5) were selected as release medium. Nevertheless, the drugs were released from enteric-coated capsules under mild alkaline condition, and thus sink condition experiment for medium was performed in phosphate buffer (Cui et al., 2015; Xue et al., 2014). Preparation method resembles the Divalproex Sodium Delayed-Release Tablets (USP32). In brief, monopotassium phosphate and sodium hydrate were dissolved in 5000 ml deionized water, pH was adjusted to 7.4 ± 0.1 with HCl (0.08 M), and the solution was then diluted to 6000 ml. In phosphate buffer above, 936 mg valproic acid standard sample was precisely weighed and dissolved into the 500 ml (7 folds of raw material drug amounts equivalent to standard amount be dissolved in the same volume of release medium) (Pradhan et al., 2014; Singh and Kim, 2000). The experimental results showed that DS could well be dissolved in release medium;

Table 2 The *in vitro* release result of DS in HCl at 2 h.

Batch	1	2	3	4	5	6	Mean
20150507	6.76	5.06	6.70	4.50	6.25	4.95	5.71
20150509	6.57	5.60	6.48	6.83	5.97	6.22	6.28
20150511	7.58	7.19	8.20	8.15	7.31	7.56	7.66

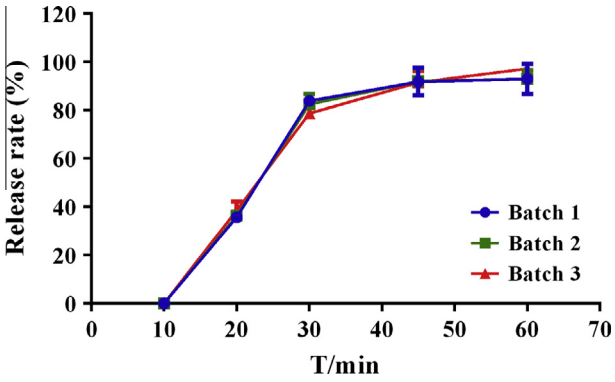

Figure 1 Release curve of DS in enteric-coated capsules in phosphate buffer. Each point represents average ± standard deviation (n = 6).

Table 3 The *in vitro* release rates of DS from enteric-coated capsules.

Batch	Release rate (%)	
	2 h in HCl	45 min in phosphate buffer
20150507	5.7	91.9
20150509	6.3	92.0
20150511	7.7	91.6

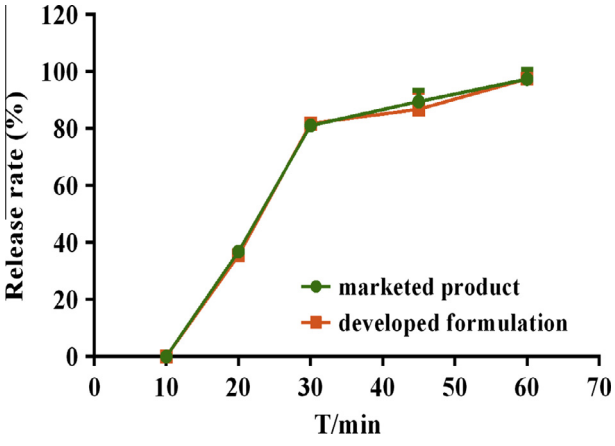

Figure 2 The release curve of the developed testing and marketed reference enteric-coated samples, with phosphate buffer (pH 7.5) as the release medium in the different stages in experiment.

therefore, the solvents could meet the release determination requirement of drugs in enteric-coated capsules.

For the drug release mechanism in matrix formulations, it can be decided by the formula: $Q = Kt^n$, where n is the release exponent value. Practically speaking, in cylindrical formulations, n value is lower than 0.45 suggesting the drug release was corresponding to the Fichian diffusion, and higher than 0.89 suggesting the drugs were released by matrix corrosion pathway (Peng et al., 2014). Whereas the characteristic parameter n is between 0.45 and 0.89, the main release mechanism of drugs from solid preparations was non-Fichian diffusion (Peppas, 2014). In this study, the n value for both the developed formulation and marketed product was 0.62 and 0.73, respectively, indicating that the drug release mechanism followed the non-Fick diffusion, which was affected by the drug diffusion and matrix corrosion (Le et al., 2014).

In conclusion, we prepared DS enteric-coated capsules, and developed an analysis method for the quantification and *in vitro* release study of DS. The results showed this assay method had highly convenience and reliability for the rapid quantitative determination of DS concentration in high-throughput release characteristic studies. Furthermore, the drugs could be well released from enteric-coated capsules in release medium within the specified time limit, and the release characteristics of the developed formulation and commercial samples were quite consistent with each other.

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