

RESEARCH ARTICLE

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## Development and validation of dissolution testings in acidic media for rabeprazole sodium delayed-release capsules

Yinhe Tan<sup>a,b,c</sup>, Xiaoqing Si<sup>c</sup>, Lulu Zhong<sup>c</sup>, Xin Feng<sup>d</sup>, Xinmin Yang<sup>c</sup>, Min Huang<sup>a</sup> and Chuanbin Wu<sup>a,b</sup>

<sup>a</sup>School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou, China; <sup>b</sup>Research and Development Center of Pharmaceutical Engineering, Sun Yat-Sen University, Guangzhou, China; <sup>c</sup>Guangdong Bidi Pharmaceutical Co. Ltd., Kaiping, China; <sup>d</sup>Department of Pharmaceutics and Drug Delivery, School of Pharmacy, University of Mississippi, Oxford, MS, USA

### ABSTRACT

Rabeprazole sodium (RAB) dissolved in acidic media is accompanied by its degradation in the course of dissolution testing. To develop and establish the accumulative release profiles of ACIPHEX<sup>®</sup> Sprinkle (RAB) delayed-release capsules (ACIPHEX<sup>®</sup> Sprinkle) in acidic media using USP apparatus 2 (paddle apparatus) as a dissolution tester, the issues of determination of accumulative release amount of RAB in these acidic media and interference of hydroxypropylmethyl cellulose phthalate were solved by adding appropriate hydrochloric acid (HCl) into dissolution samples coupled with centrifugation so as to remove the interference and form a solution of degradation products of RAB, which is of a considerably stable ultraviolet (UV) absorbance at the wavelength of 298 nm within 2.0 h. Therefore, the accumulative release amount of RAB in dissolution samples at each sample time points could be determined by UV-spectrophotometry, and the accumulative release profiles of ACIPHEX<sup>®</sup> Sprinkle in the media of pH 1.0, pH 6.0, and pH 6.8 could be established. The method was validated per as the ICH Q2 (R1) guidelines and demonstrated to be adequate for quality control of ACIPHEX<sup>®</sup> Sprinkle and the accumulative release profiles can be used as a tool to guide the formulation development and quality control of a generic drug for ACIPHEX<sup>®</sup> Sprinkle.

### ARTICLE HISTORY

Received 4 December 2015  
Revised 30 January 2016  
Accepted 27 February 2016

### KEYWORDS

Acidic medium; dissolution testing; method validation; rabeprazole sodium delayed-release capsule; release profile; stability





### Introduction

The active ingredient in ACIPHEX<sup>®</sup> Sprinkle delayed-release capsules (ACIPHEX<sup>®</sup> Sprinkle, Eisai Inc.) is rabeprazole sodium (RAB), which is chemically known as 2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridinyl]-methyl]sulfanyl]-1H-benzimidazole sodium salt. It has an empirical formula of C<sub>18</sub>H<sub>20</sub>N<sub>3</sub>NaO<sub>3</sub>S and a molecular weight of 381.42. It is very soluble in water and methanol, freely soluble in ethanol, chloroform, and ethyl acetate, and insoluble in ether and n-hexane. It is a typical proton pump inhibitor (PPI) can specifically inhibit the H<sup>+</sup>/K<sup>+</sup>-ATPase enzyme system at the secretory surface of the gastric parietal cells<sup>1,2</sup>. It is widely used in the treatment of active peptic ulcers, severe gastro-oesophageal reflux disease (GERD) and Zollinger-Ellison syndrome through suppressing the gastric acid secretion<sup>2-4</sup>. Because it rapidly degrades in acid media and is more stable under alkaline conditions<sup>3</sup>, RAB is usually prepared into enteric-coated dosage forms such as pellets, capsules, and tablets.

A predictive *in vitro* dissolution (or release) testing can serve as a useful tool during formulation development by providing discriminative *in vitro* data to guide the rational selection of desired formulation features<sup>5</sup>. A high-quality solid oral formulation should exhibit excellent effects in different physiological or pathological conditions of the human body after oral administration. Therefore, dissolution testing should be carried out under physiology conditions if possible. The pH of gastrointestinal tract (GI) fluids varies considerably along the length of the GI. Gastric fluid is strongly acidic, normally exhibiting a pH within the range 1–3.5 in healthy person in the fasted state. Following the ingestion of a meal, the

gastric juice is buffered to a less acidic pH, which is dependent on meal composition. Typical gastric pH values following a meal are in the range 3–7<sup>6,7</sup>. Depending on meal size, the gastric pH returns to the lower fasted-state value within 2–3 h. Thus, only a dosage form ingested with or soon after a meal will encounter these higher pH values, which may affect the chemical stability of a drug, drug dissolution or absorption<sup>6</sup>. Therefore, an aqueous dissolution medium with pH range from 1.0 to 6.8 should be utilized frequently. Dissolution testing of enteric-coated preparation was conducted in two steps, which are acidic step with 0.1 N hydrochloric acid (HCl) and next basic step with pH 6.8 phosphate-buffered solution (PBS). However, enteric-coated polymers, such as Eudragit L30D-55, hydroxypropylmethyl cellulose phthalate (HPMCP) start to dissolve when the pH value of the medium is above 5.5. Little information is available in the current literature with respect to the dissolution testing of enteric-coated preparations in media with pH range from 5.5 to 6.5, especially pH 6.0. Therefore, the medium of pH 6.0 was designed to use in the dissolution testing for ACIPHEX<sup>®</sup> Sprinkle.

Many analytical methods reported in the literatures were used to determined preparations of RAB like spectrophotometry<sup>8-13</sup> and spectrophotometric methods<sup>14,15</sup>, electrochemical methods<sup>16,17</sup> and chromatography such as high-performance liquid chromatography (HPLC) methods<sup>18-22</sup>, thin layer chromatography (TLC) method<sup>23</sup>, and high-performance thin layer chromatography (HPTC) methods<sup>11,24</sup>. A number of works describing the determination of rabeprazole in biological fluids<sup>25-31</sup> and dissolution testing for enteric-coated tablets<sup>3,4,32</sup> were also reported in the literatures. However, it

**CONTACT** Min Huang  [huangmin@mail.sysu.edu.cn](mailto:huangmin@mail.sysu.edu.cn)  School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou, China; Chuanbin Wu  [chuanbin\\_wu@126.com](mailto:chuanbin_wu@126.com)  School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou, China

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is difficult to use these analytical methods, especially common HPLC, to directly determine the accumulative release amount of RAB in acidic media due to its degradation in the course of dissolution.

The simultaneous detection of theophylline (TP) and cellulose acetate phthalate (CAP) in PBS by ultraviolet (UV) analysis had been carried out<sup>33</sup>. The advantage of this method is without the need for purification steps. However, not only two ingredients (such as TP and CAP) exist in the formulation of enteric-coated preparations and the others probably interfere with UV analysis of active principle ingredient, which restricts the use of the method of simultaneous detection. HPMCP is an enteric-coated ingredient of ACIPHEX<sup>®</sup> Sprinkle and can dissolve in PBS (pH 6.0–6.8), there is a strong UV absorbance because of benzene rings and double bonds in its structure, which could interfere with the determination of RAB using UV-based methodologies. The other ingredients such as diacetylated monoglycerides and hydroxypropyl cellulose in the formulation of ACIPHEX<sup>®</sup> Sprinkle may also interfere this UV analysis. Therefore, HPMCP cannot use the method of simultaneous detection<sup>33</sup> with RAB and need to be removed from the dissolution sample solution of ACIPHEX<sup>®</sup> Sprinkle. The difference of its solubility between acidic medium and alkaline medium may be utilized for removing it.

For ACIPHEX<sup>®</sup> Sprinkle, it is a challenge to develop and establish the dissolution profiles in the media of pH 1.0, pH 6.0, and pH 6.8 because of instability of RAB in these acidic media, drug degradation accompanying dissolution and interference from its degradation productions and HPMCP. To the best of authors' knowledges, there was no detailed dissolution testing in acidic media for solid dose forms of RAB, including ACIPHEX<sup>®</sup> Sprinkle, reported in the current literatures.

Therefore, the aim of this research is to solve the issues of determining the accumulative release amount of RAB in acidic dissolution medium and removing the inference of HPMCP. The dissolution samples of ACIPHEX<sup>®</sup> Sprinkle in acidic dissolution media was treated with appropriate HCl under centrifugation to form a solution of degradation products of RAB, which is of a considerably stable UV absorbance at the wavelength of 298 nm. In the way, the dissolution amount of RAB could be indirectly determined, and meanwhile the inference of HPMCP could be excluded. Certainly, accumulative release profiles established in the media of pH 1.0, pH 6.0, and pH 6.8 can be as an assessment tool for the formulation and process development as well as quality control of a generic drug of ACIPHEX<sup>®</sup> Sprinkle.

## Materials and methods

### Materials

RAB standard substance, 96.7% purity, was purchased from China Nation Institutes for Food and Drug Control. Three batches (No.: 005802 006418, 006418) of ACIPHEX<sup>®</sup> Sprinkle containing 10 mg RAB were purchased from Eisai Inc. It contained granules (or pellets) of RAB in a hard hypromellose capsule. Inactive ingredients are colloidal silicon dioxide, diacetylated monoglycerides, ethylcellulose, hydroxypropyl cellulose, hypromellose phthalate, magnesium oxide, magnesium stearate, mannitol, talc, titanium dioxide, carrageenan, potassium chloride, FD&C Yellow, No. 6 (in the 10 mg capsule), and gray printing ink. HPMCP was purchased from Shin-Etsu Chemical Co. Ltd. All other inactive ingredients were obtained from different local distributors. Sodium phosphate dibasic dodecahydrate and sodium hydroxide (NaOH) was purchased from National Pharmaceutical Group Chemical Reagent Co. Ltd. Tribasic sodium phosphate, HCl and phosphoric acid

were obtained from Guangzhou Chemical Reagent Factory. All the reagents were of analytical grade and water was purified using Millipore<sup>®</sup> system.

### Methods

#### Preparation of dissolution media

An FE20 pH meter (Mettler Toledo, Switzerland) was used to determine the pH value of 0.1 N HCl (pH 1.0), PBS (pH 6.0), and PBS (pH 6.8) used as dissolution media. 0.1N HCl, PBS (pH 6.8) were prepared per the specifications of the United States Pharmacopeia (USP) edition 36 while PBS (pH 6.0) was prepared as follow: place 1000 mL of 0.05M disodium hydrogen phosphate solution in a 2000 mL volumetric beaker, then add appropriate volume of 0.1 N HCl and adjust, if necessary, with 2 N HCl or 2 N NaOH to a pH of  $6.0 \pm 0.05$ .

#### Preparation of RAB standard solution

RAB standard substance (10 mg) was precisely weighed using MS105DU electronic weighing balance (Mettler Toledo, Switzerland) and dissolved in 0.1N HCl, PBS (pH 6.8), or methanol (for medium of pH 6.0) to form standard stock solution. The solution diluted with the dissolution medium was prepared into 1 mL solution containing 10  $\mu$ g RAB as standard solution before using.

#### Determining the stable UV absorbance wavelength

The prepared standard solutions were placed in  $37.0^\circ\text{C} \pm 0.5^\circ\text{C}$  water bath, and sampled 9.0 mL each at time points of 0, 30, 60, 90, 120 min. After cooled to room temperature, the sample solution was respectively added 0, 1.0, 1.2 mL 1.0 N HCl solution (corresponding to the media of pH 1.0, pH 6.0, and pH 6.8) to adjust pH to 1.0 and mixed uniformly. Then, a UV scan was performed on sample solutions at the wavelength range of 200–400 nm to determine the wavelength with stable UV absorbance. A UV-2450 UV-Vis spectrophotometer (Shimadzu, UV Probe 2.33 software) with a spectral bandwidth of 1 nm and a pair of 3.0 cm matched quartz cells were employed for all spectroscopic measurements.

#### Removing the interference of inactive ingredients

To remove the influence of HPMCP of the dissolution in acidic media, a series of control tests were designed and performed as follows. HPMCP, ACIPHEX<sup>®</sup> Sprinkle, and RAB were dissolved in PBS (pH 6.8) as sample solutions, respectively. The inactive ingredients prepared as per ACIPHEX<sup>®</sup> Sprinkle formulation was also dissolved in PBS (pH 6.8). These solutions were adjusted to pH 1.0 using 1.0 M HCl and stood for 30 min. Subsequently, ZB037 table-top low speed centrifuge (Xiangyi Centrifuge Instrument Corporation, China) was used to separate the precipitates. The centrifugation parameters were set at a speed of 4000 rpm for 30 min. Finally, the supernatant of HPMCP, ACIPHEX<sup>®</sup> Sprinkle, RAB, and inactive ingredients were obtained as test sample solutions. Concentration of these sample solutions was equivalent to 10  $\mu$ g/mL of RAB standard solutions. These sample solutions were carried out a UV scan with wavelength at the range of 200–400 nm. The same tests were repeated in PBS (pH 6.0).

#### Dissolution testing conditions

The dissolution test using the paddle method was performed in a RC806-mode dissolution test apparatus (Tianjin Tianda Tianfa Technology Co. Ltd., China), in accordance with the USP general

specification. Twelve-vessel dissolution unit was used to analyze the test samples by using the developed method. The dissolution testing followed the procedure predetermined by the United States Food and Drug Administration (US FDA) using USP apparatus 2 (Method A). The paddle speed was 75 or 60 rpm, and the temperature of the dissolution medium was maintained  $37.0 \pm 0.5^\circ\text{C}$  by covering the vessel. After 10 mL dissolution solution was drawn from dissolution vessel, the equal volume of fresh isothermal medium was added immediately. After ACIPHEX<sup>®</sup> Sprinkle were added in dissolution vessels in the media of pH 1.0, pH 6.0, pH 6.8, the rest dissolution conditions were as follows.

(a) In the medium of pH 1.0, place 750 mL of 0.1N HCl in the vessel for 120 min at 75 rpm, and 10 mL dissolution solution was sampled at time points of 10, 15, 20, 25, 30, 45 min.

(b) In the medium of pH 6.0, place 1000 mL of PBS (pH 6.0) for 120 min at 75 rpm and 10 mL dissolution solution was sampled at time points of 20, 30, 35, 40, 45, 50, 60, 70 min.

(c) In the medium of pH 6.8, place 750 mL of 0.1N HCl for 120 min at 75 rpm at acid stage and subsequently at buffer stage place 250 mL of 0.20 M tribasic sodium phosphate that had been equilibrated to  $37 \pm 0.5^\circ\text{C}$  was added into the acid medium as the US FDA recommended dissolution test condition for delayed-release dosage forms. Adjust, if necessary, with 2 N HCl or 2 N NaOH to a pH of 6.8. The dissolution test was continued for 45 min at 60 rpm. 10 mL dissolution solutions were sampled at time points of 10, 15, 20, 25, 30, 45 min.

#### Analytical procedures

The dissolution samples were filtered and dealt with 1.0 N HCl and removed from the interference of HPMCP as the section of removing the interference of inactive ingredients. The prepared solutions were then carried out to detect the UV absorbance at 298 nm. The RAB standard solutions were used as control and the corresponding dissolution media adjusted to pH 1.0 using 1.0 N HCl as blank control. The average cumulative drug release with standard deviations (SD) was calculated for each time point and media. The dissolution profiles were drawn by using the dissolution data versus time.

#### Validation of analytical method

The analytical method was validated according to the International Conference on Harmonization (ICH) Q2 (R1) guidelines for validation of analytical procedures for parameters such as linearity, specificity, accuracy, precision, LOD and LOQ for the analyte.

#### Linearity and range

Three replicated, seven different concentration levels of test solutions were prepared from RAB standard substance in the dissolution media. The pH value of these test solutions was adjusted to 1.0 as the section of determining the stable UV absorbance wavelength. The concentration levels ranged from 1 to 15% of analyte concentration (W/V: 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, and  $1.2\ \mu\text{g/mL}$ ) for 0.1N HCl and from 10 to 150% of analyte concentration (W/V: 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, and  $15.0\ \mu\text{g/mL}$ ) for PBS (pH 6.0 or 6.8). The linearity was evaluated by mean of UV absorbance (A) versus concentration (C), which was calculated by linear regression analysis.

#### Specificity

Specificity of the method was checked by a blank solution of inactive ingredients in the ACIPHEX<sup>®</sup> Sprinkle formulation. The inactive ingredients were prepared as per their common pharmaceutical dosage. Three aliquots of the inactive ingredients (58.3 mg)

were added to a 250 mL volumetric flask, dissolved in the media of pH 1.0, pH 6.0, and pH 6.8 and added corresponding medium to scale mark, respectively. These solutions named correspondingly placebo solution A (pH 1.0), B (pH 6.0), and C (pH 6.8) were treated as the section of removing the interference of inactive ingredients. The supernatants were collected as blank solutions of inactive ingredients. RAB standard solutions and the placebo solutions were carried out a UV scan with wavelength at the range of 200–400 nm.

#### Accuracy

The accuracy parameter was determined by the recovery test, which consisted of adding and dissolving known amounts of RAB into the placebo solution A, B, or C and following of the required dilution with corresponding dissolution media. This test was conducted by three different levels contrast to label amount of 10 mg RAB in ACIPHEX<sup>®</sup> Sprinkle. Dissolution concentration levels of RAB were designed 5%, 8% and 10% (W/W) for 0.1N HCl while 50%, 80%, and 100% (W/W) for PBS (pH 6.0 and pH 6.8) according to the property of enteric-coated preparations. Appropriate placebo solution A added 0.5, 0.8, 1.0 mL RAB standard solution ( $0.25\ \text{mg/mL}$ ) with each triplicate was prepared into 50, 80,  $100\ \mu\text{g/mL}$  RAB solution, respectively. In the same way, placebo solution B and C with each triplicate were prepared into 0.5, 0.8,  $1.0\ \text{mg/mL}$  RAB solution, respectively. The analytical procedure of amounts of RAB in these solutions was determined as the section of analytical procedures. The percent recovery is defined that the determined amount of RAB is divided by that of the added amounts multiplied by 100%. Test sample in three replicate sample preparations and the percent recoveries (mean  $\pm$  RSD of three replicates) of RAB in drug-placebo form were calculated.

#### Precision

The UV absorbances of RAB standard solution ( $10\ \mu\text{g/mL}$ ) in the media of pH 1.0, pH 6.0, and pH 6.8 at 298 nm were determined with six replicates, respectively. The results were expressed as mean  $\pm$  RSD of the determinations.

#### Stability of solutions

Solution stability was tested by allowing the standard solutions and prepared dissolution samples to stand exposed to room light and ambient room temperature for 2.0 h. The standard solutions were prepared into test sample solutions as described in the section of determining the stable UV absorbance wavelength. The dissolution sample solutions were withdrawn from dissolution media at 45 min. The test procedures of the sample solutions were dealt with as the section of analytical procedures and prepared into test solutions. The UV absorbance of these test solutions at 298 nm was determined at intervals of 0, 0.5, 1.0, 1.5, 2.0 h, and different percentages were compared to the value of initially prepared original solutions.

#### Sensitivity

For the sensitivity study, the limit of detection (LOD) and limit of quantification (LOQ) were estimated by determination of signal-noise ratios of 3:1 and 10:1, respectively, by determining a series of dilute solutions with known concentrations.

#### Results

In the present study, the method of centrifugal sedimentation, degradations of RAB using HCl and a UV-spectrophotometry were

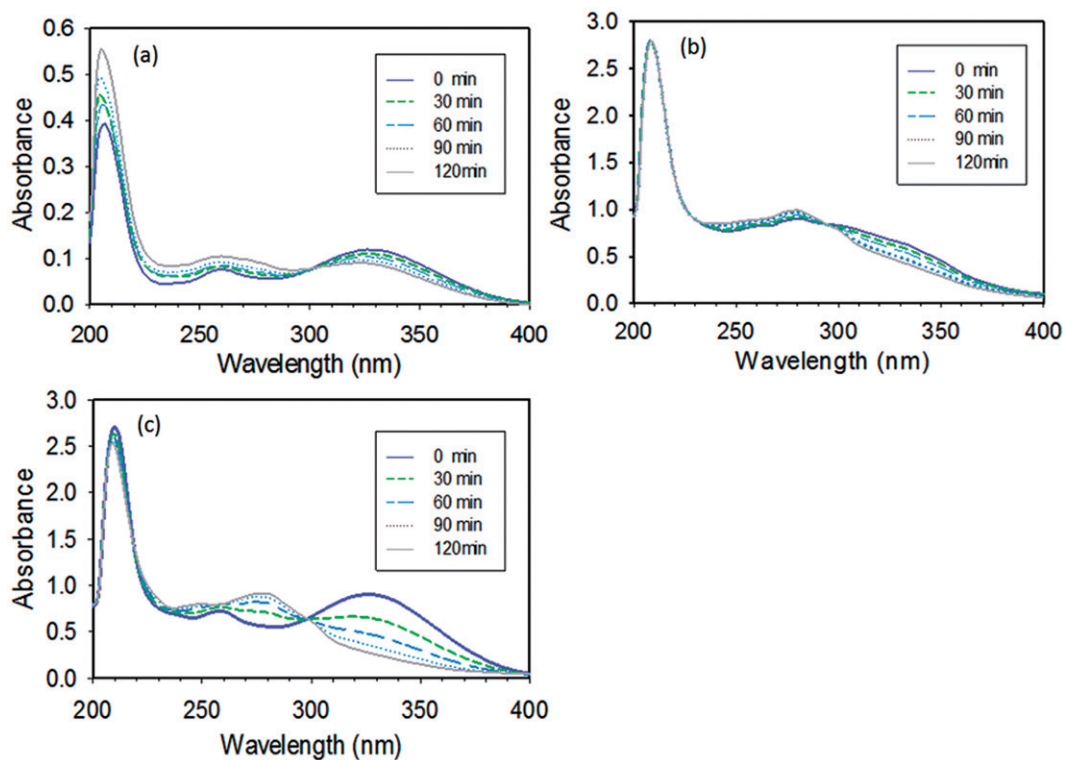


Figure 1. UV absorption spectrograms of RAB and its degradation within 120 min in the media of pH 1.0 (a), pH 6.0 (b), and pH 6.8 (c).

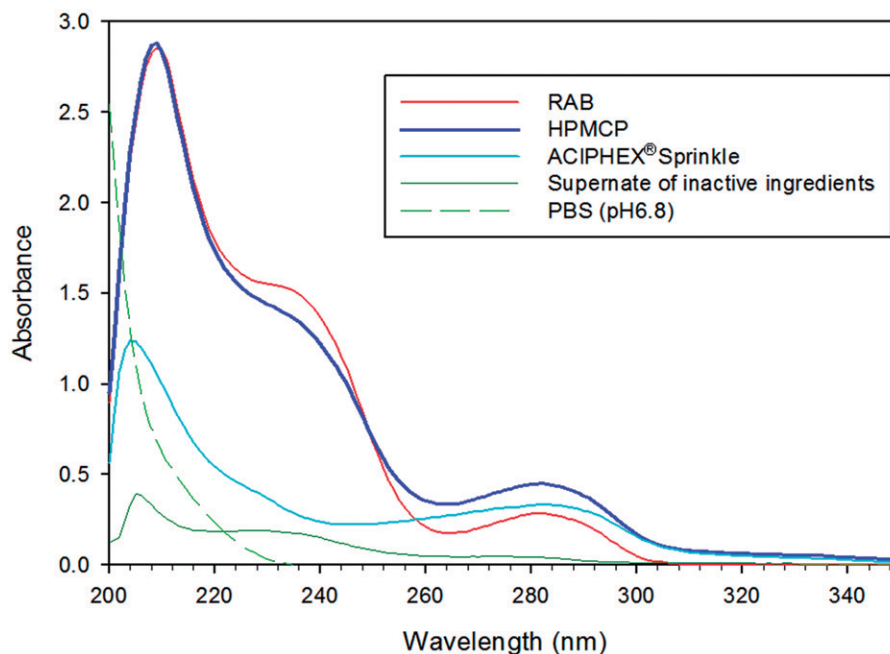


Figure 2. UV absorption spectrograms of HPMCP, ACIPHEX® Sprinkle, RAB, supernatant of inactive ingredients in PBS (pH 6.8).

developed for determination of samples containing RAB dissolved from ACIPHEX® Sprinkle in the dissolution media of pH 1.0, pH 6.0, pH 6.8. The developed methods were validated as per ICH guidelines for different parameters with the desired results.

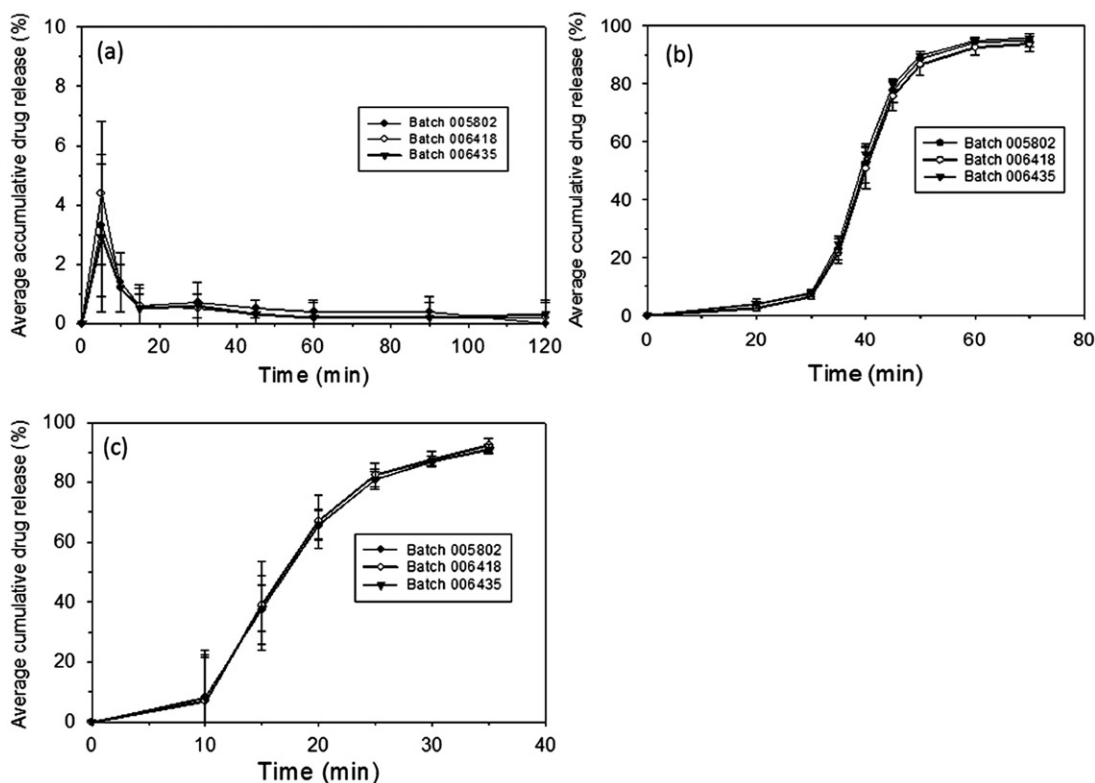
#### Determining the stable UV absorbance wavelength

Degradation solutions of RAB standard solutions, dealt with a water bath at  $37.0 \pm 0.5^\circ\text{C}$  and 1.0 N HCl, were carried out a UV scan with wavelength at the range of 200–400 nm. A stable UV absorbance of these solutions at 298 nm was found as shown in Figure 1.

Therefore, the wavelength at 298 nm was selected in subsequent UV detection.

#### Removing interference of inactive ingredients

When HPMCP is dissolved in PBS (pH 6.0 or 6.8), there is a strong UV absorbance at 200–300 nm as shown in Figure 2, which could heavily interfere with the determination of RAB in the media. However, there is almost no UV absorbance at 298 nm in supernatant of inactive ingredients containing HPMCP (Figure 2). The results indicated that when the medium of pH 6.8 containing



**Figure 3.** The release profiles of ACIPHEX<sup>®</sup> Sprinkle in the media of pH 1.0 (a), pH 6.0 (b), and pH 6.8(c).

**Table 1.** Linearity and ranges of RAB standard solutions between absorbance and concentration.

Acidic dissolution media	Calibration ranges ( $\mu\text{g/mL}$ )	Regression equations	Correlation coefficients
0.1N HCl (pH 1.0)	0.10–1.52	$A = 0.0732C - 0.0019$	1.000
PBS (pH 6.0)	0.86–12.94	$A = 0.0779C - 0.0002$	0.9995
PBS (pH 6.8)	0.91–13.64	$A = 0.0720C + 0.0004$	1.000

HPMCP were adjusted to pH 1.0 using 1.0 N HCl, HPMCP could be separated out from the solution to form floccules by standing for 30 min and be removed off by centrifugation at speed of 4000 rpm for 30 min. The same results were observed to occur in the medium of pH 6.0 (spectrograms not shown).

### Dissolution testing

Dissolution testing followed the procedure predetermined using USP apparatus 2 (paddle apparatus). The results of ACIPHEX<sup>®</sup> Sprinkle dissolution testing in the media of pH 1.0, pH 6.0, and pH 6.8 were shown in Figure 3. The average accumulative release percentage of RAB from ACIPHEX<sup>®</sup> Sprinkle in 0.1N HCl was far less than 10% up to the USP specification of delayed-release (enteric-coated) preparations. There was an apparent phenomenon of delayed-release when RAB was released from ACIPHEX<sup>®</sup> Sprinkle in the media of pH 6.0 and pH 6.8, especially pH 6.0 and the average accumulative release percentage of RAB was more than 90% up to release platform. The accumulative release profiles in acidic media demonstrated that it was reliable for the method of adjusting the pH value of dissolution sample solutions to 1.0 coupled with centrifugation to indirectly determine the amount of RAB using UV detection. In fact, it is important and discriminative for enteric-coated preparations to dissolve in the medium of pH 6.0. So it is necessary to research the specific dissolution features of RAB from ACIPHEX<sup>®</sup> Sprinkle in the medium of pH 6.0.

### Validation of analytical method

#### Linearity and ranges

The results were shown in Table 1. For three acidic dissolution media, the linearity of the calibration plot for the method was obtained over the calibration ranges tested. The regression equations for absorbance of RAB standard solutions were founded and all of the correlation coefficients obtained were more than 0.999, which indicated a high degree of correlation between A and C of the analyte.

#### Specificity

For three acidic dissolution media, the result in Figure 4 indicates that there is no interference at 298 nm between placebo solutions and RAB standard solution as there is virtually no appreciable UV absorbance exhibited at 298 nm for the placebo solution A, B, and C. Therefore, the method is specific.

#### Accuracy

Recovery studies were carried out at three levels and three determinations were made at each levels and percentage recovery was calculated. The results are mentioned in Table 2 that RAB mean recovery is ranged from 97.5% to 102.0% and RSD is ranged from 1.2% to 3.7% in the dissolution media. From the data obtained, it indicated that the recovery of RAB standard drug within the limits is accurate.

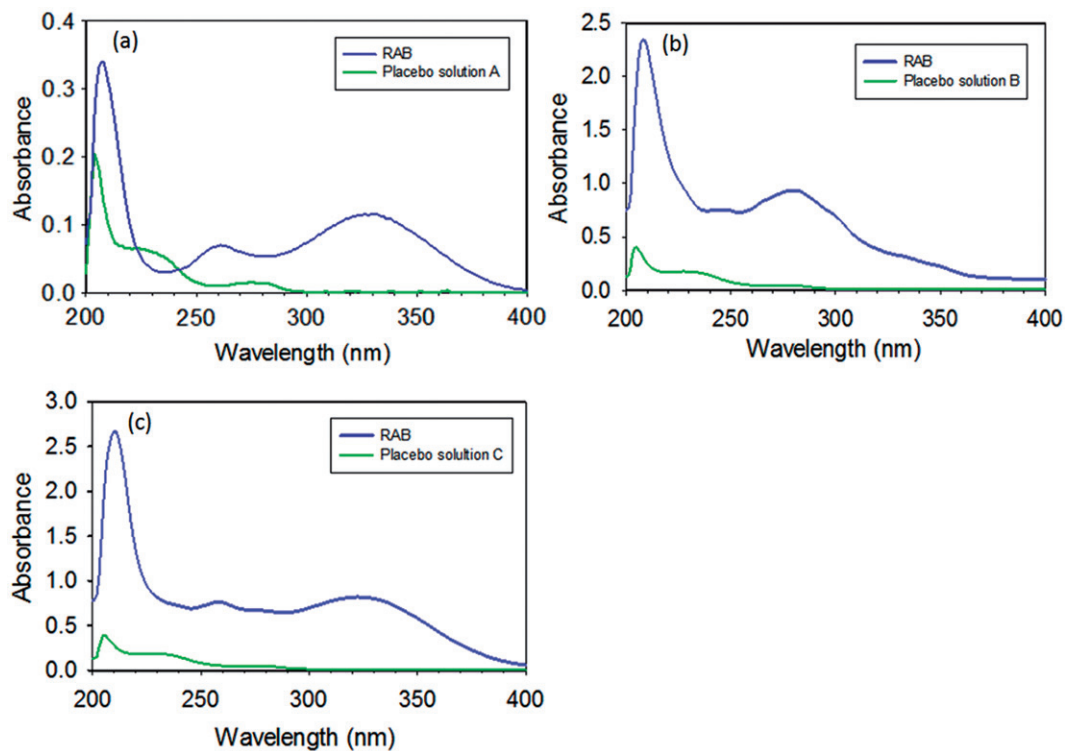


Figure 4. UV absorption spectrograms of RAB standard solution versus placebo solution A (a), B (b), and C (c).

Table 2. Percent recovery of RAB in the media of pH 1.0, pH 6.0, and pH 6.8.

Dissolution media	Levels(%)	Determined amounts (mg)	Added amounts (mg)	Recovery(%)	Mean recovery(%)	RSD (%)		
pH 1.0	5	0.126	0.123	102.4	102.0	3.7		
		0.119	0.123	96.7				
		0.119	0.123	96.7				
	8	0.209	0.196	106.6				
		0.209	0.196	106.6				
		0.198	0.196	101.0				
		0.245	0.245	100.0				
10	0.252	0.245	102.9					
	0.256	0.245	104.5					
	pH 6.0	50	0.50	0.51	98.0	98.0	1.2	
			0.49	0.51	96.1			
			0.50	0.51	98.0			
80	0.81	0.84	96.4					
	0.82	0.84	97.6					
	0.83	0.84	98.8					
100	1.01	1.02	99.0					
	1.00	1.02	98.0					
	1.02	1.02	100.0					
	pH 6.8	50	0.47	0.49	95.9			97.5
0.47			0.49	95.9				
0.48			0.49	98.0				
80		1.19	1.23	96.7				
		1.21	1.23	98.4				
		1.22	1.23	99.2				
100		1.71	1.72	99.4				
		1.65	1.72	95.9				
		1.69	1.72	98.3				

#### Precision

Mean  $\pm$  RSD of absorbance of the determinations with six replicates in the media of pH 1.0, pH 6.0, and pH 6.8 are  $0.071 \pm 0.73\%$ ,  $0.666 \pm 0.09\%$ ,  $0.632 \pm 0.17\%$ , respectively. The results show that the precision of the method is excellent.

#### Stability of solution

Results of stability of RAB standard solutions and dissolution sample solutions are shown in Table 3. It indicates that these solutions

are relatively stable within 2.0 h because of their RSD of percentage absorbances determined at 298 nm at time points of 0, 0.5, 1.0, 1.5, and 2.0 h are less than 0.8%.

#### Sensitivity

For RAB in the media of pH 1.0, pH 6.0, and 6.8, it was found that the LOD and LOQ by the proposed method were approximately  $0.03 \mu\text{g/mL}$  and  $0.10 \mu\text{g/mL}$ , respectively.

**Table 3.** Stability of the standard solutions and dissolution sample solutions.

Solutions with different pH values		Percentage compared to the initial value of prepared original sample solutions from 0 to 2.0 h (%)					RSD(%)
		0 h	0.5 h	1.0 h	1.5 h	2.0 h	
Standard solutions	pH 1.0	100	99.8	99.7	99.7	99.5	0.18
	pH 6.0	100	101.1	100.9	101.5	99.9	0.70
	pH 6.8	100	100.0	99.5	98.1	99.1	0.79
Dissolution sample solutions	pH 1.0	100	99.9	99.5	99.8	99.4	0.26
	pH 6.0	100	101.2	101.0	101.3	99.8	0.70
	pH 6.8	100	100.0	99.6	98.4	99.1	0.68

## Discussion

In the study, standard stock solutions of RAB in 0.1N HCl and PBS (pH 6.8) kept clear and no color change was observed over several hours. However, when the standard stock solutions were prepared into the concentrations of 100, 50, 40  $\mu\text{g/mL}$  using PBS (pH 6.0), these solutions turned into black soon, which followed by black precipitates appearing in approximately 30 min. While the RAB standard substance was dissolved in methanol and diluted with PBS (pH 6.0) into the concentrations of 1000, 200, 100, 50  $\mu\text{g/mL}$ , the solutions kept clear and no color change in several hours. Additionally, it is worth noting that there is no UV absorbance of methanol at 298 nm, which provides negligible inference in the UV detection of RAB. Therefore, methanol was chosen as the solvent of stock solutions while PBS (pH 6.0) was used as diluents. It was also found that RAB standard solutions prepared with above media can keep stability with a relatively stable absorbance within 2.0 h, after which the value of absorbance could change with time. This indicated that standard solutions of RAB should be prepared when use or used in the effective period by validation.

If UV detection was used to directly determine the amount of RAB in dissolution samples of ACIPHEX<sup>®</sup> Sprinkle, there was a heavy interference for the strong UV absorption of HPMCP, which was an important ingredient of the formulation of ACIPHEX<sup>®</sup> Sprinkle. HPMCP could not dissolve in a solution of pH 1.0, but dissolve in a solution of pH > 5.5. Therefore, there were two purposes when the dissolution samples in the medium of pH 6.0 or pH 6.8 were adjusted pH value to 1.0 by adding 1.0 N HCl solution into them. One is that RAB in acidic media was degraded into substances with a stable UV absorbance at 298 nm and the other is that HPMCP could be precipitated to form floccules and separated from the solutions by centrifugation. Therefore, the interference of HPMCP to UV detection in the study could be removed off.

The dissolution (or release) testing is a very important testing of solid oral dosage form performance that can be a rich source of information for quality control, formulation, process development and, most importantly, for evaluation of performance *in vivo*<sup>6,34</sup>. This test may be considered as an indicator of potential drug dissolution and absorption characteristics of a product in humans as well as demonstration of bioequivalence from batch-to-batch<sup>4</sup>. Additionally, dissolution is a requirement for regulatory approval for product marketing<sup>32</sup>. In this study, dissecting the dissolution property of ACIPHEX<sup>®</sup> Sprinkle in acidic media can be used to guide the formulation and process development and quality control of a generic drug for ACIPHEX<sup>®</sup> Sprinkle.

Drug solubility and solution stability are important properties to be considered when selecting the dissolution medium<sup>32</sup>. It is still important for good water solubility drugs to keep the stability in physiology dissolution media with pH range from 1.0 to 6.8, although some research<sup>3,32</sup> have reported that high pH media such as pH 7.5 PBS, pH 8.0 Tris buffer, and pH 9.0 borate buffer

were used for dissolution testing to assess the enteric properties of RAB-coated tablets. High pH dissolution media can be used for quality control of oral solid dosage form of RAB, but little *in vitro-in vivo* correlation can be afforded from the aspect of Biopharmaceutics Classification System (BCS). Generally, a higher pH should not exceed pH 8.0<sup>35</sup>. Therefore, a high pH dissolution medium is not an optimal choice but an expedient one. It is well known that RAB is unstable in acidic media, especially low pH media. However, it is found that degradation of RAB in acidic media is of a stable UV absorbance at 298 nm, which can indirectly determine the amount of RAB. Based on this principle, the amount of RAB could be determined by UV detection after dissolution samples of ACIPHEX<sup>®</sup> Sprinkle in acidic dissolution media were withdrawn. Therefore, it was not necessary to pay close attention to the complex degradation of RAB and their quantities in acidic dissolution media and the issue of determining the accumulative amount of RAB in these acidic media was also solved.

In general, mild agitation conditions should be maintained during dissolution testing to allow maximum discriminating power and to detect products with poor *in vivo* performance<sup>35</sup>. Using the paddle method, the common stirring speed is 50–75 rpm<sup>36</sup>. Enteric pellets belong to multiple-unit pellet system of drug delivery, are of dosage polydispersity and can be evenly dispersed in the GI tract that could be improved the bioavailability<sup>37</sup>. In this study, some enteric pellets adhered together at bottom of dissolution vessels and became black during dissolution testing when the stirring speeds of 50–60 rpm were employed in the medium of pH 6.0 in preliminary tests. This phenomenon would disappear with the stirring speed being increased to 75 rpm. Meanwhile, the phenomenon also appeared in the stirring speeds of 50 rpm but disappeared that of 60 rpm in pH 6.8 medium. Therefore, according to the properties of dosage form of enteric pellets and the phenomenon of dissolution, the stirring speed was set at 60 rpm in pH 6.8 medium, which is agreed with dissolution conditions of ACIPHEX<sup>®</sup> Sprinkle in FDA-Recommended Dissolution Methods Database<sup>38</sup>. Finally, the stirring speeds of paddle in dissolution profile testing in the media of pH 1.0, pH 6.0, and pH 6.8 were optimized to 75 rpm, 75 rpm, and 60 rpm, respectively.

In accord with previous experiments, there is a lag time for enteric-coated preparations to dissolve in the media of pH > 5.0 because of the slow dissolution of the enteric polymer and the dissolution of the drug through the dissolving polymer layer<sup>5,39</sup>. Although RAB is very soluble in water, results from this study show that a significant delay (approximately 30 min) in drug release from enteric-coated pellets of ACIPHEX<sup>®</sup> Sprinkle in the medium of pH 6.0, which is agreed with the literatures reported previously<sup>40–42</sup>. This may validate the method of dissolution testing in the study is reliable.

Though the analytical method for RAB in ACIPHEX<sup>®</sup> Sprinkle dissolution samples in this study is an indirect determination method, in fact it is rapid and simple to solve the puzzle of determining the accumulative release amount of RAB in acidic dissolution media, which cannot be solved by other analytical methods including HPLC. Furthermore, it was validated to be of good linearity, specificity, accuracy, precision, LOD and LOQ for the analyte and enough to dissect the dissolution property of ACIPHEX<sup>®</sup> Sprinkle in acidic media and guide the formulation and process development and quality control of a generic drug for ACIPHEX<sup>®</sup> Sprinkle.

## Conclusions

In conclusion, dissolution testing developed and validated for ACIPHEX<sup>®</sup> Sprinkle were considered satisfactory. It was carefully

studied in order to guarantee the UV absorbance stability of degradations of RAB at 298 nm during all analysis time ranges. The conditions that allowed the determination of dissolution amount of RAB were acidic media, USP apparatus 2 (paddle apparatus) and stirring speed of 60 or 75 rpm. The interference of HPMC in dissolution sample of acidic media can be removed by adding 1.0 N HCl into the sample coupled with centrifugation. The method is demonstrated to be adequate for quality control of ACIPHEX<sup>®</sup> Sprinkle and the release profiles in the media of pH 1.0, pH 6.0, and pH 6.8 can be used as a tool to guide the formulation development and quality control of a generic drug for ACIPHEX<sup>®</sup> Sprinkle. Moreover, a consideration with respect to solving the issues of quantification of drug dissolution, which is unstable in physiological dissolution medium and there exists a heavy interference of ingredients, is also provided.

### Disclosure statement

The authors declare that they have no conflicts of interest to disclose.

### Funding information

The authors would like to thank Guangdong Provincial Department of Science and Technology of China for its financial support (Project No.: 2013090800012).

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