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Original Paper

Determination of the enantiomeric impurity in S-(−)pantoprazole using high performance liquid chromatography with sulfobutylether-beta-cyclodextrin as chiral additive

A new and accurate HPLC method using sulfobutylether-beta-cyclodextrin (SBE-beta-CD) as chiral mobile phase additive (CMPA) was developed and validated for the determination of R-(+)pantoprazole in S-(−)pantoprazole. The influences of type and concentration of CD, ACN content and buffer pH of mobile phase on the resolution and retention of enantiomers were investigated. A baseline resolution of pantoprazole enantiomers was achieved on a Spherigel C18 column (150 mm × 4.6 mm, 5 μm) using ACN and 10 mM phosphate buffer (pH 2.5) containing 10 mM SBE-beta-CD (15:85 v/v) as mobile phase with a flow rate of 0.9 mL/min at 20°C. The detection wavelength was set at 290 nm. The method was extensively validated in terms of accuracy, precision and linearity according to the International Conference on Harmonisation (ICH) guidelines and proved to be robust. The LOD and LOQ for R-(+)pantoprazole were 0.2 and 0.5 μg/mL, respectively, with 5 μL injection volume. A good linear relationship was obtained in the concentration range of 0.5–6.0 μg/mL with r^2 >0.999 for R-(+)pantoprazole. The percentage recovery of the R-(+)pantoprazole ranged from 92.1 to 101.2 in bulk drug of S-(−)pantoprazole. The method is capable of determining a minimum limit of 0.05% w/w of R-enantiomer in S-(−)pantoprazole bulk samples.

Keywords: CMPA / Enantiomeric impurity / HPLC / Pantoprazole enantiomers / Sulfobutylether-beta-cyclodextrin

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1 Introduction

Pantoprazole, 5-(difluoromethoxy)-2-[(3,4-dimethoxy-2-pyridyl) methylsulfinyl]-1H-benzimidazole is one of the proton-pump inhibitors developed for suppression of gastric acid secretion by inhibition of the H^+/K^+-ATPase. It is a chiral drug due to asymmetric substituted sulfur atom and is used clinically as a racemic mixture [1]. However, pharmacological studies have shown that the S-(−) pantoprazole is more potent than R-(+)pantoprazole and R,S-(±)pantoprazole in inhibiting the gastric acid secretion and in reducing the gastric and duodenal ulcers [2]. S-(−)pantoprazole is therefore being developed as a single enantiomer drug. According to the International Conference on Harmonisation (ICH) guidelines, chiral identity, enantiomeric impurity and chiral assay tests may be needed in drug substance and product specifications [3]. It is specifically necessary to develop a method for resolving the enantiomers of pantoprazole, quantitative determination of R-(+)pantoprazole in bulk drug of S-(−)pantoprazole and evaluating the purity of S-(−)pantoprazole.

Chiral HPLC is one of the most powerful tools available for analyzing enantiomeric mixtures. Current chiral HPLC may be either direct methods which utilize chiral stationary phases (CSP) or chiral mobile phase additives (CMPA), or indirect methods which involve derivatization of samples. HPLC methods based on CMPA offer the advantages of flexibility, a wide range of possible additives available and lower cost compared with the equivalent CSP. One group of the widely used CMPA is the CD derivatives [4]. The negatively charged sulfobutylether-beta-cyclodextrin (SBE-beta-CD) is one of the popular beta-CD derivatives and has been applied successfully as

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Abbreviations: CMPA, chiral mobile phase additive; **CSP,** chiral stationary phase; **DAD,** diode array detector; **ICH,** International Conference on Harmonisation; **SBE-beta-CD,** sulfobutylether-beta-cyclodextrin; **SF**C, supercritical fluid chromatography

chiral selector in both CE [5 – 10] and HPLC [11]. It provides superior resolving power for cationic enantiomers. A thorough literature search has revealed that a few LC methods involving the use of direct injection and column switching technique were reported for determination of pantoprazole enantiomers in plasma samples [12 – 15]. And the enantiomeric resolution by CE using BSA as chiral selector was reported [16]. Nozal *et al.* [17, 18] compared LC with supercritical fluid chromatography (SFC) for the separation of pantoprazole enantiomers and other proton-pump inhibitors on amylose based Chiralpak AD CSP. So far to our knowledge, no chiral HPLC method using CMPA was reported for quantitative determination of the chiral impurity in bulk drug of S-(−)pantoprazole. In the current investigation, we developed for the first time an HPLC method using SBE-beta-CD as chiral additive for the determination of enantiomeric impurity in bulk drug of S-(−)pantoprazole. The influences of the composition of mobile phase on resolution and retention of enantiomers were investigated. The method was validated and applied to the optical purity assessment.

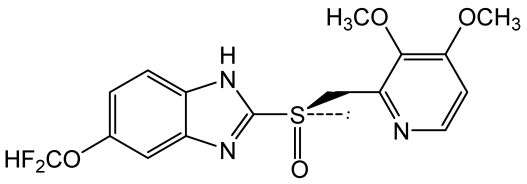
2 Experimental

2.1 Apparatus

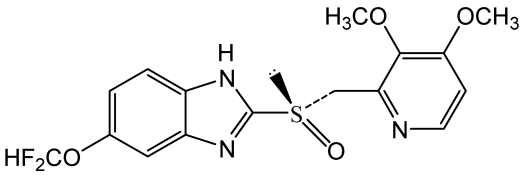
All enantioseparations were carried out on Agilent (Agilent Technologies, USA) 1100 series HPLC system comprising a quaternary pump, autosampler, thermostatted column compartment, diode array detector (DAD) and solvent cabinet with degasser. Agilent chemstation was used for data processing. The Spherigel C18 column (150 mm × 4.6 mm, 5 μm) was obtained from Dalian Johnsson Science & Technological Corporation (Liaoning, China).

2.2 Materials

S-(−)Pantoprazole and its R-enantiomer (optical purity ≥99.97%) were prepared according to patented procedures [19] by School of Pharmaceutical Engineering, Shenyang Pharmaceutical University (Liaoning, China), and their chemical structures are given in Fig.1. Beta-CD and HP-beta-CD (average degree of substitution 3.8) were purchased from Shanghai Chemicals Reagents Plant (Shanghai, China), SBE-beta-CD (average degree of substitution 6, Batch no.: 060201-4, 060901-12, 061002-12) was purchased from Shandong Xinda Fine Chemical (Shandong, China), ACN, methanol and isopropanol of HPLC grade and other reagents of analytical grade were purchased from Yuwang Reagents Plant (Shandong, China), water was purified by double distillation.



S-(-)Pantoprazole



R-(+)Pantoprazole

Figure 1. Structures of S-(−)pantoprazole and R-(+)pantoprazole.

2.3 Chromatographic conditions

Chromatographic separation was achieved on a Spherigel C18 column (150 mm × 4.6 mm, 5 μm) with ACN and 10 mM phosphate buffer (adjusted to pH 2.5 with 10% phosphoric acid) containing 10 mM SBE-beta-CD (15:85, v/v) as the mobile phase and a flow rate of 0.9 mL/min at 20°C. The injection volume was 5 μL, and the detection was carried out at a wavelength of 290 nm using DAD.

2.4 Preparation of stock and standard solutions

Stock solutions of S-(−)pantoprazole (2 mg/mL) and R-(+)pantoprazole (0.1 mg/mL) were prepared by dissolving 200.1 mg S-(−)pantoprazole and 10.0 mg R-(+)pantoprazole in 100 mL volumetric flasks separately with methanol. The R-(+)pantoprazole stock solution of 5.0 mL was transferred into a 10 mL volumetric flask and then diluted with methanol to obtain a solution of 50 μg/mL. Aliquots of 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mL of this solution of R-(+)pantoprazole were transferred into 10 mL volumetric flasks and 5.0 mL of S-(−)pantoprazole stock solution was added into each flask and diluted to the mark with methanol. The final concentrations of standard solution were 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 μg/mL of R-(+)pantoprazole with 1.0 mg/mL of S-(−)pantoprazole. All flasks of standard solutions were wrapped with aluminum foil and kept in the refrigerator at 4°C.

2.5 Validation of the method

2.5.1 Linearity of R-(+)pantoprazole

The linearity evaluation was performed with a solution of S-(−)pantoprazole (1.0 mg/mL) spiked with seven work-

ing solutions of R-(+)pantoprazole ranging from 0.5 to 6.0 μg/mL. The peak area and concentration of R-(+)pantoprazole were subjected to linear regression analysis to calculate calibration equation and correlation coefficient. The linearity was checked on three consecutive days in the same concentration range from the same stock solution. The percentage RSD of the slope and y-intercept of the calibration curves were calculated.

2.5.2 LOD and LOQ of R-(+)pantoprazole

The LOD and LOQ represent the concentration of the analyte that would yield a S/N of 3 and 10, respectively. The LOD and LOQ of R-(+)pantoprazole were determined by injecting a series of dilute solutions spiked to 1.0 mg/mL S-(−)pantoprazole bulk drug.

2.5.3 Method precision

Method precision was determined by measuring the repeatability and intermediate precision (day to day precision) of retention times and peak areas for pantoprazole enantiomers. In order to determine the repeatability of the method, replicate injections (n = 6) of 1.0 mg/mL S-(−)pantoprazole spiked with 0.5 μg/mL (LOQ) and 3.0 μg/mL (0.3% of S-(−)pantoprazole) R-(+)pantoprazole were carried out. The intermediate precision was evaluated over 3 days by performing six consecutive injections each day.

2.5.4 Method accuracy

The recovery experiment was conducted to determine the accuracy of the method, which was performed by spiking R-(+)pantoprazole at 0.05 (LOQ), 0.1, 0.3 and 0.5% levels in the solution of 1.0 mg/mL S-(−)pantoprazole in triplicate. The recovery was calculated by dividing the found amount by the added.

2.5.5 Method robustness

To determine the robustness of the method, experimental conditions were purposely altered and the chromatographic resolution of pantoprazole enantiomers was evaluated. The effect of flow rate was investigated by changing in 0.1 units from 0.8 to 1.0 mL/min. The effect of ACN content in mobile phase on resolution was studied by varying from 14 to 16%, while the other mobile phase components were held constant as stated in Section 2.3. The effect of column temperature on resolution was studied at 18 and 22°C instead of 20°C. Also, three different batches of SBE-beta-CD were investigated in order to validate the robustness.

3 Results and discussion

3.1 Method development

The aim of this work is to develop a chiral HPLC method for accurate quantification of R-(+)pantoprazole in bulk drug of S-(−)pantoprazole. A solution of 0.1 mg/mL racemic mixture prepared in methanol was used for examing the chiral resolution in the method development. The influences of CD type and concentration were examined during method optimization. Beta-CD, HP-beta-CD and SBE-beta-CD were tested as the chiral additive of the HPLC mobile phase. No chiral recognition was observed with native beta-CD and HP-beta-CD. Significant resolution of the enantiomers could be achieved only with anionic SBE-beta-CD. The influence of SBE-beta-CD concentration in the range of 0–12.5 mM on the separation of enantiomers was investigated in 10 mM phosphate buffer of pH 2.5 with 15% ACN. Figure 2 shows that the resolution of enantiomers was improved steadily with increasing concentration of SBE-beta-CD. The best resolution was achieved with 12.5 mM SBE-beta-CD due to greatest interaction between the analyte and the additive. However, higher concentration of SBE-beta-CD led to increased backpressure and decreased column efficiency, 10 mM SBE-beta-CD was therefore chosen for further study with which a baseline separation of the two enantiomers was achieved.

Acetate and phosphate buffer solutions were evaluated and phosphate buffer solution supplied better enantioseparation than acetate buffer solution. The effect of 10 and 50 mM phosphate buffer (pH 2.5) on resolution was investigated in mobile phase containing 15% ACN and 10 mM SBE-beta-CD. There was no obvious difference in resolution (Rs = 2.1) but the retention time using the 50 mM phosphate as buffer was slightly increased than when using 10 mM phosphate, therefore, 10 mM phosphate buffer was finally chosen. The pH influence of 10 mM phosphate buffer containing 10 mM SBE-beta-CD on the resolution and retention of enantiomers was

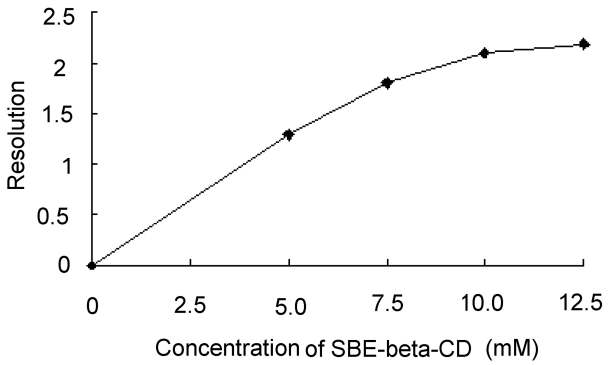


Figure 2. Effect of SBE-beta-CD concentration on resolution of pantoprazole enantiomers

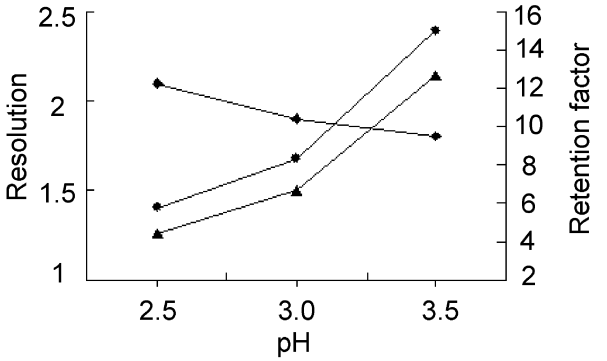


Figure 3. Effect of pH value on resolution and retention of pantoprazole enantiomers. ■: Resolution of pantoprazole enantiomers. ▲: Retention factor of R-(+)pantoprazole. ●: Retention factor of S-(−)pantoprazole.

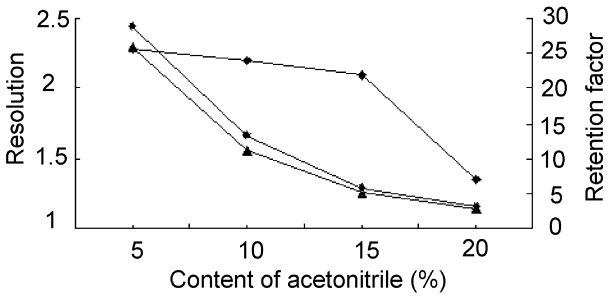


Figure 4. Effect of ACN content on resolution and retention of pantoprazole enantiomers. ■: Resolution of pantoprazole enantiomers. ▲: Retention factor of R-(+)pantoprazole. ●: Retention factor of S-(−)pantoprazole.

investigated with 15% ACN as the organic modifier. As shown in Fig. 3, the decrease in buffer pH led to both a concomitant decrease in retention factors and increase in resolution, and the shortest retention and best resolution were achieved at pH 2.5. Since pantoprazole has a pKa value of 3.9 for the protonation of the N-pyridine[16], it exists mainly as the cationic form at pH 2.5. The electrostatic interaction between the cationic analyte and the negatively charged CD promotes the chiral recognition and reduces the retention on the column.

ACN, methanol and isopropanol at various contents were used as the organic modifiers in an attempt to improve the resolution, and the highest resolution within shortest retention time was achieved with ACN in the mobile phase. The content of ACN used in these experiments was 5 – 20%. As shown in Fig.4, when the ACN content was increased from 5 to 15% the resolution was slightly decreased. A resolution more than 2.0 was obtained at 15% of ACN. However, further increasing the content up to 20% yields a significant reduction in the chiral resolution. On the other hand, ACN content lower than 15% led to peak broadening and long retentions. As a compromise for higher resolution and shorter retention, 15% ACN was chosen for analysis.

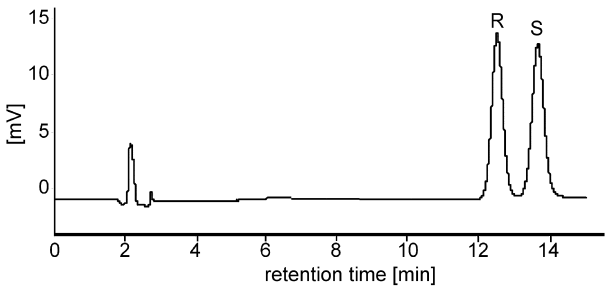


Figure 5. Chromatogram of enantioseparation of pantoprazole. Chromatographic condition: Spherigel C18 column; mobile phase, ACN: 10 mM phosphate buffer (pH 2.5) containing 10 mM SBE-beta-CD (15:85,v/v); flow rate, 0.9 mL/min; detector wavelength, 290 nm; column temperature, 20°C; peaks: R, R-(+)pantoprazole; S, S-(−)pantoprazole.

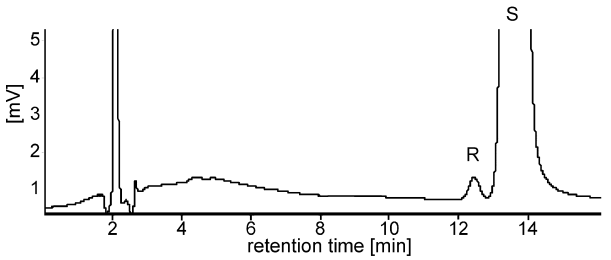


Figure 6. Chromatogram of 1.0 mg/mL S-(−)pantoprazole spiked with 0.3% R-(+)pantoprazole. Chromatographic conditions are the same as in Fig. 5.

A representative chromatogram of enantioseparation under the optimized conditions is shown in Fig. 5. A baseline resolution (Rs = 2.1) between two enantiomers within 15 min and ideal peak shape with the asymmetric factor 0.98 was obtained. R-(+)Pantoprazole was eluted prior to the S-(−)pantoprazole, which avoids its "smearing" under main compound peak. This is an advantage over previously reported method [15] for the determination of the pantoprazole enantiomers. A typical chromatogram of the 1.0 mg/mL S-(−)pantoprazole spiked with 0.3% R-(+)pantoprazole is shown in Fig. 6.

3.2 Method validation

A good linearity was obtained for R-(+)pantoprazole over the concentration range of 0.5 to 6.0 μg/mL with the linear regression equation $y = 4454x + 567.0$ ($r^2 = 0.9991$) where y is peak area of R-(+)pantoprazole and x is concentration of R-(+)pantoprazole. The percentage RSD of the slope and y-intercept of the calibration curve were 3.6 and 4.2, respectively. The correlation coefficient showed a good correlationship between the peak area and concentration of R-(+)pantoprazole in the presence of S-(−)pantoprazole.

The LOD and LOQ concentrations were estimated to be 0.2 and 0.5 μg/mL for R-(+)pantoprazole in 1.0 mg/mL S-

Table 1. Precision data

Spiking level of the R-(+) pantoprazole (%)	Parameter	RSD (%)	
		R-(+)panto-prazole	S-(−)panto-prazole
Repeatability (n = 6)			
0.3	Retention time	0.28	0.27
	Peak area	2.6	1.4
0.05 (LOD)	Retention time	0.36	0.28
	Peak area	3.6	1.4
Inter-day precision (n = 3)			
0.3	Retention time	0.32	0.27
	Peak area	2.9	1.6
0.05 (LOD)	Retention time	0.39	0.29
	Peak area	4.6	1.5

Table 2. Recovery data

Added (μg/mL)	Recovered (μg/mL)	%Recovery	%RSD
0.520	0.479	92.1	4.2
1.040	0.984	94.6	3.3
3.120	3.086	98.9	1.2
5.200	5.262	101.2	1.5

(−)pantoprazole, when S/Ns of 3 and 10 were used as the criteria.

The precision results are presented in Table 1. The repeatability study for the retention times of both enantiomers was better than the RSD of 0.4%, and the RSD for the peak area of R-(+)pantoprazole at LOQ level was within 3.6% and at higher level was within 2.6%. The intermediate precision study, the RSD was in the same order of magnititude as those obtained for intra-assay. The accuracy of the method was demonstrated with good percentage recovery which ranged from 92.1 to 101.2 determined by spiking R-(+)pantoprazole to 1.0mg/mL S-(−)pantoprazole at 0.05, 0.1, 0.3, and 0.5% levels (Table 2).

The robustness of a method is the ability of the method to remain unaffected by small changes in parameters such as flow rate, the mobile phase composition and the column temperature. The chromatographic resolution of pantoprazole enantimers was used to evaluate the method robustness under modified conditions. In all cases as described in Section 2, the resolution was found to be 2.08 ± 0.03, which demonstrated that the developed method is robust.

Compared with validated methods for analysis of pantoprazole enantiomers reported in the literature, this newly developed method has a short run time, good enantioresolution and linearity. Particularly, a reported CE [16] method using BSA as chiral selector has a rather poor LOD of 0.04 mg/mL and a total run time over 20 min. HPLC [12 – 15] and SFC [17, 18] methods used relatively expensive chiral columns. The present method with a LOD of 0.5 μg/mL is capable of determining a minimum limit of 0.05% w/w of R-enantiomer in S-(−)pantoprazole bulk sample, which meets the requirement for impurities (0.05 – 0.1%) in new drug substance according to the ICH guidelines.

Table 3. Results for quantification of R-(+)pantoprazole in bulk samples

Batch no.	%R-(+)pantoprazole	%RSD (n = 3)
Batch-1	0.27	1.2
Batch-2	0.27	1.4
Batch-3	0.29	1.2

3.3 Application

Three batches of synthetic S-(−)pantoprazole bulk samples were analyzed for the content of R-enantiomer. About 10 mg of each test sample was weighed, transferred and dissolved to a 10 mL volumetric flask. The volume was made up to the mark with methanol. The impurity results of the three batch samples of bulk S-(−)pantoprazole are shown in Table 3, which demonstrates the practical applicability of this method.

4 Concluding remarks

An HPLC method with achiral column and mobile phase containing SBE-beta-CD as the chiral additive was described for the resolution of pantoprazole enantiomers and determination of R-(+)pantoprazole. The method was completely validated and was applicable to the quantitative determination of chiral impurity in bulk drug samples of S-(−)pantoprazole.

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