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Cyclodextrins' effect on the enatioseparation of some PPIs and capillary electrophoresis method development for determining rabeprazole enantiomers

Yusung Choi[1,#], Thuy-Vy Pham[1,#], Xuan-Lan Mai[1], Quoc-Ky Truong[2], Thi-Anh-Tuyet Le[1], Thi-Ngoc-Van Nguyen[3], Gunhee Lee[1], Jong-Seong Kang[4], Woongchon Mar[5], and Kyeong Ho Kim[1,★]

[1]*College of Pharmacy, Kangwon National University, Chuncheon 24341, Korea*
[2]*Pharmacy Faculty, Pham Ngoc Thach University of Medicine, Vietnam*
[3]*Pharmacy Faculty, Can Tho University of Medicine and Pharmacy, Vietnam*
[4]*College of Pharmacy, Chungnam National University, Daejeon 34134, Korea*
[5]*College of Pharmacy, Seoul National University, Seoul 08826, Korea*
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Abstract Over the past decades, chiral switch of the proton pump inhibitors (PPIs) has been received widespread attention in therapeutic advantages as well as pharmaceutical analysis. In present study, the influence of cyclodextrins (CDs) on the chiral separation of four common PPIs (lansoprazole, omeprazole, pantoprazole, and rabeprazole) was investigated. The results demonstrated that capillary electrophoresis (CE) with dual CDs as a chiral selector system is a possible and promising method for the enantioseparation of these PPIs. Rabeprazole, which is the most challenging and acid-labile candidate among four PPIs, was selected for further development of the technique. To optimize CE condition, the effects of capillary parameters and background electrolytes on the enantioseparation were investigated. Finally, the best chiral separation was acheived by using sulfobutyl ether-β-CD, and γ-CD as dual chiral selectors. The developed CE method not only provided the effective chiral separation but also showed the good stability of rabeprazole. The proposed method was successfully validated according to the International Conference on Harmonization guideline and effectively applied to determine rabeprazole enantiomers in commercial rabeprazole tablets, with recoveries ranging from 97.17 % to 103.29 % of the label content.

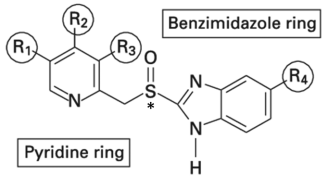
Key words: Capillary electrophoresis, Enatioseparation, Cyclodextrins, Rabeprazole enantiomers, Proton pump inhibitors

1. Introduction

Proton pump inhibitors (PPIs) like lansoprazole (LAN), omeprazole (OME), pantoprazole (PAN), and rabeprazole (RAB) are used as a primary treatment for various peptic acid disorders, such as gastroesophageal reflux disease (GERD), duodenal gastric ulcers, and Zollinger–Ellison syndrome. These PPIs function by

[#]These authors contributed equally in this study

★ Corresponding author
Phone : +82-(0)33-250-6918 Fax : +82-(0)33-259-5631
E-mail : kyeong@kangwon.ac.kr



	R_1	R_2	R_3	R_4
Omeprazole	CH_3	OCH_3	CH_3	OCH_3
Pantoprazole	H	OCH_3	OCH_3	$OCHF_3$
Lansoprazole	H	OCH_2CF_3	CH_3	H
Rabeprazole	H	$OCH_2CH_2CH_2OCH_3$	CH_3	H

Fig. 1. Chemical structures of surveyed PPIs.

suppressing gastric acid secretion by interacting with H^+/K^+-ATPase in gastric parietal cells. Most PPIs have an asymmetric sulfur atom in their chemical structures and can exist as two enantiomers (*Fig.* 1).[1] Recently, there has been a increasing interest in the pharmaceutical industry for the development of enantiomerically pure gastric acid secretion inhibitors. For example, dexlansoprazole has been marketed by Takeda Pharmaceuticals, and generic versions of esomeprazole are available worldwide. The *S*-(−) form of PAN was also developed by Emcure Pharmaceuticals Ltd. Although RAB is clinically administered in the racemate form, some studies suggested higher therapeutic effects of *R*-(+)-RAB.[2,3] Subsequently, the *R*-(+) form of RAB (dexrabeprazole) was developed by Emcure Pharmaceuticals Ltd. in 2007.

Nowadays, the enantioseparation of chiral drugs plays a key role in pharmaceutical analysis as enantiomers of racemic drugs have different pharmacodynamic and/ or pharmacokinetic properties; one of the enantiomers can be pharmacologically active while the other can be inactive, or even be toxic.[4] Although pharmaceutical and clinical analyses depend primarily on high-performance liquid chromatography (HPLC), capillary electrophoresis (CE) was introduced as a powerful alternative technique. Compared to HPLC, CE is an efficient, flexible, and selective technique that allows faster separation and lower consumption of solvents, samples, and chiral selectors.[5] In order to perform chiral separation, one or more chiral selectors are added to background electrolytes (BGE) based on the effect of the chiral selector on the mobility of the analyte.[6] Among different types of chiral selectors, cyclodextrins (CDs) are the most frequently used due to the variety in their cavity diameters and their substituents, their high solubility in aqueous solvents, and their low UV absorbance.[7]

From the review of the literature, a number of HPLC methods were successfully developed for the enantioseparation of PPIs.[8-12] However, while some CE methods using CDs have been reported for separating the enantiomers of OME, PAN, and LAN,[13-18] there was limited CE methods published to determine of RAB enantiomers. Ma *et al.* tested the possibility of using an ephedrine-based ionic liquid to resolve RAB enantiomers using nonaqueous CE. However, the resolution obtained was only about 0.87, and the results were not validated (19).[19] Papp *et al.* successfully separated two RAB enantiomers, but the stability of solution was not demonstrated, which is an important requirement when developing CE method, especially for highly acid-labile compound like PPIs.[18,20] In addition, internal standard was not applied in this published method, which could lead to the poor precision and recovery. Because in CE, tiny volumes (5–50 nL) are normally injected by inserting the capillary into a sample vial and then pressurizing the vial to introduce sample solution into the capillary. The loop injectors are not available for almost CE instruments, so the injection repeatability in CE is not good. These problem can be efficiently solved by addition of an internal standard component.[21]

In this study, the influences of different types of CDs on the enantioseparation of OME, LAN, PAN, and RAB were investigated to provide an experimental perspective of the technique. Since RAB is the most challenging and acid-labile PPI,[22] and the insufficiency has been observed in the published CE methods for its chiral separation, we focused on developing the CE method for determining RAB enantiomers. The proposed method were validated according to the International Conference on Harmonization (ICH) guideline, and applied to analyse some RAB commercial products in order to confirm the applicability of the method in routine analysis.

2. Experimental

2.1. Chemicals and reagents

RAB and PAN were provided by Shinpoong Pharmaceutical Co. Ltd (Ansan, Korea). OME was supplied by Dr. Reddy's Laboratory (Hyderabad, India). LAN was provided by Daewoong Pharmaceutical (Seoul, Korea). Esomeprazole magnesium trihydrate was supplied by CTCBIO Inc. (Hwaseong, Korea). The purity of these standards was more than 98 %. β-CD (β-CD), methyl-β-CD (M-β-CD) degree of substitution (DS) 12 (molecular weight (MW) 1303.3), 2-hydroxypropyl-β-CD (HP-β-CD) DS 4.2 (MW 1380), sulfated-β-CD (S-β-CD) DS 13 (MW 2462.3), and heptakis (2,3,6-tri-O-methyl)-β-CD (HEP-β-CD) DS 21 (MW 1429.6) were obtained from Sigma-Aldrich (Missouri, USA). γ-CD was obtained from TCI (Tokyo, Japan). Carboxymethyl-β-CD (CM-β-CD) DS 3.5 (MW 1541.2), and acetyl-β-CD (AC-β-CD) DS 7 (MW 1430) were obtained from Wacker (Munich, Germany). Sulfobutyl ether-β-CD (SBE-β-CD) DS 6.1 (MW 2242.1) was obtained from Cydex (California, USA). Other reagents and chemicals emloyed in this study were of analytical grade and supplied from Sigma-Aldrich unless otherwise indicated. Commercial pharmaceutical tablets containing 10 mg of RAB were purchased from local pharmacies. Water was purified in our laboratory using an Aqua Max water purification system from Young Lin Instrument Co., Ltd. (Anyang, Korea).

2.2. Instrumentation

All experiments were performed on an HP^{3D} CE system (Hewlett-Packard, Waldbronn, Germany) equipped with an on-column diode array detector (DAD). Instruments were controlled and data was acquired with HP^{3D} CE ChemStation software. Uncoated fused-silica capillaries with 56 cm of length and 50 μm of internal diameter (Agilent Technologies, Waldbronn, Germany) were used for chiral separation. The capillary was thermostated at 16–30 °C. The pH of the solution was measured by a SevenEasy pH meter (Mettler Toledo, Columbus, OH, USA).

A new capillary was successively treated with 1 M sodium hydroxide (NaOH), 0.1 M NaOH, 0.1 M phosphoric acid, and water for 10 min each. At the beginning of the working day, the capillary was washed with water and 0.1 M NaOH for 5 min each, after that flushing with water for 10 min. Prior to injection, the capillary was washed with water and 0.1 M NaOH for 2 min each, followed by rinsing with BGE for 5 min. At the end of the working day, the capillary was successively washed with water, 0.1 M NaOH, water for, 0.1 M phosphoric acid, and water for 10 min each. Sample solutions were introduced into capillary by hydrodynamic injection at 50 mbar for 7 sec.

2.3. Preparation of standard solutions

Due to the acid-lability of PPIs, all standard and sample solutions were prepared in 0.1 M NaOH. LAN, OME, PAN, RAB, and internal standard (IS) esomeprazole stock solutions were prepared by dissolving the corresponding accurately weighed compounds in 0.1 M NaOH to attain final concentrations of 1000 μg/mL. All stock solutions were stored at 4 °C.

Working solutions of LAN, OME, PAN, and RAB were prepared daily in 0.1 M NaOH. For preliminary experiments, 500 μg/mL solutions of each surveyed PPIs were prepared in 0.1 M NaOH. RAB calibration curves were prepared by taking appropriate aliquots of RAB racemate and IS stock solutions and diluting with 0.1 M NaOH. Each sample contained 100 μg/mL of IS and an appropriate concentration of RAB racemate. All solutions were filtered through a 0.45 μm pore size membrane and sonicated before use.

2.4. Preparation of pharmaceutical samples

Twenty tablets of each preparation were weighed and finely powdered. Each weighed portion (equivalent to 50 mg RAB racemate) was transferred into a 100 mL volumetric flask containing about 50 mL of 0.1 M NaOH. The solution was sonicated for 15 min for complete dissolution, and the remaining volume was made up with 0.1 M NaOH. The sonicated solution was thoroughly mixed and pre-filtered through filter paper. 4 mL of the tablet solution and 1 mL of the IS stock solution were transferred to a 10 ml volumetric

flask and making up the remaining volume with 0.1 M NaOH, yielding a final solution containing 200 μg/mL of RAB racemate and 100 μg/mL of IS. This solution was filtered through a 0.45 μm pore size membrane and introduced to the capillary electrophoresis system for separation. The peak area ratio of each enantiomer to IS was calculated. The amount of each RAB enantiomer was obtained using a modeled obtained from regression.

2.5. Method validation

The developed method was validated according to the ICH guidelines using esomeprazole at 100 μg/mL concentration as an IS. The following parameters were evaluated: specificity, limit of detection (LOD), limit of quantitation (LOQ), system suitability, linearity, precision, accuracy, robustness, and stability.

3. Results and Discussion

3.1. Influence of cyclodextrins on enantioseparation of PPIs

As mentioned above, CDs are very versatile and efficient chiral selectors that are widely used for developing CE methods. Therefore, various types of cyclodextrins were investigated to determine suitable conditions for enantioseparation of PPIs, including native CDs (β-CD, γ-CD), neutral CDs (M-β-CD, A-β-CD, HP-β-CD, HEP-β-CD), and negatively charged CDs (CM-β-CD, S-β-CD, SBE-β-CD) at 20 mM concentration. The cyclodextrins were dissolved in a 90 mM phosphate buffer (pH 7.0) and electrophoretic tests were conducted at a constant voltage of 25 kV and 20 °C capillary temperature.

As shown in *Table* 1, only the use of SBE–β-CD led to a slight enantioseparation in the surveyed PPIs. However, it was impossible to obtain satisfactory resolution with this selector. We conducted further investigation using dual chiral selector systems because the use of a single CD did not successfully separate the two enantiomers of each PPI.

From a review of the literature, the charged CD derivatives retain a self-electrophoretic mobility that is absent in native and neutral CDs. Among the charged CDs, SBE–β-CD was also considered to have high potential for chiral CE separation.[23] This was also confirmed from the results mentioned above. Therefore, SBE–β-CD was combined with other

Table 1. Effect of chiral selectors on enantioseparation of LAN, OME, PAN, RAB

Chiral selector		Concentration of chiral selector (mM)	R_s			
			LAN	OME	PAN	RAB
Single chiral selector	β-CD	20	-[a]	-	0.94	-
	γ-CD	20	-	-	-	-
	M-β-CD	20	-	-	-	-
	AC-β-CD	20	-	-	-	-
	CM-β-CD	20	-	-	-	-
	HP-β-CD	20	-	-	-	-
	HEP-β-CD	20	-	-	-	-
	S-β-CD	20	-	-	-	-
	SBE-β-CD	20	0.27	0.66	0.57	0.37
Dual chiral selectors (combine with 30 mM of SBE-β-CD)	β-CD	20	1.51	0.50	0.43	0.57
	γ-CD	20	0.89	-	-	1.48
	M-β-CD	20	1.90	0.57	0.89	-
	AC-β-CD	20	1.18	1.28	0.88	0.49
	HP-β-CD	20	1.31	-	0.74	-
	S-β-CD	20	1.62	0.43	1.47	0.71
	CM-β-CD	20	1.36	-	0.83	0.47
	HEP-β-CD	20	1.56	0.45	1.36	0.50

[a]not separated.

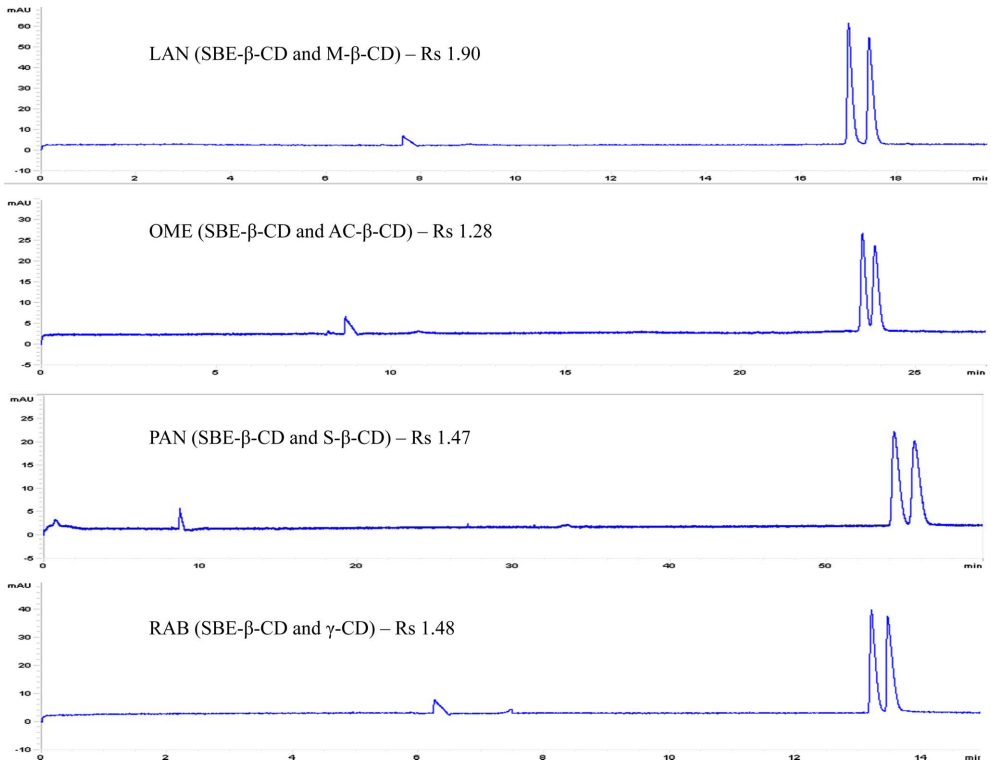


Fig. 2. Electropherograms of four PPIs with their corresponding dual CDs. CE condition: BGE: phosphate buffer (pH 7.0, 90 mM) containing 30 mM SBE-β-CD and 20 mM corresponding CD; 25 kV; 20 °C; hydrodynamic injection (7.0 s at 50 mbar); detection at 280 nm.

CDs to create dual systems. In *Table* 1, the possible resolutions of LAN, OME, PAN, and RAB were observed with a combination of SBE-β-CD and M-β-CD, AC-β-CD, S-β-CD, and γ-CD, respectively. *Fig.* 2 shows the resolved enantiomer peaks using dual CD systems. The combination of SBE-β-CD and γ-CD was selected to optimizing electrophoretic condition for RAB enantioseparation in further experiments.

3.2. Optimization of CE conditions for enantioseparation of RAB

CE is usually used to analyze charged compounds, thus the role of pH in the medium where separation occurs is important. The effective charge and, consequently, mobility of an analyte both depend directly on pH. Electroosmotic flow (EOF) in a bare silica capillary, self mobility of charged chiral selectors, and chiral recognition ability also depend on pH.[23] To the best of our knowledge, RAB was degraded by acid hydrolysis and oxidation with hydrogen peroxide.[24] Thus, the effect of buffer pH was studied using various pH values (6.0, 6.5, 7.0, 7.5, and 8.0) with a 90 mM phosphate buffer containing 30 mM SBE-β-CD and 20 mM γ-CD as BGE; CE was performed at 25 kV and 20 °C. As shown in *Fig.* 3(a), the migration time decreassed at higher pH values due to accelerated EOF. At pH values ranging from 6.0 to 7.0, the resolution between enantiomers increased and the peak shape became sharper. From pH 7.0 to 8.0, the resolution decreased as analyte-CDs interaction occured within a shorter period. Therefore, pH 7.0 was chosen as the optimized pH value due to the suitable migration time and resulting enantiomer separation.

The influence of the BGE's ionic strength on the enantioselectivity was evaluated by varying the phosphate concentration from 30 to 110 mM,

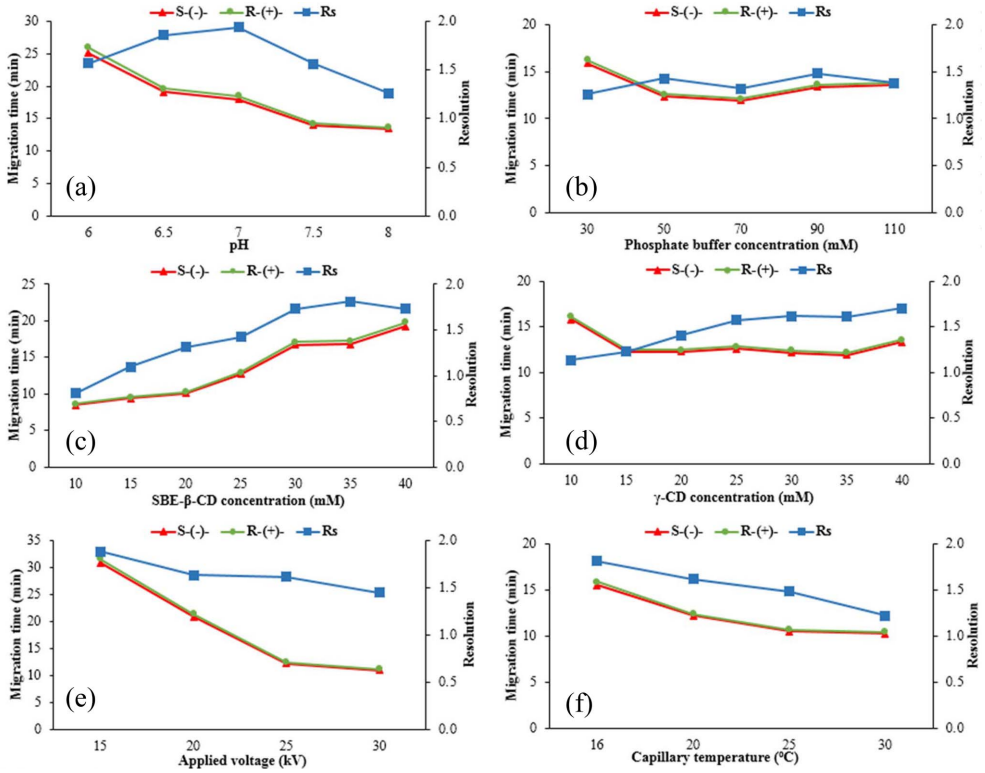


Fig. 3. Effect of (a) pH, (b) phosphate buffer concentration, (c) SBE-β-CD concentration, (d) γ-CD concentration, (e) applied voltage, and (f) capillary temperature on migration time and resolution of RAB enantiomers.

maintaining 30 mM SBE-β-CD and 20 mM γ-CD as BGE; CE was performed at 25 kV and 20 °C. When the buffer concentration increased from 30 to 50 mM, the resolution improved slightly while the migration time was reduced. No significant variations in migration time and resolution were witnessed at concentrations ranging from 50 to 90 mM. The best resolution was observed at 110 mM, but the analysis time was longer than 15 min. The concentrated buffer also generated higher current that lead to significantly decreased peak responses. Thus, 50 mM was chosen as the optimal buffer concentration (*Fig.* 3(b)).

At optimized pH and buffer concentration, the effect of SBE-β-CD on enantioseparation of RAB was studied for concentration ranging from 10 to 40 mM. As shown in *Fig.* 3(c), an increased SBE-β-CD concentration generated higher current, extended the migration time, and improved the resolution. The resolution decreased significantly when the SBE-β-CD concentration reached 40 mM. Many experimental studies have also indicated that a further increase in the chiral selector concentration above the optimum concentration may lead to decreased resolution.[25-27] The higher current was attributed to a decreased peak height. As a consequence, the optimal SBE-β-CD concentration was determined to be 30 mM based on the measured resolution and migration times.

The influence of γ-CD was also investigated at concentration ranging from 10 to 35 mM. As shown in *Fig.* 3d, the migration time gradually decreased and the resolution of RAB enantiomers increased at higher γ-CD concentration, but the best resolution was obtained when 30 mM of γ-CD was used. Thus, 30 mM was selected as optimum as the optimum γ-CD concentration for enantioseparation of RAB.

The effect of applied voltage on the chiral sepraration was assessed in the 15 to 30 kV range in 5 kV increments. Raising the voltage led to shorter migration

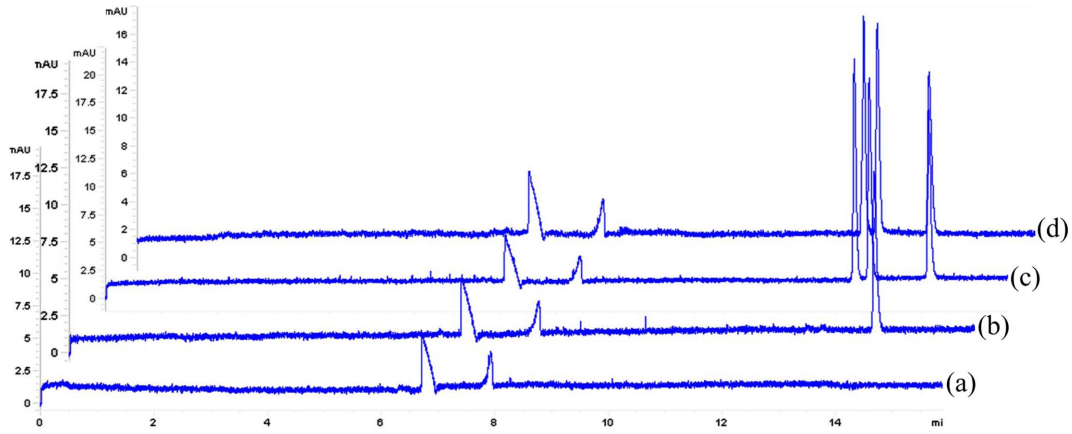


Fig. 4. Typical electropherograms of (a) blank (0.1M NaOH), (b) IS solution, (c) standard solution, (d) sample solution. CE condition: BGE: phosphate buffer (pH 7.0, 50 mM) containing 30 mM SBE-β-CD and 30 mM γ-CD; fused-silica capillary 56 cm, 50 μm i.d.; 25 kV; 16 °C; hydrodynamic injection (7.0 s at 50 mbar); detection at 280 nm. Peak 1: *S-(-)*-RAB. Peak 2: *R*-(+)-RAB. Peak 3: IS.

time, sharper peaks, and lower resolution (*Fig.* 3(e)), but also resulted in higher current and increased Joule heating, which might degrade the compounds. A significant reduction in peak areas was detected when 30 kV voltage was applied. Therefore, 25 kV was selected as the optimum applied voltage.

Controlling the capillary temperature in CE plays a key role to avoid unwanted changes in EOF, viscosity, efficiency, electrophoresis mobility, and solute-CD interaction. The effect of temperature was investigated in the 16 to 30 °C range. Increasing the capillary temperature leaded to the decreased migration time and poorer resolution (*Fig.* 3(f)). As a consequence, all experiments were conducted at 16 °C, providing the best compromise between resolution and runtime.

Electrophoresis was conducted in a buffer containing of 50 mM phosphate adjusted to pH 7.0, 30 mM SBE-β-CD, and 30 mM γ-CD with 25 kV applied voltage and 16 °C capillary temperature. Under this optimized condition, desirable RAB enantiomer separation was observed with resolution between two RAB enantiomers about 2.47.

3.3. Method validation

3.3.1. Specificity

Specificity is defined as the ability to discriminate the analyte from any other interfering substances. Typical electropherograms of a blank sample, the working standard, and an experimental sample are shown in *Fig.* 4. No peaks can be seen at the migration times for both RAB enantiomers and IS, confirming the specificity of the optimum method. The peak homogeneity (peak purity) of RAB enantiomers and IS were also evaluated using a DAD. The results show that the analyte peak cannot be attributed to more than one component.

3.3.2. System suitability

A system suitability test was performed to evaluate the reproducibility of the system. System suitability was evaluated by performing six replicate injections and measuring the migration time, peak area ratios, resolution (Rs), theoretical plate number (N), and symmetry factor (A_s) of the relevant compounds.

The migration time of *S*-(−)-RAB was 13.77 min, that of *R*-(+)-RAB was 14.05 min, and that of IS was 15.17 min (*Fig.* 4(c)). The mean value of N for *S*-(−)-RAB, *R*-(+)-RAB, and IS were 276501, 228508, and 200200, respectively; the corresponding A_s values were 0.65, 0.60, and 0.41, respectively. The Rs value between *S*-(−)-RAB and *R*-(+)-RAB was 2.47, and the Rs value between *R*-(+)-RAB and IS was 8.71. The relative standard deviation (RSD) value of the migration time ratio between *S*-(−)-RAB and IS and

that between *R*-(+)-RAB and IS were 0.27 % and 0.21 %, respectively. The RSD value of the peak area ratio between *S*-(−)-RAB and IS and that between *R*-(+)-RAB and IS were 1.52 % and 1.07 %, respectively. The obtained RSDs for the migration time ratios and peak area ratios were lower than 2 %, which is acceptable according to the common ICH acceptance criteria.

3.3.3. Linearity and sensitivity

Linearity was studied using six concentrations of racemate RAB ranging from 40 to 800 μg/mL (equivalent to 20 to 400 μg/mL for each enantiomer) with IS included at a fixed concentration of 100 μg/mL. Six replicate injections of each solution were applied and the calibration curves (RAB enantiomers peak area to IS peak area ratio versus concentration) showed good linearity with a coefficient of determination (R^2) above 0.9994 for both enantiomers (*Table* 2).

The LOD and LOQ were 5 μg/mL and 20 μg/mL for each RAB enantiomer when signal-to-noise ratios of 3 and 10 were used as evaluation criteria, respectively.

3.3.4. Precision

The precision of the proposed method was evaluated at three different concentrations (80, 100, and 120 μg/mL for each enantiomer) for (1) six replicate injections in one day (intra-day), and (2) triplicates for three consecutive days (inter-day) under the optimized conditions.

The precision results are reported in *Table* 3. The intra-day RSD value ranged from 1.21 % to 1.54 % and from 0.97 % to 1.42 % for *S*-(−)-RAB and *R*-(+)-RAB, respectively. The inter-day RSD value ranged from 1.26 % to 1.48 % and from 1.03 % to 1.86 % for *S*-(−)-RAB and *R*-(+)-RAB, respectively.

3.3.5. Accuracy (Recovery)

Accuracy was expressed as the recovery rate as evaluated by adding known amounts of the standard solutions at low, medium, high levels (80 %, 100 %,

Table 2. Linearity and sensitivity data of the proposed method

	S-(−)-RAB	*R*-(+)-RAB
Limit of detection (μg/mL)	5	5
Limit of quantitation (μg/mL)	20	20
Linearity range (μg/mL)	20 – 400	20 – 400
Intercept ± SD (n=6)	0.0096 ± 0.0005	0.0098 ± 0.0007
Slope ± SD (n=6)	0.0506 ± 0.0332	0.0427 ± 0.0483
Correlation coefficient (R^2)	0.9994	0.9997

Table 3. Precision and recovery (accuracy) of the proposed method

Parameters	Nominal conc. (μg/mL)	*S*-(−)-RAB			*R*-(+)-RAB		
		Measured conc. (mean ± SD μg/mL)	Recovery (%)	RSD (%)	Measured conc. (mean ± SD μg/mL)	Recovery (%)	RSD (%)
Intra-day precision (n=5)	80	80.53 ± 0.98	100.67	1.21	79.18 ± 0.96	98.98	1.21
	100	98.61 ± 1.52	98.61	1.54	97.77 ± 1.39	97.77	1.42
	120	119.91 ± 1.63	99.93	1.36	117.56 ± 1.15	97.97	0.97
Inter-day precision (n=11)	80	80.46 ± 1.13	100.57	1.40	80.30 ± 1.49	100.38	1.86
	100	99.14 ± 1.47	99.14	1.48	98.05 ± 1.51	98.05	1.55
	120	120.32 ± 1.51	100.27	1.26	117.78 ± 1.21	98.15	1.03
Recovery (n=3)	80	79.65 ± 0.74	99.56	0.74	79.69 ± 0.88	99.62	0.88
	100	100.23 ± 1.71	100.23	1.71	100.35 ± 0.80	100.35	0.80
	120	119.82 ± 1.24	99.85	1.24	118.52 ± 0.75	98.77	0.76

120 % of the known amount, respectively). All experiments were performed in triplicate. The developed method exhibited good accuracy with overall average recovery of 99.88 % for *S*-(−)-RAB and 99.58 % for *R*-(+)-RAB, respectively, with RSD less than 1.71 %. The results are shown in *Table* 3.

3.3.6. Robustness

Robustness was obtained by evaluating small variations in some electrophoretic parameters, such as the electrolyte solution pH (7.0 ± 0.2), SBE-β-CD concentration (30 ± 5 mM), γ-CD concentration (30 ± 5 mM), capillary temperature (16 ± 2 °C), and applied voltage (25 ± 2 kV). Standard solutions containing 200 μg/mL of RAB racemate and 100 μg/mL of IS were used in six replicate injections. These minor changes in the optimal conditions hardly affected the peak area ratio of the RAB enantiomers, and the RSD values for the peak ratios between RAB enantiomers and esomeprazole (IS) were less than 2 %.

3.3.7. Stability of solutions

The stability of racemate RAB in NaOH solution was studied by placing the samples in tightly capped volumetric flasks at 4 °C or at room temperature. The samples were analyzed and the peak areas were compared.

RAB enantiomers were found to be stable in NaOH for a week when stored at 4 °C and 2 days at room temperature with RSD values under 2 % (*Table* 4). No degradation products were detected.

Table 4. Stability data of RAB solutions

Condition	Time	Peak Area (mAU*s)		
		S-(−)-RAB	R-(+)-RAB	IS
	Initial	58.21	59.04	63.04
4 °C	Day 1	59.76	60.29	64.82
	Day 2	58.61	59.70	63.29
	Day 7	59.17	60.27	64.55
	RSD (%)	1.14	0.99	1.39
Room temperature	Day 1	57.32	58.46	62.83
	Day 2	56.40	57.59	61.32
	RSD (%)	1.58	1.26	1.50

Table 5. Application of the proposed method to pharmaceutical formulations

Drug	Label content (mg)		Found (mg) ± SD (n=6)	
	S-(−)-RAB	*R*-(+)-RAB	*S*-(−)-RAB	*R*-(+)-RAB
A	5	5	5.01 ± 0.08	5.00 ± 0.08
B	5	5	5.09 ± 0.06	5.09 ± 0.07

3.4. Method application

This analytical method was used to quantify the content of each RAB enantiomer in tablets from two different manufacturers. Tablets A and B contained 10 mg of RAB racemate. The measured content of *S*-(−)-RAB in the formulations ranged from 5.01 to 5.09 mg, while the measured content of *R*-(+)-RAB ranged from 5.00 to 5.09 mg. RSD values for both enantiomers in all products were less than 2.0 % (*Table* 5).

4. Conclusions

In conclusion, the results demonstrate that CE with dual cyclodextrins as a chiral selector system is a possible and promising method for the separation of enantiomers in surveyed PPIs including LAN, OME, PAN and RAB, with the possible resolutions were 1.90, 1.28, 1.47, and 1.48, respectively. Since RAB is the most challenging candidate among four PPIs and the published CE methods for its enantioseparation have been insufficiently developed and validated, this present study investigated a modified and effective CE method for the chiral separation of RAB. The effects of capillary-ralated parameters such as capillary temperature, and applied voltage, as well as BGE including pH, buffer concentration, and CDs concentration on the enantioseparation were all investigated. Finally, the best chiral separation was performed on a 56-cm long, 50-μm wide bare fused-silica capillary using a BGE containing of 50 mM phosphate adjusted to pH 7.0, 30 mM SBE-β-CD, and 30 mM γ-CD with 25 kV applied voltage and 16 °C capillary temperature. With the suitable sample preparation process and optimal CE condition, the developed method not only achieved the desirable RAB enantiomer separation but also demostrated the

good stability of RAB solution in 0.1M NaOH. The proposed method is satisfactory the requirements of ICH in analytical method validation. The method was also successfully applied to commercial tablets without any interference from excipients. Thus, this method can be used for the routine quality control of RAB enantiomers in pharmaceutical products.

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Conflict of Interest

The authors of this manuscript have no financial and personal relationship with other people or organizations that could influence their work.

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Authors' Positions

Yusung Choi : Graduate Student
Thuy-Vy Pham : Graduate Student
Xuan-Lan Mai : Graduate Student
Quoc-Ky Truong : Researcher
Thi-Anh-Tuyet Le : Graduate Student
Thi-Ngoc-Van Nguyen : Associate Professor
Gunhee Lee : Graduate Student
Jong-Seong Kang : Professor
Woongchon Mar : Professor
Kyeong Ho Kim : Professor