Research Article

Chiral separation of lansoprazole and rabeprazole by capillary electrophoresis using dual cyclodextrin systems

Novel capillary electrophoresis methods using CDs as chiral selectors were developed and validated for the chiral separation of lansoprazole and rabeprazole, two proton pump inhibitors. Fourteen different neutral and anionic CDs were screened at pH 4 and 7 in the preliminary analysis. Sulfobutyl-ether-β-CD with a degree of substitution of 6.5 and 10 at neutral pH proved to be the most suitable chiral selector for both compounds. Various dual CD systems were also compared, and the possible mechanisms of enantiomer separation were investigated. A dual selector system containing sulfobutyl-ether-β-CD degree of substitution 6.5 and native γ-CD proved to be the most adequate system for the separations. Method optimization was carried out using an experimental design approach, performing an initial fractional factorial screening design, followed by a central composite design to establish the optimal analytical conditions. The optimized methods (25 mM phosphate buffer, pH 7, 10 mM sulfobutyl-ether-β-CD/20 mM γ-CD, +20 kV voltage; 17°C temperature; 50 mbar/3 s injection, detection at 210 nm for lansoprazole; 25 mM phosphate buffer, pH 7, 15 mM sulfobutyl-ether-β-CD/30 mM γ-CD, +20 kV voltage; 18°C temperature; 50 mbar/3 s injection, detection at 210 nm for rabeprazole) provided baseline separation for lansoprazole ($R_s = 2.91$) and rabeprazole ($R_s = 2.53$) enantiomers with favorable migration order (in both cases the S-enantiomers migrates first). The optimized methods were validated according to current guidelines and proved to be reliable, linear, precise, and accurate for the determination of 0.15% distomer as chiral impurity in dexlansoprazole and dexrabeprazole samples.

Keywords:
Chiral separation / Dual cyclodextrin system / Experimental design / Lansoprazole / Rabeprazole

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Additional supporting information may be found online in the Supporting Information section at the end of the article.

1 Introduction

Lansoprazole (2-[[3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methylsulfanyl]-1H-benzimidazole), and rabeprazole (2-[[4-(3-methoxypropoxy)-3-methylpyridin-2-yl]methylsulfanyl]-1H-benzimidazole) are benzimidazole-derivative proton pump inhibitors (PPIs). These drugs suppress gastric acid secretion through interaction with (H⁺/K⁺)-ATP-ase in gastric parietal cells and proved to be effective in the treatment of duodenal and gastric disorders. Both compounds are chiral, possessing an asymmetric sulfoxide group in their molecular structure (Fig. 1). Lansoprazole and rabeprazole have mainly been used in the therapy as racemic mixtures but in some countries the enantiopure forms (dexlansoprazole and dexrabeprazole) are also available. The enantiopure form of PPIs display therapeutic advantages, such as superior metabolic and pharmacokinetic profile, as compared to their racemates [1].

These facts necessitate the development of new methods for the enantiomeric analysis of lansoprazole and rabeprazole. Concerning the separation techniques available, CE has become an attractive alternative over the widespread chromatographic techniques, being superior in solvent, chiral selector and analyte consumption, short analysis time, rapid method development, and high separation efficiency [2].
In CE, the most frequently used chiral selectors are CDs; cyclic oligosaccharides with a hydrophobic cavity, and hydrophilic outside rim [3, 4]. Their chiral discrimination process usually involves an inclusion of a hydrophobic moiety of the chiral analyte in the CD cavity, along with lateral interactions of the hydroxyl or other polar groups of the CD with polar moieties of the guest molecule [5]. Although this is the general case, several unusual binding modes were also described [6] or proposed [7]. External complex formation of the β-blocker talinolol was observed in aqueous (with heptakis(2,3-di-O-methyl-6-sulfo)-β-CD) as well as in non-aqueous (with heptakis(2,3-di-O-acetyl-6-sulfo)-β-CD) environments using NMR spectroscopy [6]. No evidence of complexation between the topical fungicide enilconazole enantiomers and heptakis(2-O-methyl-3,6-di-O-sulfo)-β-CD was established using NMR spectroscopy, although CE experiments evidenced enantiorecognition using this CD as chiral selector. The authors proposed the formation of a shallow external complex between the enantiomers of the analyte and the chiral selector, which is difficult to detect by NMR spectroscopy [7].

Dual selector systems have widely been used in chiral CE since 1994 when the first study applying a combination of a charged and a neutral CD was published [8]. The combination of sulfobutyl-ether-β-CD with neutral CD derivatives has also proved its applicability for more than 20 years [9].

Rapid and efficient method development in CE can be achieved through a direct method by simply dissolving the chiral selectors in the buffer electrolyte [2]. This way, the chiral selector forms transient diastereomeric complexes with the enantiomers of the analyte. The subsequent separation can be rationalized upon two mechanisms: difference in complex stability (thermodynamic factor) and difference in complex mobility (kinetic factor). Generally, both mechanisms contribute to the separation, but in some cases, one of the factors predominates in the enantiomeric separation [10].

Only few studies were published on the chiral analysis of lansoprazole and rabeprazole by CE. A CE method was developed and validated for the chiral separation of pantoprazole, omeprazole, and lansoprazole, using bovine serum albumin as chiral selector, in a phosphate buffer at pH 7.4; however, this method has low sensitivity due to strong UV absorbance of the buffer system [11]. A validated CE method for enantioselective determination of lansoprazole from pharmaceutical formulations was published, using β-CD as chiral selector dissolved in phosphate buffer at pH 2.2 [12]. The use of copper (II)-L-histidine complex and HP-β-CD as dual chiral selector system in a phosphate buffer for enantiomeric separation of pantoprazole, lansoprazole, omeprazole, and tenatoprazole has also been reported [13]. A dual CD system (30 mM SBE-β-CD/20 mM methyl-β-CD) was used for the determination of enantiomeric purity of dexlansoprazole; the method was validated and applied for determination from capsules, with an analysis time of around 20 min [14]. The only published enantiomeric separation method by CE of rabeprazole used an ephedrine-based chiral ionic liquid, which served as both the chiral selector and the BGE in a non-aqueous CE system [15].

Based on previously published articles regarding chiral separation of PPI by CE and on other studies regarding CD complexation of PPIs [16, 17]; CD derivatives seem the most promising candidates as chiral selectors for the enantioseparation of lansoprazole and rabeprazole. Moreover, SBE-β-CD was applied for the chiral separation of the structurally similar pantoprazole in CE [18, 19] as well as in HPLC [20] as chiral mobile phase additive.

The aim of our study was to develop new, simple, rapid, and cost-effective methods for the enantiomeric quality control of dexlansoprazole and dexrabeprazole using CD-modified CE.

Experimental design-based methodologies have shown a steadily growth in analytical chemistry [21], including chiral CE as well [22]. In order to optimize the analytical conditions, a multivariate approach was used; a fractional factorial design was used in the screening process, followed by a central composite design (CCD) for the optimization.

2 Materials and methods

2.1 Reagents and samples

Racemic lansoprazole and rabeprazole sodium were United States Pharmacopeia reference standards (Rockville, USA). R-lansoprazole and R-rabeprazole were purchased from Beijing Mesochem Technology (Beijing, China).

Phosphoric acid (85%), disodium hydrogenophosphate, and sodium dihydrogenophosphate were purchased from Merck (Darmstadt, Germany). Sodium hydroxide and methanol were purchased from Lach Ner (Neratovice, Czech Republic), while DMSO was product of Sigma–Aldrich Hungary (Budapest, Hungary).

The ultrapure water used throughout the study was prepared by a Milli-Q Direct 8 Millipore system (Milford, MA, USA). All reagents were of analytical grade.

Three classes of CDs were used as chiral additives, as follows: native neutral CDs (α-CD, β-CD, γ-CD), derivatized neutral CDs (hydroxypropyl-β-CD – HP-β-CD DS 3 (M1 = 1309.0), 4.5 (M1 = 1396.4) and 6.3 (M1 = 1497.6); randomly methylated β-CD – RAMEB (M1 = 1303.4); derivatized ionizable CDs (carboxymethyl-α-CD - carboxymethylated-α-CD DS 3.5 (M1 = 1212.9); carboxymethyl-β-CD-carboxy-methylated-β-CD DS 3.5 (M1 = 1375.1);
The obtained results were evaluated in terms of resolution ($R_s$) obtained by the $R_s = 2(t_2 - t_1)/(w_1 + w_2)$ equation, where the migration times ($t_1$ and $t_2$) and the peak-widths ($w_1$ and $w_2$) were marked for the first- and second-migrating enantiomer, respectively.

In the process of method optimization, Design Expert 7.0 statistical software (Stat-Ease, Minneapolis, USA) was used for constructing the experimental plans and data evaluation.

3 Results and discussions

3.1 Preliminary analysis

A variety of neutral and anionic CDs, being also different in cavity size, type, and degree of substitution (DS), applied in the concentration range of $1–30 \text{mM}$ was screened at pH 4.0 and pH 7.0.

Both compounds of interest are amphoteric with an acidic $pK_{a1} \sim 9$ for both compounds and with a basic $pK_{a2} \sim 4$ and 4.9 in the case of lansoprazole and rabeprazole, respectively. However, it is established that the stability of PPIs decreases in acidic medium, leading to significant degradation [23].

At pH 4.0, both charged and uncharged forms of lansoprazole and rabeprazole are present in significant amount in the BGE, giving to the analyte its own effective electrophoretic mobility. Under neutral conditions, both compounds exist practically in the uncharged form only, migrating with the EOF. Under these circumstances, the enantiomeric separation capability of eight anionic CDs and their dual systems formed with neutral CDs at pH 7, and of eight anionic CDs and seven uncharged CDs at pH 4 was investigated.

In acidic conditions, no chiral separation of rabeprazole was achieved, while lansoprazole exhibited partial enantiomeric resolution with some neutral CDs ($\beta$-CD and HP-$\beta$-CDs), as expected based of previous studies [12, 14].

In neutral conditions, chiral recognition occurred for both compounds using SBE-$\beta$-CDs of DS of 6.5 and 10. Lansoprazole could also be partially separated by SBE-$\beta$-CD, DS 4. Considering the good separation capability of SBE-$\beta$-CD against non-ionized forms of both analytes, and the relative instability of the PPIs in acidic conditions, further studies were conducted using neutral BGE buffers.

Although the best resolution values were obtained when using SBE-$\beta$-CD DS 10, the application of SBE-$\beta$-CDs with lower DS also has some practical advantages, such as lower ionic strength and the concomitant better peak shapes, and shorter analysis time.

Based on these observations, the optimization of the analytical method was carried out using SBE-$\beta$-CD DS 6.5/\(\gamma\)-CD dual selector system.

3.2 Method optimization

3.2.1 Screening design

In order to assess the influence of experimental variables on the separation, a 2$^{6-1}$ fractional factorial design with four additional centre point measurements (twelve experimental runs in total) was carried out. Two experimental responses were monitored: the resolution value between the two enantiomers.
and the analysis time (represented by the migration time of
the second migrating enantiomer).

The effect of the six factors on the experimental responses
was evaluated, using the following ranges: concentration of
SBE-β-CD 3–10 mM (factor A), concentration of γ-CD 15–
35 mM (factor B), buffer concentration 25–50 mM (factor C),
buffer pH 6.5–7.5 (factor D), applied voltage 15–25 kV (fac-
tor E) and system temperature 10–20°C (factor F). Each factor
had two levels (coded as −1 and 1), and 4 additional injections
at the centerpoint of the experimental plan were carried out
(each factor being set at 0 level) for better estimation of exper-
imental error and to increase the number of degrees of freedom
of the model. The experimental plan and results obtained
are summarized in the Supporting Information Table S1.

A simple first order regression model was applied for this
design:

\[
Y = \beta_0 + \beta_1 \cdot A + \beta_2 \cdot B + \beta_3 \cdot C + \beta_4 \cdot D + \beta_5 \cdot E
+ \beta_6 \cdot F
\]

(1)

where, \(Y\) represents experimental response, \(A, B, C, D, E,\) and \(F\) are the experimental factors to be optimized, \(\beta_0\) is
the intercept, \(\beta_{1-6}\) are coefficients.

In order to estimate the significance of the regression
coefficients of the model ANOVA was carried out. The insignifi-
cant model terms were deleted one by one, with parallel
revaluation of the model after each deleted term.

The following regression equations were obtained:

for lansoprazole

\[
\text{Analysis time (t)} = +8.95 + 1.29 \cdot A - 3.66 \cdot E - 0.75 \cdot F
\]

(2)

\[
\text{Resolution (R)} = +1.22 + 0.31 \cdot A - 0.16 \cdot B + 0.11 \cdot C
+ 0.11 \cdot E - 0.14 \cdot F
\]

(3)

Both models showed good performance indicators for
resolution and analysis time: \(R^2 = 0.9886, R^2_{\text{adj}} = 0.9837\) and
\(R^2 = 0.9865, R^2_{\text{adj}} = 0.9731.\)

and for rabeprazole

\[
\text{Analysis time (t)} = +7.94 + 0.78 \cdot A - 0.61 \cdot B
- 1.69 \cdot E - 1.56 \cdot F
\]

(4)

\[
\text{Resolution (R)} = +1.16 + 0.23 \cdot A + 0.052 \cdot B
- 0.083 \cdot E - 0.12 \cdot F
\]

(5)

Excellent performance indicators were obtained in both
cases for resolution and analysis time: \(R^2 = 0.9873, R^2_{\text{adj}} = 0.9788,\) and \(R^2 = 0.9943, R^2_{\text{adj}} = 0.9905.\)

In the case of lansoprazole, three factors had significant
influence on both experimental responses: concentration of
SBE-β-CD, applied voltage and system temperature.

In the case of rabeprazole four of six parameters had sig-
ificant effect: concentration of SBE-β-CD, concentration of
γ-CD, system temperature and applied voltage. From these,
the first three were chosen for further optimization.

### 3.2.2 Optimisation design

Response surface methodology was carried out using a CCD
with three variables for both compounds separately, based on
the results of the screening design.

The CCD consisted of a two-level factorial design \((2^3),\) with
6 axial points \((\alpha = 1.4),\) and 6 central points, a total
of 20 experiments. The rest of the parameters, considered
to be less important, were held constant in the optimization
process (25 mM phosphate buffer, pH 7.0, 20 mM γ-CD for
lansoprazole; and, 25 mM phosphate buffer, pH 7.0, applied
voltage: +20 kV for rabeprazole).

Response surface methodology experimental models are
able to estimate the interaction and second order terms.
The initial mathematical model for both CCD was the follow-
ing:

\[
Y = \beta_0 + \beta_1 \cdot A + \beta_2 \cdot B + \beta_3 \cdot C + \beta_4 \cdot AB + \beta_5 \cdot AC
+ \beta_6 \cdot BC + \beta_7 \cdot A^2 + \beta_8 \cdot B^2 + \beta_9 \cdot C^2
\]

(6)

where, \(Y\) represents experimental response (resolution and
analysis time), \(A, B\) and \(C\) are the experimental factors to be
optimized, \(\beta_0\) is the intercept, \(\beta_{1-9}\) are linear coefficients, \(\beta_{6,8}\)
are coefficients of interaction terms and \(\beta_{7,9}\) coefficients of
the quadratic terms.

Based on ANOVA the following regression models were
obtained for lansoprazole:

\[
\text{Resolution (R)} = 2.94 + 0.78 \cdot A - 0.21 \cdot B - 0.32 \cdot C
+ 0.20 \cdot B \cdot C - 0.52 \cdot A^2 - 0.31 \cdot C^2
\]

(7)

\[
\text{Analysis time (t)} = +10.80 + 3.58 \cdot A - 2.51 \cdot B
- 1.98 \cdot C - 1.37 \cdot A \cdot B - 1.33 \cdot A \cdot C
+ 1.09 \cdot B \cdot C
\]

(8)

Both models showed good performance indicators for
resolution and analysis time: \(R^2 = 0.9473, R^2_{\text{adj}} = 0.9229,\) and
\(R^2 = 0.9633, R^2_{\text{adj}} = 0.9464.\)

In the case of rabeprazole, the following models were
calculated:

\[
\text{Resolution (R)} = +1.48 + 0.58 \cdot A + 0.22 \cdot B
- 0.13 \cdot C - 0.14 \cdot B \cdot C + 0.18 \cdot C^2
\]

(9)

\[
\text{Analysis time (t)} = +7.07 + 2.28 \cdot A - 0.84 \cdot B
- 1.25 \cdot C - 1.20 \cdot A \cdot B + 1.03 \cdot B^2
\]

(10)

also possessing good performance indicators \((R^2 = 0.9390,\)
\(R^2_{\text{adj}} = 0.9172\) and \(R^2 = 0.9387, R^2_{\text{adj}} = 0.9168\) for resolution
and analysis time, respectively).
According to the F values obtained during statistical analysis, the concentration of SBE-β-CD had the greatest influence on the resolution and analysis time, in the case of both compounds. 3-D response surface plots are shown in the Supporting Information Fig. S1 and S2 for both responses for lansoprazole and rabeprazole, respectively.

The obtained $R^2$ and $R^2_{adj}$ values indicate that all four models fit the experimental results. Based on the above models, Derringer’s desirability functions were used for both compounds to predict the global optimum of both experimental responses. In this approach, experimental results are transformed in desirability values on a scale between 0 and 1, 0 representing the most undesirable and 1 the most desired outcome of each response. In our case, resolution had to be enhanced, and analysis time had to be minimized, both responses having the same weight. Global desirability was calculated as geometric mean of the individual desirability values, and then the overall optimum was searched in the experimental space.

Based on the CCD, the following optimal conditions were obtained for lansoprazole: 10 mM SBE-β-CD, +20 kV applied voltage and 17°C system temperature. The analogous data for rabeprazole were: 15 mM SBE-β-CD, 30 mM γ-CD, and 18°C. The optimized conditions provided baseline
separation for lansoprazole \((R_s = 2.91 \pm 0.05)\) and rabeprazole \((R_s = 2.53 \pm 0.04)\) enantiomers. The migration order of the enantiomers was determined by injecting racemate samples enriched with \(R\)-lansoprazole and \(R\)-rabeprazole, respectively. The \(S\-(\text{-})\)enantiomers of both compounds eluted first.

The CCD experimental plans and results are summarized in the Supporting Information Table S2 and S3 for lansoprazole and rabeprazole, respectively.

Representative electropherograms obtained after applying the optimized analytical conditions are presented in Fig. 2A and B for lansoprazole and rabeprazole, respectively. To verify the robustness of the optimized system a Plackett-Burman design was performed: where 12 experiments were carried out by modifying the values of six analytical parameters within the following ranges: voltage \(\pm 1\) kV, temperature \(\pm 1^\circ\)C, buffer pH \(\pm 0.2\), buffer concentration \(\pm 3\) mM, SBE-\(\beta\)-CD concentration \(\pm 0.5\) mM and \(\gamma\)-CD concentration \(\pm 1\) mM. As experimental responses, the migration times of both enantiomers and resolution were followed. Statistical analysis (ANOVA) revealed no significant correlation between the analysed factors and responses in the studied ranges, which demonstrates the robustness of the method. Plackett-Burman designs with the experimental result are summarized in the Supporting Information Table S4 and S5 for lansoprazole and rabeprazole, respectively. The method was applied further for the determination of chiral impurities in eutomer samples.

### 3.3 Method validation

Our methods were validated according to International Council of Harmonization (ICH) guideline, based on precision, linearity, accuracy, limits of detection (LOD) and limit of quantification (LOQ) for the determination of \(S\)-lansoprazole and \(S\)-rabeprazole in \(R\)-lansoprazole and \(R\)-rabeprazole samples.

Method sensitivities were determined by sequentially diluting sample solutions. The LOD and LOQ for the \(S\)-enantiomers were determined at 3:1 and 10:1 signal-to-noise ratios in the presence of solution of \(R\)-enantiomers, respectively. The LOQ of both \(R\)-enantiomers were 6 \(\mu\)g/mL corresponding 0.15% impurity in 4000 \(\mu\)g/mL sample of eutomer, while LODs were 2 \(\mu\)g/mL, corresponding 0.05% chiral impurities. Representative electropherograms with optimal parameters containing 0.15% chiral impurities are presented in Fig. 3A and B for dexlansoprazole and dexrabeprazole, respectively.

Linearity of the methods was investigated in the range of 6–60 \(\mu\)g/mL (0.15–1.5%) \(S\)-lansoprazole and \(S\)-rabeprazole in 4000 \(\mu\)g/mL, \(R\)-lansoprazole and \(R\)-rabeprazole samples, respectively performing three replicate injections at six concentration points. A linear regression curve was obtained by plotting peak areas versus corresponding concentration using the least square method and slope, intercept, and

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lansoprazole</th>
<th>Rabeprazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>6–60 (\mu)g/mL ((0.15–1.5%))</td>
<td>6–60 (\mu)g/mL ((0.15–1.5%))</td>
</tr>
<tr>
<td>Regression equation</td>
<td>(y = 0.215 + 0.875)</td>
<td>(y = 0.316 + 0.975)</td>
</tr>
<tr>
<td>Coefficient of determination</td>
<td>(r^2 = 0.9994)</td>
<td>(r^2 = 0.9992)</td>
</tr>
<tr>
<td>LOD</td>
<td>2 (\mu)g/mL</td>
<td>2 (\mu)g/mL</td>
</tr>
<tr>
<td>LOQ</td>
<td>6 (\mu)g/mL</td>
<td>6 (\mu)g/mL</td>
</tr>
<tr>
<td>Accuracy (Recovery %)</td>
<td></td>
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<tr>
<td>6 (\mu)g/mL ((0.15%))</td>
<td>98.12 (\pm) 2.12 (96.12 \pm 1.72)</td>
<td></td>
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<tr>
<td>24 (\mu)g/mL ((0.60%))</td>
<td>101.01 (\pm) 5.72 (99.12 \pm 2.12)</td>
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<tr>
<td>40 (\mu)g/mL ((1%))</td>
<td>97.12 (\pm) 1.01 (100.45 \pm 1.89)</td>
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<tr>
<td>Intra-day Precision (RSD%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.15%</td>
<td>3.48</td>
<td>3.55</td>
</tr>
<tr>
<td>0.60%</td>
<td>2.12</td>
<td>1.02</td>
</tr>
<tr>
<td>1%</td>
<td>1.99</td>
<td>0.98</td>
</tr>
<tr>
<td>Inter-day Precision (RSD%)</td>
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<tr>
<td>0.15%</td>
<td>5.48</td>
<td>3.78</td>
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<tr>
<td>0.60%</td>
<td>2.56</td>
<td>1.45</td>
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<tr>
<td>1%</td>
<td>1.78</td>
<td>0.89</td>
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</tbody>
</table>
correlation coefficients were also determined. The following equations were calculated: 
\[ y = 0.215x + 0.875 \quad (r^2 = 0.9994) \]
for lansoprazole and 
\[ y = 0.316x + 0.975 \quad (r^2 = 0.9992) \]
for rabeprazole.

Precision (repeatability based on RSD% of migration time and peak area) and accuracy (calculated as recovery%) were estimated at three levels of the impurities, i.e., 0.15, 0.60, 1% in the presence of 4 mg/mL dexlansoprazole and dexrabeprazole, respectively. The validation data including accuracy and precision values are summarized in Table 1 for both drugs. RSD values of intra-day and inter-day precision were below 5.5% and accuracy between ±6%.

According to obtained results, our optimized method proved to reliable, linear, precise and accurate for the determination of 0.15% distomers in dexlansoprazole and dexrabeprazole samples.

4 Concluding remarks

Two rapid, new and cost-effective CE methods with CDs have been developed for the determination of enantiomeric purity of two PPIs, dexlansoprazole and dexrabeprazole. In order to discriminate between enantiomers of lansoprazole and rabeprazole 14 different CDs were screened at two different pHs. Various dual systems were also compared and a dual CD system containing SBE-\(\gamma\)-CD DS 6.5 and native \(\gamma\)-CD proved to be the most adequate for both compounds.

Method optimization was carried out by multivariate approach, performing first a rapid screening for significant factors by fractional factorial design, followed by response surface type optimization designs (CCD). The optimized circumstances were used for method validation and determination of chiral impurities in the eutomers.

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5 References


