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Chiral resolution of pantoprazole sodium and related sulfoxides by complex formation with bovine serum albumin in capillary electrophoresis

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Abstract

The separation of enantiomers of pantoprazole sodium, omeprazole and lansoprazole by capillary zone electrophoresis using bovine serum albumin (BSA) as the chiral selector is described. Baseline separation of the three structurally related drugs was obtained after optimization of the most important experimental parameters. For this purpose, influences such as BSA concentration, pH and concentration of 1-propanol as organic modifier on the separation were investigated. Increasing concentrations of BSA improved the chiral resolution but lowered the sensitivity of the detection system. Discrimination of the enantiomers was observed only in a narrow pH range of 7–8. An optimum of pH 7.4 was a good compromise in terms of enantio-resolution and peak shape. 1-Propanol when added to the buffer system, improved the peak shape of the analytes and the resolution. The optimized method has been validated for pantoprazole sodium and is useful for routine analysis.

Keywords: Enantiomer separation; Pharmaceutical analysis; Buffer composition; Pantoprazole; Sulfoxide; Omeprazole; Lansoprazole; Albumin

1. Introduction

Chiral resolution of enantiomers is one of the most successful fields of application of capillary electrophoresis. Ease of operation, rapid method development and high separation efficiencies are striking features of this technique. In recent years a number of different principles for chiral separation were developed to broaden the range of application. Depending on the chiral selector used for the discrimination of the enantiomers, six main principles exist so far: (i) host–guest complexation by cyclodextrins [1–3] or crown ethers [4,5], (ii) ligand-exchange complexation [6], (iii) solubilization by optically active micelles [7,8], (iv) complexation by macrocyclic peptides [9] or (v) oligosaccharides [10] and (vi) complex formation with proteins.

Host–guest complexation of aromatic functional groups by cyclodextrins and various derivatives thereof, is by far the most often used and probably the best understood separation principle for this purpose. Although sophisticated optimization schemes for the cyclodextrin system have been reported [11,12] to simplify method development, many separation problems cannot be solved by this system. Protein-based stationary phases are widely used for chiral separation in HPLC. Although they have a broad applicability, chromatography columns

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are expensive and sensitive, and method optimization can be time-consuming. The successful application of proteins as chiral selector in capillary electrophoresis (CE) has been described by several research groups [13–17].

Pantoprazole sodium (briefly: pantoprazole), omeprazole and lansoprazole (Fig. 1) are representatives of the proton pump inhibitor (PPI) class of drugs [18]. A common feature of these 2-[(2-pyridylmethyl)sulfinyl]benzimidazole derivatives is their chiral center at the sulfoxide group, that is generated by oxidation of the corresponding sulfide precursors. Since this oxidation step is performed without chiral reagents, one might expect racemic products, i.e. 1:1 mixtures of the respective enantiomers. Nevertheless, regulatory authorities demand the experimental proof of this sound expectation. Therefore, analytical techniques are required to determine the enantiomeric ratio of racemic compounds. In general, polarimetry is the method of choice for measuring enantiomeric ratios. However, this technique suffers from two main disadvantages:

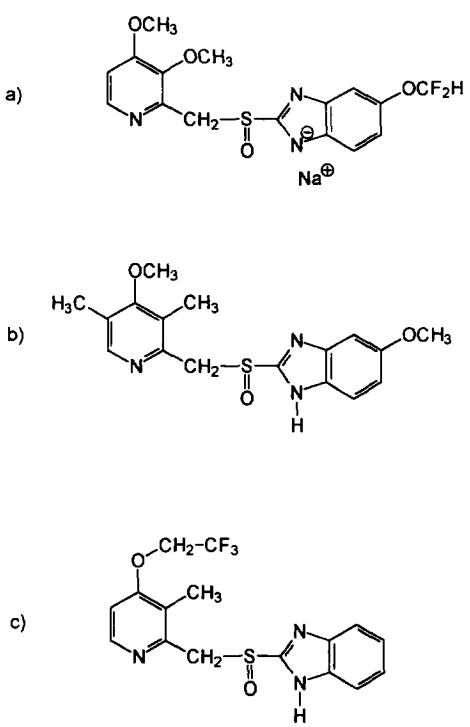

Fig. 1. Chemical structures of (a) pantoprazole, (b) omeprazole and (c) lamsoprazole.

(i) the impact of chiral impurities from synthesis on optical rotation is not taken into account and (ii) the sensitivity in detecting small variations of the enantiomeric ratio is often low. Thus, a chiral separation technique seems to be favourable for this purpose. In preliminary investigations, chiral separation of pantoprazole by capillary electrophoresis, using various cyclodextrins, was examined but failed [19]. Because of their broad applicability, protein- and cellulose-based stationary phases are widely used in HPLC for chiral separations. For pantoprazole and related sulfoxides, a complete baseline separation of the enantiomers could not be obtained [20–23] yet. The present paper describes the enantioseparation of these drugs using bovine serum albumin (BSA) as chiral selector. For pantoprazole, experimental factors, such as concentration of BSA, pH and buffer modifiers, are systematically investigated.

2. Experimental

2.1. Instrumentation

All experiments were performed using a Grom CE-System 100 (Fa. Grom, Herrenberg, Germany) capillary electrophoresis instrument equipped with a 66 cm (effective length 50 cm)×50 μm I.D. open tube fused silica capillary. After each analysis, the capillary was rinsed with 0.1 *M* NaOH for 3 min followed by rinsing with buffer for 2 min. If not otherwise stated, the following standard separation method was used: the buffer system consisted of 10 m*M* potassium phosphate, pH 7.4, with 40 μ*M* BSA and 7% (v/v) 1-propanol. Analytes were detected at 290 nm. Injection was accomplished in the electrokinetic mode using 5–8 kV for 7 s. In general, 1.0 mg pantoprazole was dissolved in 10 ml bidistilled water, and 5 mg of both omeprazole and lansoprazole were dissolved in 2 ml 1-propanol followed by dilution to 5 ml with bidistilled water, respectively. In order to improve reproducibility, all experiments were performed at a constant current of 35 μA and at field strengths of approx. 300 V/cm. The individual enantiomers of the racemate, pantoprazole, were identified by spiking experiments with the pure enantiomers.

UV spectra were measured in the range of 190 to 400 nm using a Lambda-16 UV–Vis spectrometer from Perkin-Elmer (Überlingen, Germany).

2.2. Chemicals

All chemicals purchased were of the highest quality. Acetone, potassium dihydrogenphosphate, dipotassium hydrogenphosphate, sodium chloride and 1-propanol were purchased from Merck (Darmstadt, Germany). Bovine serum albumin, fraction V was from Serva Feinbiochemica (Heidelberg, Germany). 2-Propanol was obtained by Riedel-de Haen. (±)-Pantoprazole sodium and enantiomers [24], (±)-omeprazole and (±)-lansoprazole were synthesized at Byk Gulden (Germany).

3. Results and discussion

The successful resolution of many enantiomers, using various proteins as chiral selectors in capillary electrophoresis, encouraged us to investigate BSA for this purpose. The UV absorption of proteins below 330 nm extremely limits their applicability as buffer additive in capillary zone electrophoresis (CZE). Thus, a proper selection of the wavelength for detection and of the protein concentration are indispensable for obtaining sufficient sensitivity. Fig. 2 shows the UV spectra of pantoprazole and of BSA. Taking into account an extinction coefficient of 15.543 L mol^{-1} cm^{-1} at 290 nm (the absorbance maximum for pantoprazole) compared to a corresponding value of approx. 25.000 L mol^{-1} cm^{-1} at 290 cm^{-1} for BSA, the concentration of the protein in the buffer system should be as low as possible for maximal sensitivity.

3.1. Effect of the concentration of BSA on enantiomeric resolution of pantoprazole

The influence of the BSA concentration on the resolution of (±)-pantoprazole was studied in the concentration range of 15–105 μ*M*. The resolution and the separation factor of both enantiomers improves steadily with increasing concentration of the

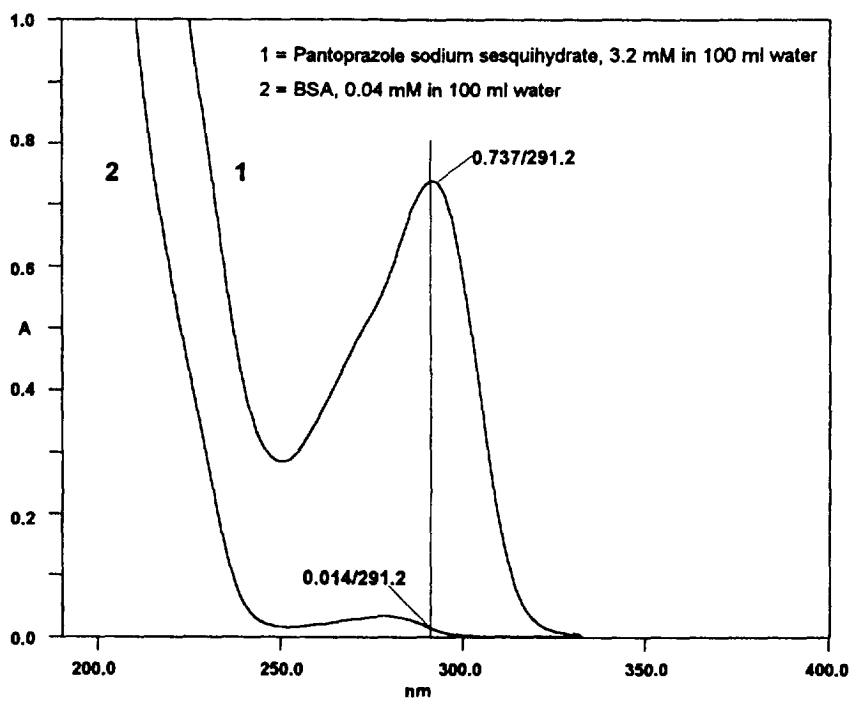

Fig. 2. UV spectra of 3.2 m*M* pantoprazole (1) and 40 μ*M* BSA (2) in water.

protein (see Fig. 3). Interestingly, migration times and peak shapes are only slightly influenced by the selector concentration. Furthermore, the electro-osmotic flow (EOF), which can be calculated from the negative peak at approx. 15 min., is basically independent from the protein concentration, an indication that adsorption of the protein on the silica surface has almost no influence on the EOF. Similar findings are reported by other authors [25]. However, the baseline noise increases with increasing BSA concentration, by a factor of 3 going along with decreasing signal intensities of the analytes. Table 1 summarizes the experimental data obtained. The separation factor α was calculated by

$$\alpha = \frac{t_2}{t_1} \quad (1)$$

where t_1 is the migration time of the first eluting enantiomer and t_2 the migration time of the antipode.

3.2. Influence of the buffer pH on enantiomeric resolution of pantoprazole

Apart from the concentration of bovine serum

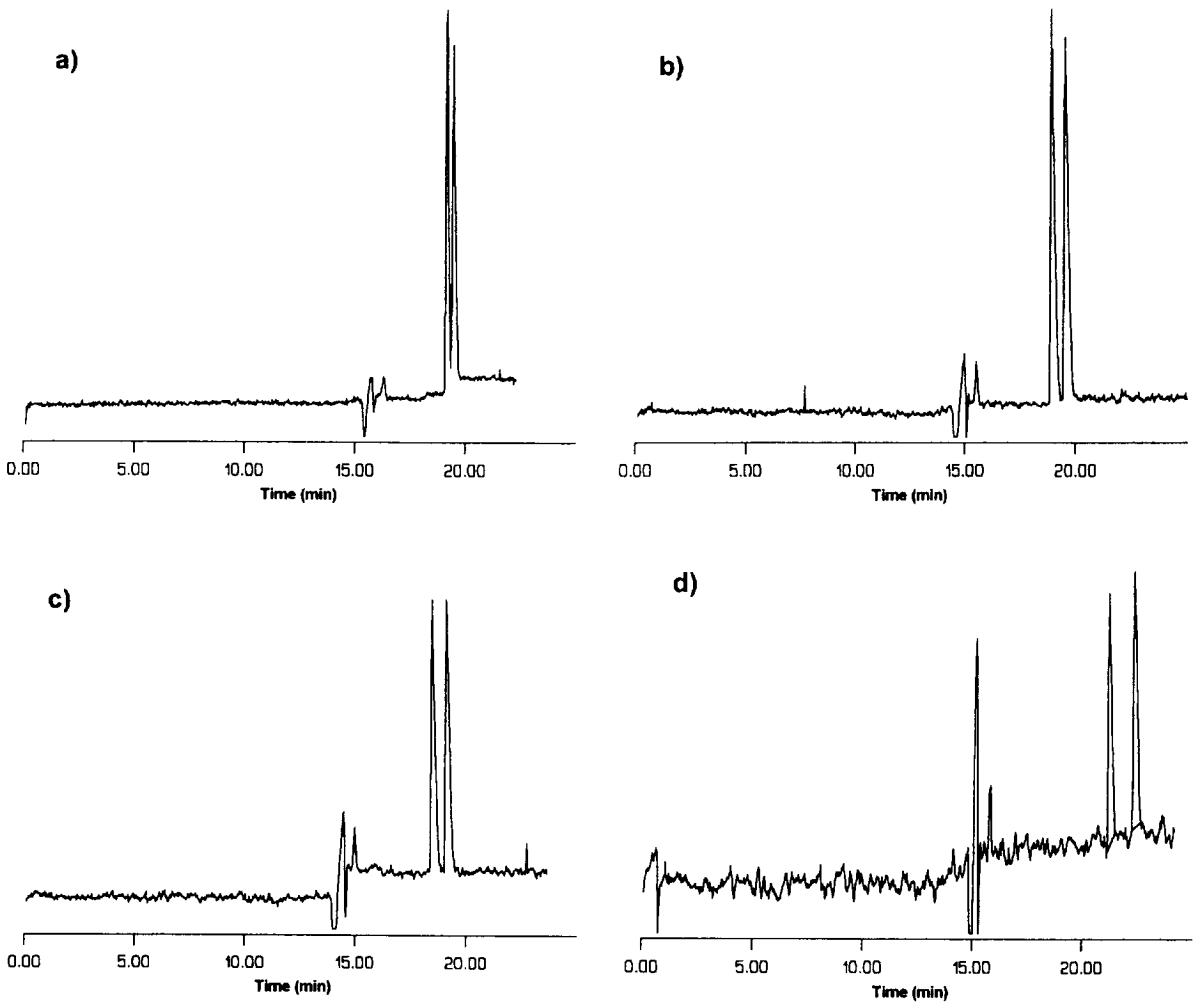

Fig. 3. Effect of BSA concentration on the chiral resolution of pantoprazole. Experimental conditions: (a) 15, (b) 30, (c) 40 and (d) 85 μM BSA. Buffer: 30 mM potassium phosphate, pH 7.4, with 5% 1-propanol. One cm of the y-axis corresponds to 3 mAU. Other conditions are given in Section 2.

Table 1
Influence of the BSA concentration on the separation of (±)-pantoprazole

BSA (μM)	Baseline noise (arbitrary units)	R_s	α	$\mu(+)\times 10^5$ (cm V^{-1} s^{-1})
0		0.0	1.0	−2.55
15	1	1.1	1.018	−3.47
30	1.5	1.5	1.032	−4.38
40	1.5	1.6	1.035	−4.70
50	1.75	2.1	1.043	−4.83
85	2.25	3.7	1.053	−5.45
105	3	4.4	1.071	−5.93

R_s = resolution, α = separation factor, $\mu(+)$ = electrophoretic mobility of (+)-pantoprazole (first eluting enantiomer). Experimental conditions as in Fig. 3.

albumin, the pH is the most important parameter for the separation of both enantiomers. In a preliminary screening program it was found that only a narrow range of the buffer pH between pH 7 and 8 could be used for method optimization. Owing to the acid-activation of pantoprazole-related sulfoxides in acidic media [18], low pH values (pH<5.5) were not investigated. BSA has an isoelectric point of about 4.7 and pantoprazole has pK values of 3.9 for the protonation of the N-pyridine and 8.2 for the deprotonation of the benzimidazole–NH, respectively. Thus, in the pH range 7–8 the protein is negatively charged and pantoprazole is uncharged or partly negative charged, therefore they have different electrophoretic mobilities. Chiral separation of both enantiomers is based on these differences in mobility and on different association constants of both enantiomers to the chiral selector. Poor separation was found for pH 7.0 to 7.2. At pH 7.3 both enantiomers are almost baseline separated in two narrow peaks (Fig. 4a). Although resolution markedly improves with higher pH values, peaks start to distort as a consequence of kinetic effects of the stronger interactions of the deprotonated negatively charged enantiomers with the protein (Fig. 4b–d). These stronger interactions with the chiral selector, as observed at higher pH values, not only cause longer migration times but also broad peaks. Chiral recognition, however, is determined by the difference in the Gibbs energy of the association of both enantiomers. Hence, optimal separation is obtained if $\Delta(\Delta G)$ is maximal while ΔG of the complex formation is minimized.

3.3. Organic modifier concentration

In several papers, organic modifiers such as 1-propanol, 2-propanol, etc. were described to significantly improve peak shapes and resolution [13,14] of chiral separations using BSA. Similar effects are known with albumin-modified stationary phases in HPLC. The addition of organic modifiers may have an effect on the three-dimensional structure of the protein and/or on protein–analyte association. While 2-propanol shows a positive effect on the separation only at portions of about 15% (results not shown here), 1-propanol significantly improves peak shape and resolution at portions of 3 to 10%. For concentrations of more than 10%, peaks become very sharp but enantiomeric resolution decreases again. Fig. 5 shows the effect of 1-propanol on the separation of (±)-pantoprazole.

3.4. Separation of omeprazole and lansoprazole

In contrast to pantoprazole sodium, both omeprazole and lansoprazole are free acids which needed to be dissolved in 1-propanol–water mixtures. While enantio-separation of lansoprazole succeeded using the standard separation technique optimized for pantoprazole with a resolution of 1.9 in approx 17 min (Fig. 6a), (±)-omeprazole could not be separated under these conditions. Baseline separation with a resolution of 1.5 was achieved at a BSA concentration of 100 μm and 7% 1-propanol, as shown in Fig. 6b. The ratio of enantiomers was 1.00±0.01 for omeprazole and 0.98±0.02 for lansoprazole, respectively.

3.5. Validation of the standard separation method for pantoprazole

For routine analysis of enantiomers, a proper validation of the separation method is required. For this purpose precision, accuracy, linearity, limit of detection (LOD) and ruggedness were investigated for the standard separation method. The reproducibility of peak areas was determined by six runs under identical experimental conditions. Relative standard deviation (R.S.D.) values of 4.9% were calculated. The main reasons for this high value might be the electrokinetic injection mode, which is known to

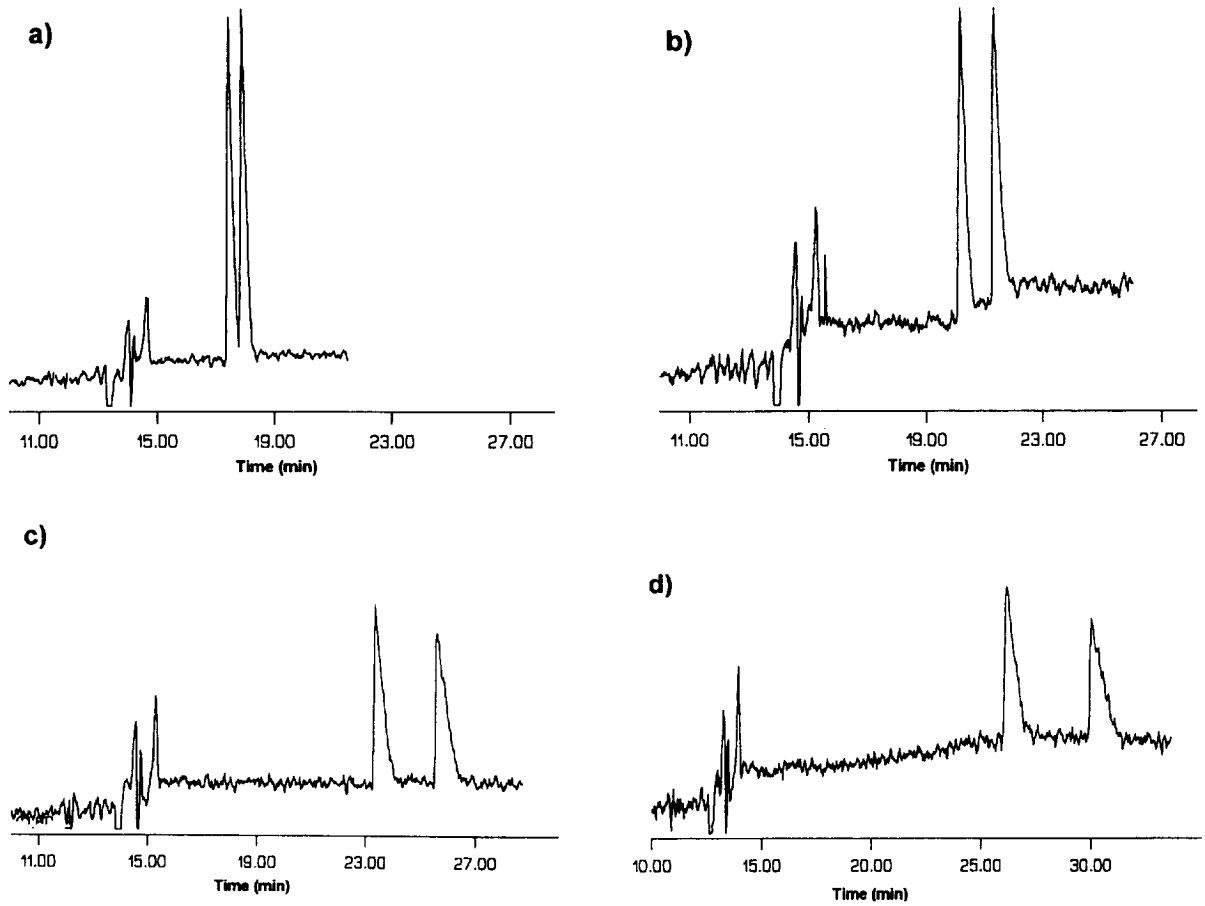

Fig. 4. Effect of pH on the separation of (±)-pantoprazole. Experimental conditions: (a) pH 7.3, (b) pH 7.4, (c) pH 7.6 and (d) pH 7.9. Buffer: 10 m*M* potassium phosphate with 55 μ*M* BSA and 5% 1-propanol. One cm of the *y*-axis corresponds to 3 mAU. For other conditions see Section 2.

show poor reproducibility, and the poor ratio of signal-to-noise and associated problems with the setting of the integration points. Thus, better results should be obtained by hydrodynamic injection. Accuracy was determined by the spiked placebo method using pure enantiomers. Theoretically expected values and experimental data correlated well within the R.S.D. of the method. In contrast to HPLC analysis [20], no bias for the ratio of the peak areas of both enantiomers was found with this method. Linearity was investigated in the range of 80 to 120% and calculated on the basis of 5 measuring points (80, 90, 100, 110 and 120% of the declared working concentration), with each point analyzed in triplicate. Linear regression analysis gave a correlation coefficient of 0.95. LOD was determined to be 0.04 mg/mL (4% of a main peak) based on a ratio of the signal-to-noise of 3 at a working concentration of 1.0 mg/mL. In terms of BSA concentration, pH and the portion of 1-propanol, the ruggedness of the method was studied in parallel to the method optimization. In addition, the quality of BSA had a dramatic influence on the separation. For instance, after applying a new batch of BSA from the same supplier, the method had to be adjusted with respect to the organic modifier.

3.6. Batch analysis of pantoprazole sodium

Several batches of pantoprazole were investigated

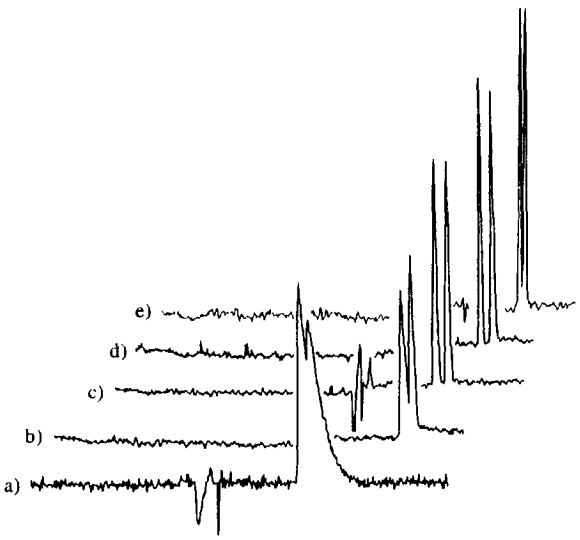

Fig. 5. Influence of the concentration of 1-propanol on the chiral separation of pantoprazole. Experimental conditions: (a) 0%, (b) 3%, (c) 5%, (d) 8% and (e) 12% 1-propanol in 30 m*M* potassium phosphate and 40 μM BSA, pH 7.4. One cm of the *y*-axis corresponds to 3 mAU. Other conditions are given in Section 2.

with the optimized CE method. The ratios of enantiomers were determined (see Table 2). Within the standard deviation it was demonstrated that the ratio of enantiomers is one, as expected for a racemate.

To summarize, the standard separation method

Table 2
Ratio of enantiomers $\bar{r}_{ex}$ of different batches of pantoprazole sodium ($n=3$)

Batch	$\bar{r}_{ex}$
1	0.99±0.03
2	0.99±0.01
3	1.00±0.01
4	1.02±0.03
5	0.99±0.02

fulfils all requirements for routine analysis. Although the validation data are worse than those obtained with HPLC [20], the CE investigation described herein represents the unique method for chiral resolution of pantoprazole, omeprazole and lansoprazole with complete baseline separation. Method development in CE was much faster, and routine analysis is much more inexpensive than in HPLC. A decisive advantage of CE over HPLC is the fact that CE does not show a systematic error in the quantitative analysis of the pantoprazole enantiomers.

4. Conclusions

CE using BSA as chiral selector is suited for routine enantio-separation of the racemate of (±)-pantoprazole and related sulfoxides, such as ome-

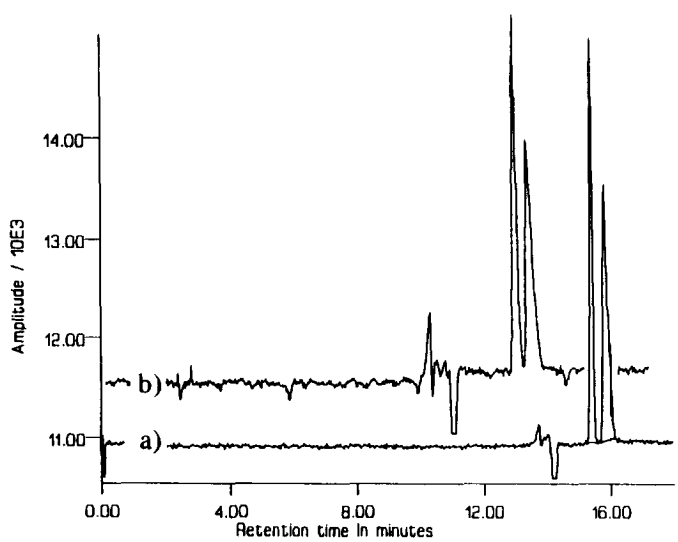

Fig. 6. Chiral separation of lansoprazole (a) and omeprazole (b) in CZE using BSA as chiral selector. Experimental conditions: (a) 40 μ*M* BSA and (b) 100 μ*M* BSA. One cm of the *y*-axis corresponds to 3 mAU. Other conditions are described in Section 2.

prazole and lansoprazole. The concentration of BSA had to be adjusted for sufficient detection sensitivity. Due to the strong background absorption of the buffer system, the limit of detection of the optimized system is rather poor. A substantial advantage of CE compared to HPLC is the simplicity to screen various types of chiral selectors and the rapid method development. While in HPLC several expensive and sensitive columns are needed for this purpose, in CE the chiral selector needs to be dissolved in the buffer electrolyte only.

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