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Determination of salbutamol enantiomers by high-performance capillary electrophoresis and its application to dissolution assays

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Abstract

Capillary zone electrophoresis was successfully applied to the chiral separation of salbutamol after addition of a suitable cyclodextrin chiral selector to the electrophoresis buffer. Parameters important in achieving enantiomeric separation are cyclodextrin type, mobile phase pH and applied field strength. In our study, salbutamol enantiomeric separation was obtained with the following conditions: heptakis (2,6-di-*O*-methyl)-β-cyclodextrin in 40 mM Tris (pH 2.5) and at 15 kV, obtaining a 3.09 resolution with migration times of 13.74 min for (*R*)-salbutamol and 13.98 min for (*S*)-salbutamol. Linearity, limit of quantitation, precision and accuracy were established using this method. The calibration curve was linear in a range of 1–40 μg ml^{-1} of racemic salbutamol (0.5–20 μg ml^{-1} of each enantiomer). This method was applied to evaluate the enantioselective release of salbutamol and taking into account the hypothesis that one enantiomer of a chiral drug would be released faster than the other from a pharmaceutical dosage form containing a racemic drug and a chiral excipient. For this purpose, matrix tablets formed by chiral excipients such as hydroxypropylmethylcellulose (HPMC) were considered. The release of the enantiomers of salbutamol from the formulations containing HPMC was found to be equivalent, with constant dissolution values (K) of $1.187 \pm 0.223\%$ min^{-n} for (*R*)-salbutamol and $1.076 \pm 0.268\%$ min^{-n} for (*S*)-salbutamol. © 1997 Elsevier Science B.V.

Keywords: Capillary electrophoresis; Chiral separation; Cyclodextrins; Salbutamol; Validation

1. Introduction

The existence of two or more forms of a compound that have identical molecular formulas but differ in the nature of the binding sequence of their atoms or in the arrangement of their atoms in space is very common among pharmacologically active substances. It has been estimated that approximately 50% of the commercially available drugs obtained by chemical synthesis have a chiral center [1]. These isomers often differ in their pharmacodynamic and/or pharmacokinetic properties because of the stereoselective interaction

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with optically active biological molecules. Unfortunately, investigators often either ignore or are unaware of the fact that many drugs are racemic. This has ominous implications and, therefore, it is appropriate to question the relevance of pharmacologic, pharmacokinetic, and pharmacodynamic data obtained from an assay which does not discriminate between enantiomers [2].

The growing awareness of the importance of stereochemistry has resulted in an increasing demand for suitable methods to determine the enantiomeric composition and purity of materials such as natural products and products of asymmetric synthesis and biotransformations [3]. Gas chromatography (GC) and high-performance liquid chromatography (HPLC) are widely used for enantioseparation [4]. During the last few years, different forms of high-performance capillary electrophoresis (HPCE) have proved to be useful for separating chiral compounds. Since enantiomers possess nearly identical physical properties, they do not usually exhibit appreciable differences in electrophoretic mobility and are not separated in a conventional capillary zone electrophoresis experiment; thus, a chiral selector becomes necessary [5]. Cyclodextrins (CD) represent a range of cyclic glucopyranoses, that have a characteristic conical shape with a hydrophobic interior cavity and a relatively hydrophilic surface. Cyclodextrins and their alkylated derivatives are able to form inclusion complexes with many substances, including racemic drugs. The complex constants are primarily determined by the size, geometry, hydrophobicity, and hydrogen-bonding ability of the analytes [6].

This paper studies the use of cyclodextrins (CDs) in salbutamol enantiomeric separation as well as the importance of parameters such as cyclodextrin type and pH in optimizing resolution. The release of salbutamol enantiomers from hydrophilic matrices elaborated with chiral excipients was also investigated to test the suitability of this HPCE method to study the enantioselective release of the drug.

2. Materials and methods

2.1. Apparatus and electropherographic conditions

A Hewlett Packard capillary electrophoresis system, equipped with a diode-array detector, was used. Capillary zone electrophoresis (CZE) was performed in a fused silica capillary (40 cm effective length, 48.5 total length and 50 μm i.d.). Separations were carried out in a constant voltage mode (15 kV) at 30°C capillary temperature. Samples were introduced by hydrodynamic injection at 50 mbars for 5 s. Before the analysis of each sample, the capillary was rinsed with 0.1 N NaOH and with the running buffer for a further 2 min.

2.2. Reagents

Tris(hydroxymethyl)aminomethane (TRIS), α-cyclodextrin, β-cyclodextrin and heptakis (2,6-di-*O*-methyl)-β-cyclodextrin were obtained from Sigma (St. Louis, MO). Salbutamol was supplied by Vencaser (Bilbao, Spain). Salbutamol (*R*)- and (*S*)-enantiomers were kindly supplied by Sepracor (Marlborough, MA). Hydroxypropylmethylcellulose (HPMC) K100 was a gift from Colorcon (Kent, UK). All other chemicals were analytical grade.

2.3. Analytical procedure

In order to optimize the method for the enantioseparation of salbutamol several factors were studied.

2.3.1. Effect of type of cyclodextrin

Electrophoresis buffers were prepared by dissolving 40 mM Tris–base in water and adjusting the pH with phosphoric acid to 2.5. Different concentrations of α-cyclodextrin, β-cyclodextrin and heptakis (2,6-*O*-methyl)-β-cyclodextrin (10, 10 and 20 mM, respectively) were dissolved in the Tris–phosphate buffer.

2.3.2. Effect of pH

Electrophoresis buffers were prepared by dissolving 40 mM Tris–base in water and adjusting the pH with phosphoric acid to 2.5 or 5. To this

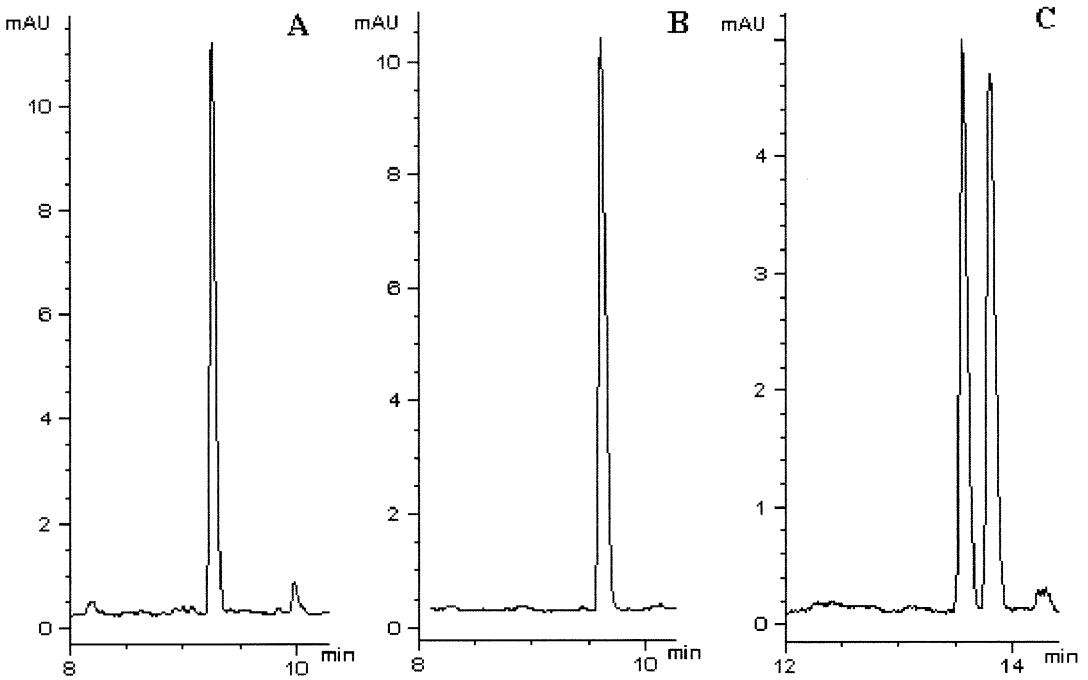

Fig. 1. Effect of cyclodextrin type on separation: (A) α-cyclodextrin; (B) β-cyclodextrin; (C) heptakis (2,6-di-*O*-methyl)-β-cyclodextrin.

solution heptakis (2,6-*O*-methyl)-β-cyclodextrin (20 mM) was addded.

2.3.3. Effect of applied field strength

Electrophoresis buffers were prepared by dissolving 40 mM Tris–base in water and adjusting the pH with phosphoric acid to 2.5. To this solution heptakis (2,6-*O*-methyl)-β-cyclodextrin (20 mM) was added. The applied field strengths studied were 15 and 30 kV.

2.4. Resolution

The resolution between the optical isomers was calculated by using the triangle method [7] and the equation:

$$R_s = 2\left(\frac{t_2 - t_1}{\omega_2 + \omega_1}\right)$$

in which t is the migration time and the peak width. Separation efficiency was obtained by calculating the number of theoretical plates using the expression [8]:

$$n = 16\left[\frac{t_R}{\omega_b}\right]^2$$

in which t_R is the retention time and b is the width of the peak base.

2.5. Identification of the enantiomers

The identification of salbutamol enantiomers was performed by injecting two solutions of salbutamol racemate (40 μg ml^{-1}) under the final conditions of our method: heptakis (2,6-di-*O*-methyl)-β-cyclodextrin in 40 mM Tris (pH 2.5) and at 15 kV, adding 20 μg ml^{-1} (*R*)-enantiomer to the first solution and to the second one the same amount of the (*S*)-enantiomer (20 μg ml^{-1}).

2.6. Validation of the analytical method

The specific requirements for the formal validation of a CE method are frequently unclear. In general, the criteria for validation applied to CE methods are similar to those employed for other

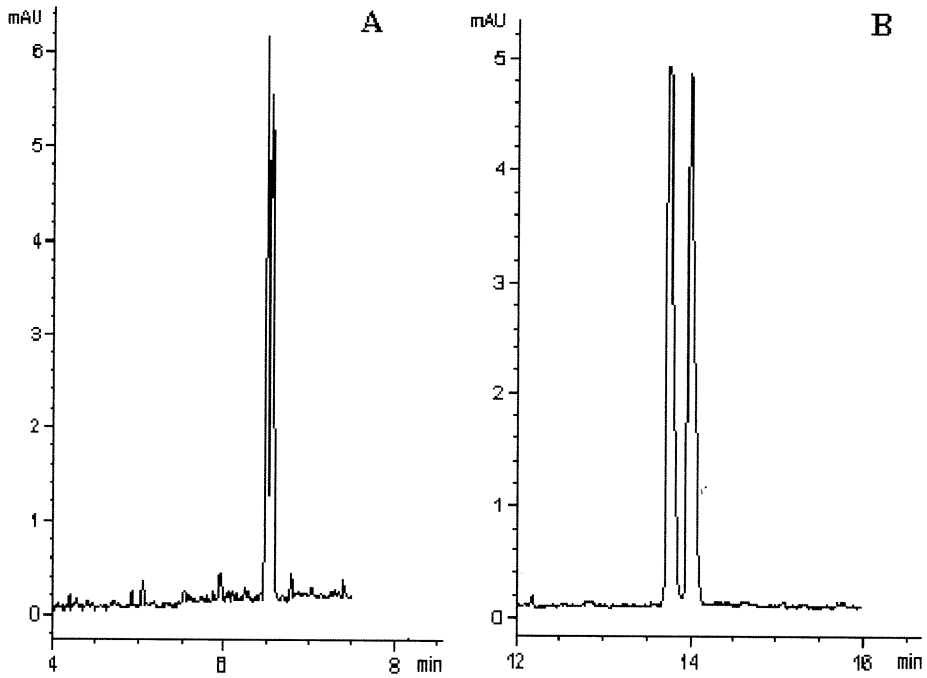

Fig. 2. Effect of buffer pH on separation: (A) pH 5; (B) pH 2.5.

quantitative techniques such as HPLC. Generally, aspects such as accuracy, precision and linearity are evaluated during validation [9–11].

2.6.1. Linearity

In order to determine linearity, three calibration curves with six standards of concentrations of 40, 20, 10, 5, 2 and 1 μg ml^{-1} of salbutamol as racemic mixture (which is equivalent to 20, 10, 5, 2.5, 1 and 0.5 μg ml^{-1} of each enantiomer) were prepared. These standards were injected in duplicate (in 3 different days) and the peak area obtained was plotted against concentration. Regression linear analysis were then performed and correlation coefficients obtained for each enantiomer. Additionally, the response factors, defined as the relationship between peak area and concentration, were calculated.

2.6.2. Limit of quantitation

The limit of quantitation was calculated as the sample concentration which produces a peak with a height 6 times the level of the baseline noise.

2.6.3. Precision

The precision of a method is defined as the closeness of agreement between independent test results obtained under prescribed conditions.

2.6.3.1. Repeatability. A repeatability assay was performed on several aliquots of a homogeneous sample analyzed independently from the beginning (sample preparation) to the end (results acquisition), and was conducted by the same analysts on the same instrument.

Six replicates were used for this analysis, at three levels of concentration: 30, 8 and 3 μg ml^{-1} (15, 4 and 1.5 μg ml^{-1} of each enantiomer), high, medium and low for the standard concentration range assayed.

2.6.3.2. Reproducibility. To assess reproducibility, three different quality control racemic concentrations of 30, 8 and 3 μg ml^{-1} (corresponding to 15, 4 and 1.5 μg ml^{-1} of each enantiomer) (high, medium and low) were injected in duplicate (in 3 different days) with the standard concentrations,

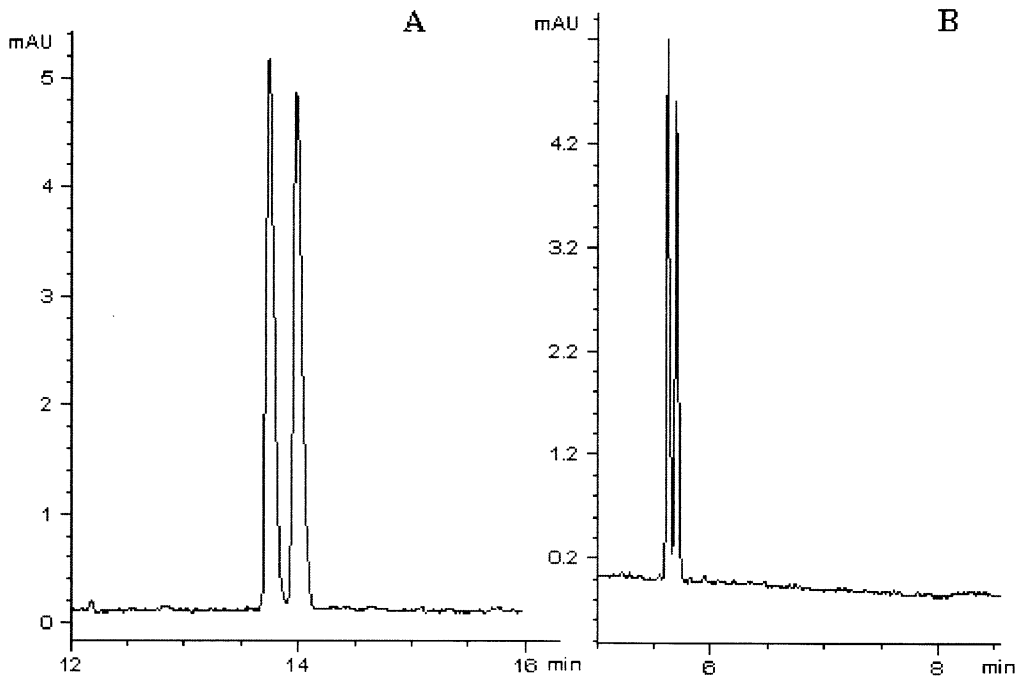

Fig. 3. Effect of the applied field: (A) 15 kV; (B) 30 kV.

and their concentration was calculated by interpolating on the standard curve.

2.6.4. Accuracy

The accuracy of a method is defined as the closeness of agreement between the test results and the accepted reference value. Accuracy was determined by calculating the mean recovery for the found concentration as a percentage of the nominal concentration in standard samples. To determine accuracy, three levels of concentration, 30, 8 and 3 μg ml^{-1} (15, 4 and 1.5 μg ml^{-1} of each enantiomer), were injected in triplicate, and the concentration was calculated by interpolating on the standard curve injected the same day.

To test the acceptability of the accuracy, a Student's *t*-test was applied to examine the statistical significance of the difference as regards to the theoretical value. The $t_{experimental}$ value was calculated by means of the following equation:

$$t_{experimental} = \frac{|100 - R|n^{1/2}}{CV}$$

where: R is mean recovery; n is number of determinations; and CV is the coefficient of variation.

2.7. Release tests

Hydrophilic matrices were elaborated according to a previously described method [12]. The release test (six replicates) were performed in water (500 ml, 37°C), using USP 23 apparatus 1 at 100 rpm. Samples (1 ml) were drawn at fixed intervals, filtered and assayed by the HPCE method.

In order to understand the mechanism of drug release from these matrices, dissolution curves were fitted according to the non-linear equation [13]:

$$\frac{M_t}{M_\infty} = Kt^n$$

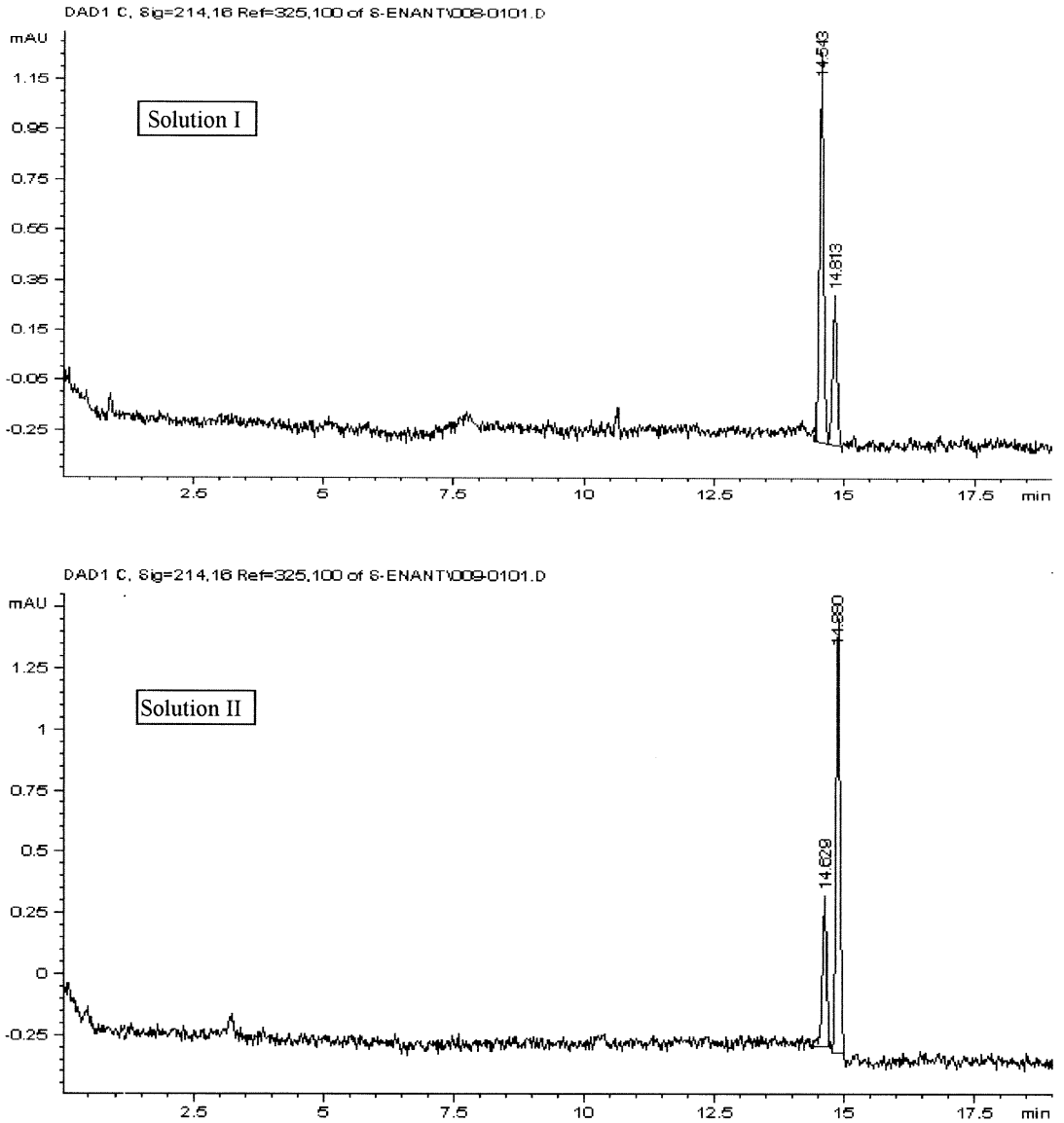

Fig. 4. Electropherograms obtained for solution I and II containing both 40 μg ml^{-1} of racemic salbutamol and 20 μg ml^{-1} of (*R*)-enantiomer and (*S*)-enantiomer, respectively.

where M_t/M_∞ is the fraction of drug released up to time t, K is a constant incorporating the structural and geometric characteristics of the release device, and n is the release exponent indicative of the release mechanism. The curves were fitted with WinNonlin [14], a non-linear regression analysis program.

3. Results and discussion

3.1. Effect of the type of cyclodextrin on enantiomeric separation

Fig. 1 shows the effect of the type of cyclodextrin on enantiomeric separation. The size of the

Table 1
Results obtained in the linearity assay

Nominal concentration (μg ml^{-1})	Concentration found (μg ml^{-1})			Response factor
	Day 1	Day 2	Day 3	
(*R*)-Salbutamol				
20	22.62	23.36	23.11	1.17
10	11.20	11.26	11.07	1.12
5	5.37	5.55	5.37	1.09
2.5	2.46	2.63	2.60	1.01
1	1.13	1.27	1.25	1.18
0.5	0.70	0.65	0.68	1.28
(*S*)-Salbutamol				
20	22.89	23.43	23.24	1.18
10	11.16	11.12	11.15	1.11
5	5.56	5.60	5.52	1.11
2.5	2.63	2.62	2.55	1.04
1	1.08	1.32	1.28	1.18
0.5	0.62	0.67	0.68	1.24

CD cavity and hydrophobic nature can be crucial to the success of the separation [5]. If it is too small, neither enantiomer will be able to enter the cavity; if it is too large, both enantiomers will be able to enter the cavity undifferentiated and no separation will occur [15]. Chemical modification of the CD exterior can affect the enantioselectivity of guest–host complex formation since they 'stretch' the cavity mouth and can change the steric qualities of the host/guest interaction [5]. According to several authors [3], α-cyclodextrin is more useful in enantiomeric separations of molecules possessing a benzyl group. On the other hand, β-cyclodextrin allows separation of compounds with a naphthyl group. Our study only obtained the enantiomeric separation of salbutamol using heptakis (2,6-di-*O*-methyl)-β-cyclodextrin, whereas no enantiomeric separation could be obtained for salbutamol using α- or β-CD.

3.2. Effect of pH

Fig. 2 shows the effect of pH on separation. The pH of the mobile phase influences the ionic state at the capillary wall, thereby altering both electro-osmotic flow and solute–capillary wall interactions [5]. A basic drug separation is better in an acid pH in which the electro-osmotic flow is low. That is why there are no interactions between the complex CD–solute and the capillary wall; the latter migrates toward the cathode at electro-osmotic flow rate, considerably increasing the migration time and improving the resolution (from 1.87 at pH 5 to 3.09 at pH 2.5).

3.3. Effect of the applied field strength

The interaction of the analytes with the CDs (and thus the enantioselectivity) is diffusion-controlled and requires sufficient separation time; a voltage increase when the electro-osmotic flow decreases affects the migration rate of the CD–solute complex. The migration rate of the CD–solute complex is higher and decreases the resolution [6]; as Fig. 3 shows, a 2.40 resolution is obtained at 30 kV, whereas a 3.09 resolution is obtained at 15 kV.

3.4. HPCE final conditions

Under the experimental conditions analyzed (chiral selector, pH and field strength), the best

Table 2
Precision of salbutamol enantiomers determined by the CE method

	Nominal concentration (μg ml^{-1})	Concentration found (μg ml^{-1})	Coefficient of variation (%)
Repeatability			
(*R*)-Salbutamol	15	16.29	1.10
	4	4.15	4.7
	1.5	1.49	7.11
(*S*)-Salbutamol	15	16.29	0.57
	4	4.17	5.84
	1.5	1.49	5.10
Reproducibility			
(*R*)-Salbutamol	15	14.94	1.87
	4	4.08	5.93
	1.5	1.54	0.98
(*S*)-Salbutamol	15	14.92	2.34
	4	4.12	7.58
	1.5	1.55	0.99

resolution was achieved for the two enantiomers of salbutamol with the following conditions: 20 mM heptakis (2,6-di-*O*-methyl)-*β*-cyclodextrin in 40 mM Tris (pH 2.5) and at 15 kV, obtaining a 3.09 resolution with migration times of 13.74 min for (*R*)-salbutamol and 13.98 min for (*S*)-salbutamol, as Fig. 3A shows. The number of theoretical plates was 258 447. Fig. 4 shows the separation of the racemate solution (40 μg ml^{-1}) to which 20 μg ml^{-1} of (*R*)- and (*S*)-salbutamol, respectively, were added. The electropherograms displayed in this figure show the different mobilities of the two enantiomers under the final conditions chosen; (*R*)-salbutamol migrates first.

3.5. Linearity and sensitivity of the method

The determined response was directly proportional to the concentration in the range 1–40 μg ml^{-1} (0.5–20 μg ml^{-1} of each enantiomer), as shown in Table 1. The regression equations obtained from the data for both enantiomers were: $y = 1.1468x - 0.0968$ with a correlation coefficient (r^2) of 0.9993 for (*R*)-salbutamol and $y = 1.1542x - 0.0911$ for (*S*)-salbutamol, with $r^2 = 0.9994$. The coefficients of variation of the slope and intercept were 1.37 and 7.99% for (*R*)-salbutamol and 0.87 and 5.25% for (*S*)-salbutamol, respectively ($n = 3$). The limit of quantitation, calculated as a signal 6 times the background level height, was 1 μg ml^{-1}.

3.6. Precision and accuracy

The results of the precision and accuracy assay are shown in Tables 2 and 3, respectively. In the repeatability assay, the coefficients of variation for three concentration samples in the range 1–40 μg ml^{-1} (0.5–20 μg ml^{-1} of each enantiomer) were between 1.10 and 7.11% for (*R*)-enantiomer and between 0.57 and 5.84% for (*S*)-enantiomer. For reproducibility, the coefficients of variation of the mean values for salbutamol observed over 3 days at concentrations of 30, 8 and 3 μg ml^{-1} (15, 4 and 1.5 μg ml^{-1} of each enantiomer) were 1.87, 5.93 and 0.98%, respectively, for the (*R*)-enantiomer and 2.34, 7.58 and 0.99% for the (*S*)-enantiomer. For these concentrations, the deviations from the amounts added were −0.55, +2.92 and +3.00%, respectively, for (*R*)-enantiomer and −0.38, +2.08 and +2.84% for (*S*)-enantiomer.

The results of the accuracy assay are displayed in Table 3. The mean recovery of salbutamol was 101.34% for (*R*)-enantiomer and 101.79% for (*S*)-enantiomer, with $t_{\text{experimental}}$ values of 0.95 and 1.49, respectively; hence, this analytical method has the required accuracy.

Table 3
Accuracy of salbutamol enantiomers determined by the CE method

	Nominal concentration (μg ml^{-1})	Concentration found (μg ml^{-1})	Mean recovery (%)
(*R*)-Salbutamol	15	14.92	99.45
	4	4.12	102.92
	1.5	1.55	103.00
(*S*)-Salbutamol	15	14.94	99.62
	4	4.08	102.08
	1.5	1.54	102.32

The value of t for $P = 0.05$ and $n-1$ degrees of freedom is 2.306.

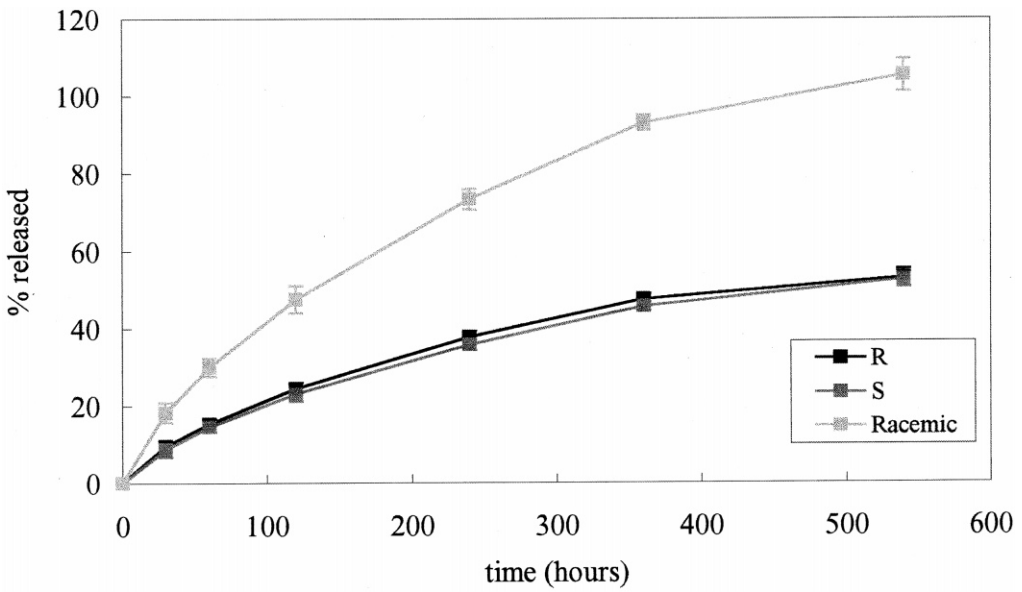

Fig. 5. Salbutamol racemate and its two enantiomers release curves from hydroxypropylmethylcellulose tablets.

3.7. Applicability of the method

Even though nowadays there are numerous studies about the influence of enantioselectivity on drug absorption, distribution, metabolism and excretion processes [16–19], very few publications about enantioselective release from solid dosage forms have been found [20–24]. The capillary zone electrophoresis method developed was later used to measure the percentages of salbutamol enantiomers released in a dissolution assay of salbutamol in hydroxypropylmethylcellulose matrix tablets. The experiment was carried out to determine whether an enantioselective release of salbutamol occurred due to the existence of numerous chiral centers in the structure of this excipient.

Fig. 5 shows the release of salbutamol enantiomers and salbutamol as a racemic mixture from hydroxypropylmethylcellulose matrix tablets. As can be seen in this figure, the release of both enantiomers is equivalent, and the percentage of total salbutamol released is similar to that obtained in previous studies [12,25].

The release curves were fitted to the non-linear equation $M_t/M_\infty = Kt^n$. The values of the dissolution constant K were $1.187 \pm 0.223\%$ min^{-n} for (*R*)-enantiomer, $1.076 \pm 0.268\%$ min^{-n} for (*S*)-

enantiomer and $2.255 \pm 0.455\%$ min^{-n} for the racemic salbutamol. These values were quite similar and, therefore, the salbutamol release from the hydrophilic matrix was not enantioselective. The n values obtained were of 0.631 ± 0.032 for the (R)-enantiomer, 0.642 ± 0.046 for the (S)-enantiomer, and 0.636 ± 0.036 for the racemate. These values obtained for the n exponent were near 0.6, indicating the existence of an anomalous (non-Fickian) transport mechanism for both enantiomers. Similar results were obtained by several authors [20–24] when studying drug release from chiral excipients.

4. Conclusions

A sensitive, selective, accurate and reproducible capillary zone electrophoresis method using heptakis (2,6-di-*O*-methyl)-β-cyclodextrin as a chiral selector has been developed for the analysis of salbutamol enantiomers. This method has been applied to the study of salbutamol enantiomer release from matrix tablets prepared with chiral excipients, as hydroxypropylmethylcellulose. No enantioselective release of the drug from these matrices was observed.

Acknowledgements

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