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Rapid determination of salbutamol in pharmaceutical preparations by chiral capillary electrophoresis

A fast and simple method of chiral capillary electrophoresis (CE) has been applied to the analysis of salbutamol in different pharmaceutical preparations. Using of a 25 mM acetate buffer (pH 5.0), containing 13.1 mg/mL carboxymethyl-β-cyclodextrin (CM-β-CD), an applied voltage of 20 kV and a temperature of 25°C, the enantiomers of salbutamol could be separated in about 2 min. Three different pharmaceutical preparations (two syrups, one oral solution, and two kind of tablets) containing a racemate of salbutamol were injected directly in the CE system, following dilution in dimethyl sulfoxide (DMSO). Appreciable differences in the retention times were observed for salbutamol enantiomers in the different formulations studied, which were attributed to the effect of the matrix components on the electrophoretic mobility. The standard addition method was used for the calibration due to the existence of matrix interferences. Finally, the stability of the enantiomers of salbutamol in the oral solution was studied calculating the enantiomeric ratio values when the solution was injected immediately after being opened in the first case and after being opened and stored in the fridge for two months in the second case.

Keywords: Chiral capillary electrophoresis / Pharmaceutical preparation / Salbutamol
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1 Introduction

Salbutamol is a β_2-adrenoceptor agonist clinically used as bronchodilator for the treatment of patients with asthma, bronchitis, emphysema and, in general, breathing diseases with bronchoconstriction [1]. The pharmacological action of salbutamol resides in the *R*-(−)-enantiomer, which moreover was found to undergo a faster metabolism in men than the *S*-(+)-enantiomer [2]. However, no information is currently available on the toxicity of inactive *S*-(+)-enantiomer and salbutamol is still commercialized and administrated as a racemate [3].

Capillary electrophoresis (CE) is an effective tool for the resolution of enantiomers, which is accomplished by supplying the background electrolyte with a chiral selector capable of discriminating between the enantiomers concerned. In fact, chiral CE has been the subject of much attention and has been applied with success to the enantiomeric separation of different chiral compounds during the last decade [4, 5]. The chiral selectors used for this purpose include cyclodextrins (CDs) and derivatives, chiral crown ethers, noncyclic oligosaccharides and polysaccharides, macrocyclic antibiotics, proteins and peptides, metal complexes, and chiral surfactants [4, 6–10].

The validation of chiral methods of analysis is of obvious importance in pharmaceutical science for determining enantiomeric purity of products, to analyze drugs formulations or to perfom formulation stability studies as it is shown by the great number of works published on this subject [5, 6, 11–18]. In the case of the quantitation of chiral bronchodilators by CE, the developed methods used β-CD derivatives as chiral selectors. Thus, Esquisabel and co-workers [16] obtained the chiral separation of salbutamol using 2,6-di-*O*-methyl-β-CD as chiral selector in 14 min approximately. This chiral method was applied to the study of salbutamol enantiomer release from matrix tablets that contained the racemic drug and a chiral excipient as hydroxypropylmethylcellulose. No enantioselective release of the salbutamol from these matrices was observed. The quantitative determination of salbutamol, clenbuterol, and tulobuterol enantiomers from a spiked sample by CE using sulfated β-CD as chiral selector has been performed by Vela and co-workers in about 20 min

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Abbreviations: ANOVA, analysis of variance; **CM-β-CD**, carboxymethylated β-cyclodextrin

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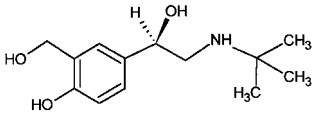
[17]. Finally, a fast enantiomeric separation, in about 2 min, of salbutamol using a neutral β-CD derivative (permethylated β-CD) or a negatively charged β-CD derivative (carboxymethyl-β-CD) as chiral selector was performed recently by our research team [18].

The main objective of this work was the application of the fast and simple chiral CE method developed by our research team for the chiral separation of salbutamol [18] to the determination of this compound in different pharmaceutical preparations where it was used as a racemate. Furthermore, since there is no information concerning the unstability of one enantiomer of salbutamol with respect to the other one in pharmaceutical formulations, the evaluation of the stability of these enantiomers in an oral solution was also performed.

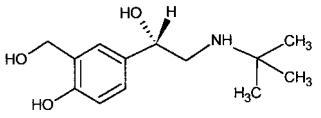
2 Materials and methods

2.1 Chemicals and samples

All reagents were of analytical grade. Dimethyl sulfoxide (DMSO), and sodium hydroxide were supplied from Merck (Darmstadt, Germany); hydrochloric acid was purchased from Panreac (Barcelona, Spain); CM-β-CD (degree of substitution 3) was obtained from Cyclolab (Budapest, Hungary). Water used to prepare solutions was purified through a Milli-Q system from Millipore (Bedford, MA, USA). All solutions were filtered through 45 μm pore size disposable nylon filters from Scientific Resources (Eatontown, NJ, USA). Salbutamol was purchased from Sigma (St. Louis, MO, USA). The structure of the enantiomers of this basic drug is shown in Fig. 1. Different pharmaceutical preparations containing salbutamol sulfate were acquired in pharmacy shops at Alcalá de Henares (Madrid, Spain). Table 1 shows the composition of the different pharmaceutical preparations studied in this work.



R(-)-Salbutamol



S(+)-Salbutamol

Figure 1. Structure of the enantiomers of salbutamol.

Table 1. Composition of the different pharmaceutical preparations studied in this work

Pharmaceutical preparation	Composition
Syrup (A)	Salbutamol sulfate, sodium saccharine and excipients (sodium citrate, citric acid monohydrate, hydroxypropylmethylcellulose, sodium benzoate, orange flavor, sodium chloride, and purified water)
Syrup (B)	Salbutamol sulfate and excipients (sodium benzoate, hypromelose, sodium saccharine, citric acid, sodium citrate, orange flavor, and purified water)
Oral solution (C)	Salbutamol sulfate and excipients (sodium benzoate, citric acid monohydrate, sodium saccharine, sodium citrate, sodium chloride, aroma of raspberry, and water)
Tablet (D)	Salbutamol sulfate and excipients (corn starch, polyvinylpyrrolidone, cellulose, sodium starch caboxymethylated, silicium dioxide, talc, magnesium estearate)
Tablet (E)	Salbutamol sulfate and excipients (sodium chloride, povidone, sodium croscarmellosa, silicagel, magnesium estearate, cellulose acetate, methylhydroxypropylcellulose, titanium dioxide, hydroxypropylcellulose, lacas rubber, industrial methylated spirit, 2-etoxyiethanol, *n*-butyl alcohol, aluminium laca of lipstick, and polydimethylsiloxano)

2.2 Apparatus

An HP^{3D} CE system (Hewlett-Packard, Waldbronn, Germany) equipped with an on-column diode array detector (DAD) and an HP 3D-CE Chemstation software was used. An uncoated fused-silica capillary from Composite Metal Services (Worcester, England) with 50 μm inner diameter (ID) and 375 μm outer diameter (OD) with an effective length of 25 cm (33.5 cm total length) was employed. Capillary temperature was set to 25°C and UV detection was performed at 230 nm. Separation voltage was 20 kV. Electrolytic solutions were degassed in an ultrasonic bath KM from Raypa (Barcelona, Spain). A 654 pH meter from Metrohm (Herisau, Switzerland) was employed to adjust the pH of the separation buffer.

2.3 Procedure

Electrolytic solutions were prepared weighing and dissolving the appropriate amount of buffer and CM-β-CD in Milli-Q water to obtain the required concentration and

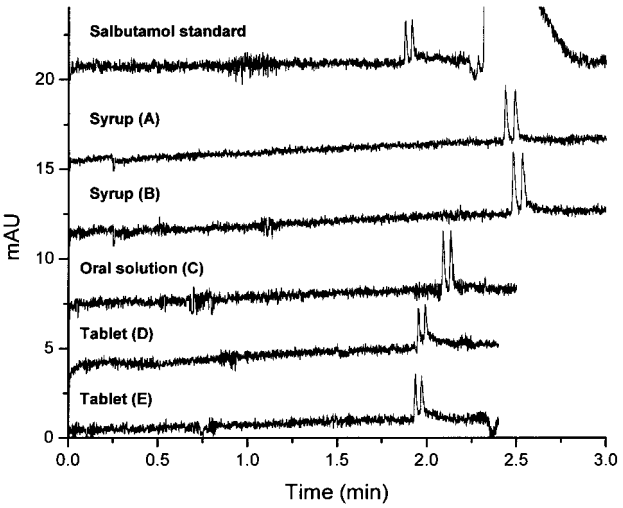


Figure 2. Separation of salbutamol enantiomers in a standard solution (0.045 mg/mL) and in dilutions of the five pharmaceutical preparations studied in this work (0.045 mg/mL in salbutamol) using the chiral CE method. Experimental conditions: 25 mM acetate buffer (pH 5) with 13.1 mg/mL CM-β-CD; temperature, 25°C; applied voltage, 20 kV; UV detection at 230 nm; injection by pressure, 30 mbar for 2 s sample followed by 30 mbar for 2 s buffer; 50 μm ID, 375 μm OD capillary of 33.5 cm length (25 cm to the detector).

adjusting the pH to the desired value with a 1 M hydrochloric acid solution and 0.1 M sodium hydroxide. Standard solutions of salbutamol used for the calibration by the external standard method were prepared by diluting with DMSO a stock solution of 10 mg/mL of salbutamol in DMSO to obtain final concentrations comprised from 0.005 to 0.120 mg/mL (0.005, 0.010, 0.020, 0.030, 0.045, 0.060, 0.080, 0.100, and 0.120 mg/mL). The determination of the salbutamol content in the different pharmaceutical preparations (two syrups, an oral solution, and two tablets) required a dilution of each formulation in a final volume of DMSO to achieve a final concentration of 0.015 mg/mL in salbutamol. For the calibration by the standard additions method, three increasing concentrations of salbutamol standard (0.010, 0.030, and 0.060 mg/mL) were added to a solution of the pharmaceutical preparation with a concentration of 0.015 mg/mL of salbutamol. The four resulting solutions were directly injected in the electrophoretic system. Nevertheless, in the case of the tablets it was necessary to include a centrifugation step to separate the insoluble excipients from the solution before the injection of the solution in the CE system. Between injections, the capillary was washed with 0.1 M sodium hydroxide (10 bar for 0.2 min), followed by the running buffer (10 bar for 0.4 min). The injection was made by pressure: 30 mbar for 2 s of sample followed by 30 mbar for 2 s of separation buffer.

2.4 Data treatment

All the experimental data were manipulated using Excel 7.0 from Microsoft Office software [19]. Regression analysis, analysis of variance (ANOVA) and comparison of regression lines for significantly different slopes and intercepts were made using Statgraphics Plus software [20].

3 Results and discussion

3.1 Separation of the enantiomers of salbutamol in different pharmaceutical preparations

In a previous work it was reported that the fast enantiomeric separation of salbutamol, in about 2 min is possible, using 25 mM acetate buffer (pH 5) containing 13.1 mg/mL CM-β-CD at a temperature of 25°C and an applied voltage of 20 kV in a capillary of 25 cm of effective length [18]. These conditions have been applied in this work to the fast chiral analysis of salbutamol in different pharmaceutical preparations: two syrups, an oral solution, and two kind of tablets. Figure 2 shows the electropherograms corresponding to a standard solution of salbutamol (0.045 mg/mL) and to five dilutions in DMSO of the different pharmaceutical preparations studied containing 0.045 mg/mL of salbutamol sulfate. It was observed that a mixture of the two enantiomers of salbutamol was detected in all cases and no interfering peaks were observed. In addition, a similar enantiomeric resolution for the salbutamol standard and the salbutamol contained in the different pharmaceutical preparations was observed. However, an increase in the migration times of the enantiomers of salbutamol detected in some of the different pharmaceutical preparations was observed with respect to the migration times of the enantiomers of the salbutamol standard. This result could be attributed to the effect of matrix components on the electroosmotic flow (EOF). According to Table 1 both syrups have a similar composition, being the difference that syrup (A) contain hydroxypropylmethylcellulose and sodium chloride instead of the hypromelose contained in syrup (B). Furthermore, the migration times observed for the salbutamol enantiomers of the oral solution are more similar to the standard solution than those of the syrups, probably due to the absence of compounds such as hydroxypropylmethylcellulose or hypromelose which can interact with the inner wall of the capillary when they are injected in the CE system [21]. On the other hand, although both kind of tablets studied have a more complex composition than the syrups or the oral solution studied, migration times of the enantiomers of salbutamol in these pharmaceutical preparations are similar to the migration times of

the enantiomers of the salbutamol standard and similar between them, although their composition is also very different. These results could be explained taking into account the insolubility observed for some excipients when these tablets were dissolved in DMSO and which were separated from the solutions injected in the CE system by centrifugation. After these results, we checked if the chiral CE method was useful for the determination of the salbutamol content in the different pharmaceutical preparations considered.

3.2 Quantitation of salbutamol in pharmaceutical preparations

The quantitation of salbutamol by the chiral CE method was performed in different pharmaceutical preparations: two syrups, an oral solution, and two kind of tablets. For the calibration, corrected total peak areas, calculated as the addition of the corrected areas corresponding to the first and the second migrating enantiomers were used (the corrected area for each enantiomer was obtained by dividing the area of each electrophoretic peak by its corresponding migration time). Although the use of corrected areas in CE is frequent [22], in this case it was totally necessary in order to increase reproducibility of calibration data and also to compensate fluctuations in electrophoretic conditions due mainly to the compositions of the different samples injected and also due to the pH of the separation buffer (pH 5.0 is a critical value where small variations of pH values produce big differences in the electrophoretic mobility) [23].

Solutions of salbutamol standard with concentrations ranging from 0.005 to 0.400 mg/mL were injected by triplicate in order to determine the linear concentration range. It was observed a linear relationship between the total corrected area and the concentration of salbutamol in the concentration range between 0.005 and 0.120 mg/mL. Therefore, solutions of salbutamol standard comprised from 0.005 to 0.120 mg/mL were prepared and injected by triplicate during three different days in the CE system in order to validate the calibration curve obtained by plotting corrected total peak area *versus* concentration. The equation obtained when the average of the corrected total peak area for the three lines considered *versus* the concentration was plotted is $y = 0.1871 + 0.0362x$ being the standard error associated to the intercept 0.0289, to the slope 0.0004, and to the calibration curve 0.0515. The ANOVA of the average of the three calibration lines considered revealed that the lack of fit was always statistically smaller than the pure error, which confirmed that a straight line was a suitable model in the concentration range employed. In addition, a correlation coefficient equal to 0.9995 indicated a relatively strong relationship between the variables. On the other hand, the sensitivity of this chiral CE method, corresponding to the slope of the calibration line, was 0.0362 $mL \cdot \mu g^{-1}$ (0.0362×10^{-3} $mL \cdot mg^{-1}$).

The slope and the standard error of the calibration curve were used to calculate the limit of detection (LOD) and the limit of quantitation (LOQ). LOD and LOQ were defined as the analyte concentrations given signals exceeding that of the intercept by 3 and 10 times, respectively, the standard error of the calibration curve [24]. The LOD calculated for salbutamol was about 4 μg/mL and the LOQ was about 14 μg/mL.

In order to evaluate the precision of the CE method (see Table 2), repeatability and reproducibility were studied. The repeatability in migration time and peak area of the enantiomers and in the corrected total peak area, was determined (as RSD) for ten consecutive injections of a dilution in DMSO of each pharmaceutical preparation with a final concentration of 0.045 mg/mL in salbutamol. RSD was less than 5.0% for the migration times of the enantiomers, less than 5.4% for peak area of the enantiomers, and less than 4.8% for corrected total peak area. On the other hand, reproducibility in peak area and migration time of the enantiomers and in the corrected total peak area was measured as the RSD obtained in four different days (injections by triplicate) for sample dilutions of 0.045 mg/mL in salbutamol. As it can be observed in Table 2, the RSD values obtained were less than 5.5% for the migration times of the enantiomers, less than 6.5% for the peak area of the enantiomers, and for the corrected total peak area RSD was less than 6.0%. It should be emphasized that RSD values for the corrected total peak area are lower than those obtained for the peak area, that is, the precision obtained for the corrected total peak areas is better than those obtained with noncorrected peak areas, therefore, corrected peak areas were used for the calibration.

Prior to the quantitative analysis, the presence or absence of matrix interferences was studied. Then, the slopes of the regression lines obtained by the external standard method and the standard addition method, which was applied to the different pharmaceutical preparations, were compared. Table 3 shows the calibration lines obtained for the variation of the corrected total peak area as a function of the concentration of salbutamol standard by the external standard method and as a function of the added concentration of salbutamol to the sample by the standard addition method. In addition, each calibration line was obtained as the average of three calibration lines obtained in three different days and each one validated by ANOVA. The comparison of these two regression lines obtained as explained above for each

Table 2. Precision in peak areas and migration times for salbutamol enantiomers and in corrected total peak area for salbutamol in five different pharmaceutical preparations by the CE method[a)]

Precision	Pharmaceutical preparation	C_s[b)]	t_1 (RSD)	t_2 (RSD)	A_1 (RSD)	A_2 (RSD)	A_t (RSD)
Repeatability[c)]	Syrup (A)	0.045	2.62 (3.06)	2.69 (3.13)	1.40 (3.24)	1.38 (3.50)	1.05 (3.39)
	Syrup (B)	0.045	2.64 (4.97)	2.71 (4.28)	1.17 (3.78)	1.16 (3.79)	0.87 (2.41)
	Oral solution (C)	0.045	2.13 (1.48)	2.18 (1.51)	1.24 (3.49)	1.17 (3.76)	1.12 (4.76)
	Tablet (D)	0.045	2.02 (2.82)	2.06 (2.86)	0.91 (5.43)	0.91 (4.90)	0.89 (3.73)
	Tablet (E)	0.045	1.92 (1.74)	1.97 (1.77)	1.00 (3.58)	1.01 (4.12)	1.03 (3.10)
Reproducibility[d)]	Syrup (A)	0.045	2.48 (3.87)	2.54 (3.94)	1.33 (4.13)	1.30 (5.99)	1.05 (4.76)
	Syrup (B)	0.045	2.60 (4.42)	2.67 (4.48)	1.25 (6.49)	1.20 (6.16)	0.93 (3.63)
	Oral solution (C)	0.045	2.14 (1.65)	2.18 (1.52)	1.17 (6.19)	1.20 (4.01)	1.10 (6.00)
	Tablet (D)	0.045	1.99 (5.45)	2.02 (5.17)	0.93 (4.46)	0.93 (5.67)	0.93 (4.83)
	Tablet (E)	0.045	1.89 (2.98)	1.94 (3.35)	0.95 (4.37)	0.94 (4.79)	0.99 (3.88)

t_1 and t_2 are the migration times corresponding to the first and the second migrating enantiomers, respectively; A_1 and A_2 are the peak areas for the first and the second migrating enantiomers, respectively; A_t is the corrected total peak area $((A_1/t_1)+(A_2/t_2))$ and the RSD value was calculated with the uncertainty of this mathematic operation [21].

a) Experimental conditions specified in Section 2.3

b) Concentration of salbutamol in a solution prepared by dilution of the commercial preparation (mg/mL)

c) RSD values determined for ten consecutive injections

d) RSD values determined for four different days (each injection was made by triplicate)

Table 3. Comparison of the calibration lines[a)] obtained by the external standard method and the standard addition method for the different pharmaceutical preparations studied

Pharmaceutical preparation	External standard method[b)]	Standard addition method[c)]
Syrup (A)	$y = 0.1871 + 0.0362x$ ($n = 9$, $r = 0.9995$)	$y = 0.7356 + 0.0420x$ ($n = 4$, $r = 0.9997$)
Syrup (B)	$y = 0.1871 + 0.0362x$ ($n = 9$, $r = 0.9995$)	$y = 0.7606 + 0.0497x$ ($n = 4$, $r = 0.9991$)
Oral solution (C)	$y = 0.1871 + 0.0362x$ ($n = 9$, $r = 0.9995$)	$y = 0.7099 + 0.0473x$ ($n = 4$, $r = 0.9999$)
Tablet (D)	$y = 0.1871 + 0.0362x$ ($n = 9$, $r = 0.9995$)	$y = 0.6913 + 0.0404x$ ($n = 4$, $r = 0.9999$)
Tablet (E)	$y = 0.1871 + 0.0362x$ ($n = 9$, $r = 0.9995$)	$y = 0.6833 + 0.0417x$ ($n = 4$, $r = 0.9995$)

a) Calibration lines obtained as the average of three calibration curves obtained in three different days (n, number of points considered for the calibration curve; r, correlation coefficient)

b) Concentration range for the salbutamol standard: 0.005–0.120 mg/mL

c) Concentration range for the salbutamol standard added to the pharmaceutical preparations (dilution containing 0.015 mg/mL of salbutamol sulfate): 0–0.06 mg/mL

calibration method and for each pharmaceutical preparation revealed that statistically significant differences ($P<0.05$) among the slopes were observed for all the pharmaceutical preparations studied. Then, the presence of matrix interferences was detected being necessary to use the standard addition method for the quantitation of salbutamol.

The salbutamol content in the different pharmaceutical preparations studied was determined from a standard addition line obtained as average of three calibration lines obtained in three different days and validated by ANOVA for each pharmaceutical preparation. Table 4 groups the salbutamol content determined by the chiral CE method and the salbutamol content calculated taking into account the salbutamol content decleared in the label of the product and the dilution in DMSO. Results show that differences in both values obtained for the syrup (B) and the oral solution (C) were 2 and 0%, respectively, whereas for the other three pharmaceutical preparations studied (syrup (A) and tablets (D) and (E)) these differences were about 17, 14, and 9%, respectively.

Finally, as the interconversion of enantiomers of a chiral compound can occur with time [11] and their stability can be different, it seems interesting to test the change in the proportion of the enantiomers in stored pharmaceu-

Table 4. Quantitative analysis of salbutamol in different pharmaceutical preparations by the chiral CE method[a)]

Salbutamol content by CE (mg/mL)[b)]	Pharmaceutical preparation	Decleared salbutamol content in pharmaceutical preparation (mg/mL)[c)]
17.5×10^{-3}	Syrup (A)	15.0×10^{-3}
15.3×10^{-3}	Syrup (B)	15.0×10^{-3}
15.0×10^{-3}	Oral solution (C)	15.0×10^{-3}
17.1×10^{-3}	Tablet (D)	15.0×10^{-3}
16.4×10^{-3}	Tablet (E)	15.0×10^{-3}

a) Experimental conditions specified in Section 2.2
b) Concentration of salbutamol determined by the chiral CE method
c) Concentration of salbutamol obtained by dilution of the pharmaceutical preparations considering the salbutamol content indicated in the label of each preparation

tical preparations with a rapid and simple method as used in this work. The oral solution (C) studied in this work is prepared by the patient from a powder by dissolving it in water and storing it in a fridge for consumption to a maximum of ten days. The chiral method was applied to study the stability of the salbutamol enantiomes in this oral solution. In this case, the enantiomeric ratio values for the oral solution (C) were calculated in order to study if there was variation in the proportion of the enantiomers of salbutamol when the oral solution was injected after being opened and after being opened and stored in the fridge for two months. The enantiomeric ratio was calculated as the corrected area of the second migrating enantiomer divided by the corrected area of the first migrating enantiomer. An enantiomeric ratio value of 0.92 was obtained when dilutions in DMSO of the oral solution (C) containing 0.040 mg/mL in salbutamol was opened and immediately injected in the CE system, and a value of 0.98 was obtained when dilutions were analyzed after the formulation was opened and stored for two months. Since the differences between both values of enantiomeric ratio can be considered within the experimental error, the results indicated that the racemate has been remained after the oral solution (C) was opened and stored during a long time.

4 Concluding remarks

The application of a fast and simple chiral CE method based on the use of a 25 mM acetate buffer at pH 5 with 13.1 mg/mL CM-β-CD at 25°C and 20 kV as the separation voltage for the quantitation of salbutamol in different pharmaceutical preparations (two syrups, an oral solution, and two kinds of tablets) containing a racemate of salbutamol was performed in this work. Although differences in the migration times were observed for the different formulations studied, which were attributed to the influence of their different composition on the electrophoretic mobility, the use of corrected total peak area enabled to compensate the fluctuations in the electrophoretic conditions. The chiral CE method was characterized by a linear concentration range from 0.005 to 0.120 mg/mL of salbutamol, LOD of salbutamol about 4 μg/mL, and acceptable values of precision (repeatability and reproducibility) in terms of migration time, peak area, and corrected total peak area. For the quantitation of salbutamol in the different pharmaceutical preparations studied the standard additions method was used for the calibration due to the existence of matrix interferences for a 95% confidence level. Finally, the stability of the enantiomers in the oral solution was studied calculating the enantiomeric ratio values obtained when it was injected after being opened and after being opened and stored in the fridge for two months obtaining no significant differences.

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