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Short communication

Determination of salbutamol in syrups by capillary electrophoresis with contactless conductivity detection (CE-C^4D)

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Abstract

This paper describes the separation and quantification of salbutamol in pharmaceutical products (salbutamol syrups) by capillary electrophoresis (CE) with contactless conductivity detection (C^4D). The system was studied by micellar electrokinetic capillary chromatography (MEKC) and free solution capillary electrophoresis (FSCE), being the latter chosen in function of best resolution and sensitivity in comparison with the MEKC method. CE-C^4D was applied to analysis of salbutamol in syrups utilizing 1.0×10^{-2} mol L^{-1} acetic acid/sodium acetate buffer (pH 4.9) as running electrolyte. Tetraethylammonium (TEA) solution was used as internal standard. The results obtained include a linear dynamic range from 7.0×10^{-5} to 3.0×10^{-4} mol L^{-1} and good repeatability (R.S.D. = 4.7% for $n = 10$ for a 7.0×10^{-5} mol L^{-1} salbutamol solution). The detection limit was calculated as 1.0×10^{-5} mol L^{-1} and the limit of quantification was estimated as 3.3×10^{-5} mol L^{-1}. For syrups analysis the reproducibility presented deviations between 1.5% and 2.5% (three different days) obtained for measurements in triplicate.
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1. Introduction

Salbutamol α^1-[(*tert*-butylamino) methyl]-4-hydroxy-*m*-xylene-α-α′-diol is a β_2-selective adrenoreceptor agonist, which actuates as a pronounced bronchodilatory, cardiac, uterine and metabolic agent. It is administered in a variety of ways, including tablets or syrup; inhalation by aerosol, injection and it is normally used in the sulfate form. Moreover, β_2-selective adrenoreceptors are drugs that potentially produce a certain amount of anabolic-like effects, depending on their route of administration. For that reason, the International Olympic Committee (IOC) restricts the use of salbutamol (albuterol) in athletes only by inhalation and, even then, it must be declared in writing to the relevant medical authority prior to the competition. Higher doses of this drug may have lipolytic effect and residues of these compounds can be toxic to humans [1–3].

So far the analytical methods reported for the determination of salbutamol in pharmaceutical formulations and biological samples include: chromatography [4,5], spectrophotometry [6–9], fluorescence and chemiluminescence [10–12], immunobiosensor [13], potentiometry [14–16], conductometry [17] and amperometry coupled with batch injection analysis (BIA) [18]. Many of these techniques are expensive or require time-consuming derivatization steps.

Capillary electrophoresis (CE) has proved to be a powerful analytical technique in several applications, including pharmaceutical and biomedical areas. There is a family of CE techniques utilized to separate and detect a diversity of compounds. The versatility and number of ways that the capillary electrophoresis can be employed suggest that almost all molecules can be separated using this powerful technique. β-Agonists, for example, has been analyzed by CE directly [19,20], utilizing solid-phase extraction (SPE) or using beta-cyclodextrin (beta-CD) [19–27] or another chiral selector [28].

In this paper is presented a new way to determine salbutamol in pharmaceutical products (syrups) by using CE with

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capacitively coupled contactless conductivity detection (C^4D). The basic idea of this detection technique is to apply an alternate voltage over a couple of cylindrical electrodes positioned outside the capillary and measure the current that flows through them. This current is a function of the solution conductivity at the gap between the electrodes [29,30]. Our and other groups have employed this technique for many applications in different matrices during the last decade [31–38]. In present study, this technique was directly applied to determine salbutamol (without any pre-treatment) rapidly and precisely in complex samples.

2. Experimental

2.1. Reagents and solutions

Standard salbutamol sulfate was kindly donated by Boehringer Ingelheim from Brazil and was utilized without further purification. Other reagents were of analytical grade purchased from Sigma or Merck and the solutions were prepared with deionised water from a Millipore® Milli-Q Plus system. Salbutamol must be stored in the dark and at low temperatures. The pharmaceutical products (syrups) utilized in this study were acquired in a local drugstore. Pre-treatment of the samples was not necessary. Just before the measurements, the samples were conveniently diluted with the running electrolyte (1.0×10^{-2} mol L^{-1} acetic acid/sodium acetate buffer (pH 4.9)).

Suitable dilutions of a 1.0×10^{-2} mol L^{-1} salbutamol stock solution were performed in order to obtain analytical standard solutions employed in the experiments. The running electrolyte chosen for the analyses was a 1.0×10^{-2} mol L^{-1} acetic acid/sodium acetate buffer (pH 4.9) while a 3.0×10^{-4} mol L^{-1} tetraethylammonium (TEA) solution was used as internal standard. Previously, other buffer solutions were investigated, searching for the best medium for salbutamol analysis in syrups, namely sodium phosphate pH 12.1 with 2.0×10^{-4} mol L^{-1} cetyltrimethylammonium bromide (CTAB) for reversion of the electroosmotic flow and a solution of boric acid + potassium hydroxide with a final pH 9.0, using TEA (3.0×10^{-4} mol L^{-1}) as the internal standard, both exploring the free solution capillary electrophoresis (FSCE) condition. Experiments of MEKC using 3.0×10^{-2} mol L^{-1} sodium borate solution (pH 9.4) and 5.0×10^{-2} mol L^{-1} sodium dodecylsulfate (SDS) were also carried out.

2.2. Capillary electrophoresis apparatus

The CE equipment interfaced to a microcomputer was built in our laboratory and details about the construction and the detector developed have been reported elsewhere [39,40]. The fused silica capillary (50 μm inner diameter, 375 μm outer diameter and 63 cm length) employed was purchased from Agilent Technologies (São Paulo, Brazil). The contactless conductometric detector was positioned at 53 cm from the beginning of the capillary. The capillary was conditioned by this sequence of 20 min flushes: water, then 0.1 mol L^{-1} NaOH, one more time water and after that the running electrolyte. Before the first injection, high voltage (28 kV) was applied for approximately 20 min. The capillary was flushed between runs with electrolyte solution for 5 min. During the experiments the temperature inside the instrument was maintained at 25 ± 1 °C.

The introduction of samples in the capillary was done exploring the hydrodynamic pressure generated by the elevation of the cathode vessel up to 10 cm and staying in that height for 25 s (FSCE mode) or for 30 s (MEKC mode). The detector was operated at 600 kHz and the most favorable separation voltage was experimentally determined for each of the different electrolytes studied. In this type of detection the electrodes are positioned outside of the capillary and high frequencies (typically 10^5–10^6 Hz) are used to reduce the effects due to the high impedance of the capillary wall. Contactless conductometric detector response is due to the difference between electrolyte and analyte conductances [29,30]. For this reason, this detection mode is being considered an interesting tool for analytical purposes.

3. Results and discussion

As mentioned above, different systems were employed in the investigation involving the salbutamol detection. The first experiments were performed in borate buffer pH 9.0 and it was observed that the apparent mobility of the species and of the electroosmotic flow (EOF) were similar (Fig. 1), indicating that in such conditions the net charge of the species is nearly null (it is in the zwitterionic form, close to isoelectric point, and also it does not complex with borate to form an anionic species). The conclusion was that at this pH the separation was not favorable, since a co-elution with the EOF marker occurs.

On the other hand, the borate buffer could be employed in the salbutamol separation by MEKC once on that circumstance this electrolyte confers a condition of neutrality. For this experiment, more concentrated solutions of the borate buffer were used in order to obtain an improved signal/noise ratio in comparison with the experiments performed in FSCE. The potential

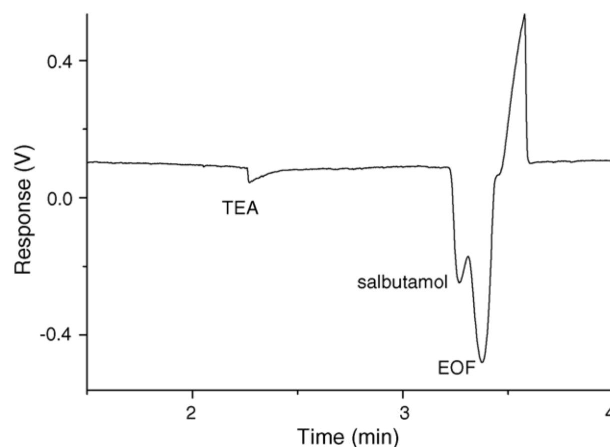

Fig. 1. Electropherogram obtained for salbutamol by using borate buffer 5.0×10^{-3} mol L^{-1} (pH 9.0). Concentration of the species: 3.0×10^{-4} mol L^{-1}. Separation voltage: 25 kV.

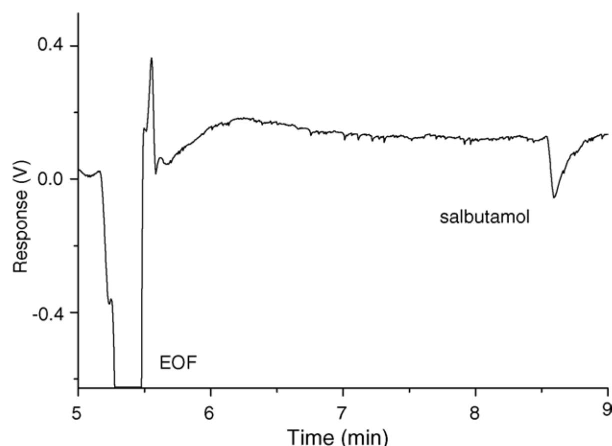

Fig. 2. Electropherogram obtained for salbutamol by using borate buffer 3.0×10^{-2} mol L^{-1} (pH 9.4) + SDS 5.0×10^{-2} mol L^{-1}. Concentration of salbutamol: 3.0×10^{-4} mol L^{-1}. Separation voltage: 15 kV.

of separation had to be significantly decreased owing to higher conductivity of the electrolyte. A consequence of this change was an increase in the running time (Fig. 2).

The detection of salbutamol was also achieved by using phosphate buffer pH 12.1 (Fig. 3). The counter-ion selected was sodium. In this case, the sensitivity is determined by the difference between the mobility of the anionic species of the electrolyte (HPO_4^{2-}, PO_4^{3-} and OH^-) and of the analyte [35]. Usually, the salbutamol sulfate is the form utilized in the syrups manufacture. An interesting characteristic in the detection of salbutamol as anion is the possibility to determine sulfate simultaneously. In addition, sugars could also be analyzed in the same syrup sample if suitable dilution is performed. The drawback of this condition was the long time necessary for each analysis.

Finally, separation of dissociated salbutamol was carried out in acetate buffer pH 4.9 in which the analyte shows positive charge. In such a running electrolyte the shape of the peaks of

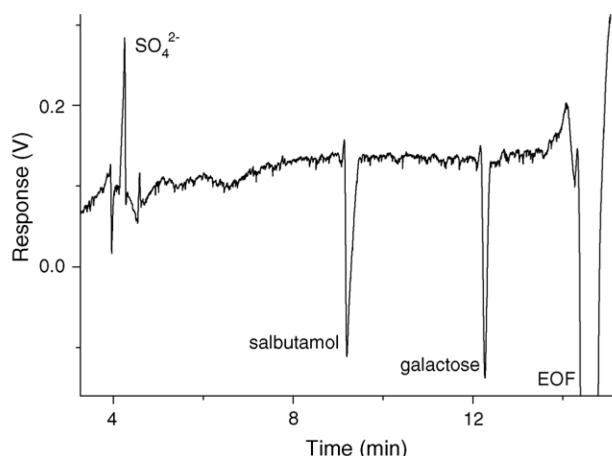

Fig. 3. Electropherogram obtained for salbutamol by using 4.5×10^{-3} mol L^{-1} sodium phosphate buffer (pH 12.1), 1.0×10^{-2} mol L^{-1} NaOH, and 4.0×10^{-2} mol L^{-1} CTAB. Concentration of species: 2.5×10^{-4} mol L^{-1} salbutamol, 5.0×10^{-4} mol L^{-1} galactose, and 5.0×10^{-4} mol L^{-1} sulfate. Separation voltage: 12 kV.

TEA (added as internal standard) and salbutamol demonstrates that the compounds have a lower mobility than that one of the buffer counter-ion (tailing peaks), but this experimental fact does not affect the quantification.

The best results in salbutamol determination were obtained by FSCE. Comparison with MEKC by considering analysis time and sensitivity is illustrated in Table 1. Each one of the electrolytes utilized in FSCE showed their advantages. However, in solutions with elevated pH, the analysis requires a significantly higher time, although this condition shows better signal–noise ratio. Since the acetate buffer provided a short analysis time and a good signal–noise ratio, this solution was elected as running electrolyte for all experiments involving the salbutamol analysis.

In order to obtain the best conditions in the capillary electrophoresis experiments, it was important to study the effect of pH and concentration of the acetate buffer solution. The dependence of the migration times of salbutamol and of the internal standard TEA on the pH was examined in the range from 4.4 to 5.4. The results demonstrated that there was better separation at pH 4.9. The effect of the concentration was verified by using acetic acid/sodium acetate (pH 4.9) solutions ranging between (1.0 and 3.0) $\times 10^{-2}$ mol L^{-1}. The results showed that, with an increase of the concentration of the running electrolyte, the peaks decreased and became broader and the noise increased. Certainly, Joule heating accounts for this behavior. Also, there was a change of the electroosmotic flow in the capillary. Considering the sensitivity, the analysis time and the resolution, 1.0×10^{-2} mol L^{-1} acetic acid/sodium acetate (pH 4.9) was considered the most suitable running electrolyte for the analyses.

The typical electropherogram of salbutamol standard solutions in this buffer can be seen in Fig. 4. There are presented the electropherograms (Fig. 4a–f) corresponding to standard solutions of salbutamol, ranging from 7.0×10^{-5} to 3.0×10^{-4} mol L^{-1}. Fig. 4(curve g) corresponds to a syrup analysis. The inset depicts the linearity of response obtained for salbutamol. All these experiments were done with solutions containing in the running electrolyte 1.0×10^{-2} mol L^{-1} acetic acid/sodium acetate solution at pH 4.9 and 3×10^{-4} mol L^{-1} of TEA. The separation voltage of 28 kV (maximum value of the voltage source) was the most favorable and was adopted for all determinations involving syrups. In such conditions the time necessary for the analyte travel the 53 cm of capillary was only 3.20 min. The total time necessary for the electroosmotic flow goes through the detector was just 4.66 min (~4 min and 40 s).

3.1. Calibration plot and measurements of repeatability and reproducibility

Under the optimized conditions, a series of experiments in triplicate was carried out utilizing standard solutions of the analyte in different concentrations to build the analytical curve. It was observed a linear relationship between the total corrected area under the peaks and the salbutamol concentration in the range from 7.0×10^{-5} to 3.0×10^{-4} mol L^{-1}.

Table 1
Comparison between the methods utilized in salbutamol detection

Electrolyte	pH	Method	Analysis time[a] (min)	EOF marker (min)	Signal/noise
Ac^-/Hac	4.9	FSCE	3.20	4.66	220
$H_3BO_3/B(OH)_4^-$	9.0	FSCE	3.27	3.37	n.c.[b]
$H_3BO_3/B(OH)_4^-$/SDS	9.4	MEKC	9.08	5.50	34
HPO_4^{2-}/PO_4^{3-}/CTAB	12.1	FSCE	12.30	14.36	250

[a] Migration time of the analyte.
[b] Not calculated.

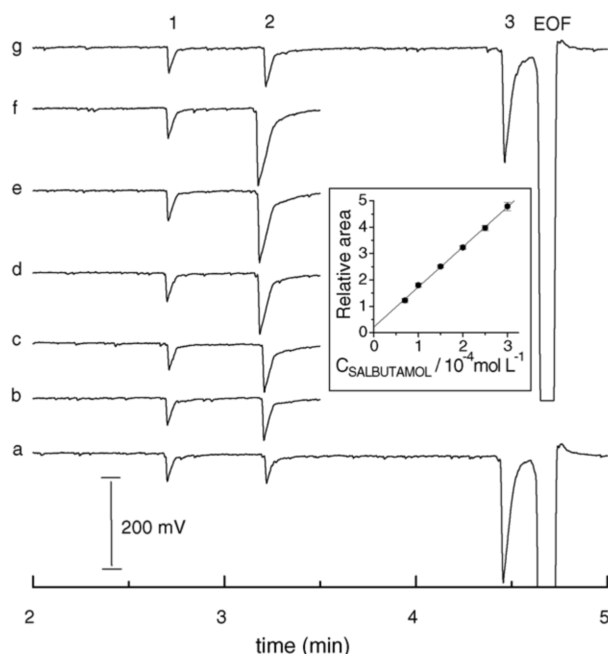

Fig. 4. Electropherograms of standard solutions of (a) 7.0×10^{-5} mol L^{-1}, (b) 1.0×10^{-4} mol L^{-1}, (c) 1.5×10^{-4} mol L^{-1}, (d) 2.0×10^{-4} mol L^{-1}, (e) 2.5×10^{-4} mol L^{-1} and (f) 3.0×10^{-4} mol L^{-1} salbutamol (a)–(f) and of a commercial syrup diluted solution (g). Running buffer: 1.0×10^{-2} mol L^{-1} acetic acid/acetate (pH 4.9). Peak 1: standard solution of TEA (3.0×10^{-4} mol L^{-1}); peak 2: salbutamol solutions (standard and sample); peak 3: system peak. Separation voltage: 28 kV; hydrodynamic injection: 10 cm, 25 s. For clarity, curves (b–f) present only the peaks correspondent to the internal standard and salbutamol. The 3 and EOF peaks in (b)–(f) were identical to the ones from curves (a) and (g).

The straight line could be represented by the equation $y = (0.21 \pm 0.05) + (15172 \pm 260)x$ (correlation coefficient equal to 0.999, for $n = 6$). The detection limit estimated for this interval was 1.0×10^{-5} mol L^{-1} (three times the standard deviation of the blank [41]) and the limit of quantification (ten times the standard deviation of the blank) was calculated as 3.3×10^{-5} mol L^{-1}.

For 10 repetitive measurements of a solution containing 7.0×10^{-5} mol L^{-1} of salbutamol, the standard deviation was calculated as 4.7%. This standard deviation was the result of experiments done in three different days and carried out by different operators. For measurements involving syrups, experiments made in triplicates (and carried out by the same operator) the relative standard deviations obtained were situated between 1.5% and 2.5%.

3.2. Determination of salbutamol in syrups

Table 2 shows the results obtained for the analysis of three pharmaceutical formulations. In this table, the nominal content of salbutamol and the average of three determinations for each sample are presented. Since there is no official method for the determination of salbutamol in syrups, the results obtained by the present method were only compared with the nominal values pointed out by the manufactures.

Significance test (null hypothesis) was applied to the results presented in Table 2, resulting experimental *t*-values between 1.8 and 4.3. These results suggest there are no evidences of systematic errors, for 2 degrees of freedom (95% of confidence interval), which the critical value of *t* is 4.3 [41].

In conclusion, the capillary electrophoresis with C^4D proved to be very suitable for the determination of salbutamol in pharmaceutical products. The proposed method has low cost, each analysis requires relatively short time, and it does not use organic solvents and generates little residues. In addition, it dispenses previous steps such as separation, extraction or filtration. The FSCE seems to be more appropriate because of improved resolution and sensitivity in comparison with the MEKC method. Good repeatability and reproducibility are also characteristics of the method utilized.

Table 2
Results obtained after analysis of three different syrup samples of salbutamol sulfate by capillary electrophoresis (CE) with contactless conductivity detection (C^4D)

Sample	Composition	Labeled value (mg/5 mL)	CE-$C^4D \pm$ S.D.[a] (mg/5 mL)
1	Salbutamol sulfate, sodium sacharine, propylparaben, methylparaben, flavor, citric acid, sodium cyclamate, dye and purified water	2.4	2.20 ± 0.19
2	Salbutamol sulfate, citric acid, glycerine, propylparaben, sodium citrate, sugar and purified water	2.4	2.35 ± 0.02
3	Salbutamol sulfate, sodium sacharine, propylparaben, methylparaben, flavor, citric acid, sodium cyclamate and purified water	2.4	2.30 ± 0.04

[a] Average ± S.D. of three determinations.

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