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Chiral separation of bupivacaine enantiomers by capillary electrophoresis partial-filling technique with human serum albumin as chiral selector☆

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Abstract

Capillary electrophoresis (CE) is a powerful technique for enantiomer separations due to its intrinsic high separation efficiencies, speed of analysis, low reagent consumption and small sample requirements. However, some chiral selectors present strong background UV absorption providing high detection limits. The present paper deals with the application of the partial-filling technique to the separation of bupivacaine enantiomers by capillary electrophoresis using human serum albumin (HSA) as chiral selector. In this procedure the cationic surfactant cetyltrimethylammonium bromide (CTAB) was used as a dinamic capillary coating in order to reduce the electro-osmotic flow and detect both bupivacaine enantiomers out of the chiral selector plug. Several experimental conditions such as CTAB concentration, pH, HSA concentration and plug length, background electrolyte concentration, temperature and voltage were studied. Under the selected conditions it is possible to detect the separated enantiomers out of the HSA plug in less than 4 min using 50 mM Tris pH 8 as background electrolyte with 50 μM CTAB, at 30 °C and using a separation voltage of 25 kV. The proposed methodology was then validated for analytical purpouses and applied to the analysis of pharmaceutical preparations commercially available. The results obtained with the proposed methodology were in good agreement with those declared by the manufacturers. The simplicity, sample throughput, accuracy, reproducibility and low cost of the proposed method make it suitable for the control of the enantiomeric composition of bupivacaine in pharmaceuticals.

Keywords: Enantiomers; Bupivacaine; Human serum albumin (HSA); Partial filling technique

1. Introduction

Most of the pharmacological processes present a high degree of stereoselectivity resulting in a difference between the activities of drugs enantiomers. In fact, very often one of them is the most active while the other may produce side-effects and even toxicity in some cases. Consequently the safety and efficiency of drugs could be improved with single-enantiomer formulations. Thus, there is a special interest within the pharmaceutical laboratories to develop these single-enantiomer formulations [1–3] and consequently a need for analytical methods to control the enantiomeric purity of drugs arises [4].

Local anesthetics agents play an important role in modern anesthesiology. Most of these drugs possess a chiral center and particularly in the case of bupivacaine and even being both enantiomers active as nerve blockers, the *R*-(+) is more toxic than the *S*-(−) form [5]. Therefore, selective and robust methods for the enantioseparation of bupivacaine are needed for the quality control of this drug in pharmaceuti-

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cal preparations. Chromatographic [6–8] and more recently electrophoretic methods have been reported for the enantioseparation of local anesthetics, cyclodextrins (CDs) being the most commonly used selectors for electrically driven separations. Among the different kinds of CDs, methyl-β-CD [9,10] and sulfated-β-CD [11] were found to be the most appropiate selectors for the separation of the enantiomers of this kind of drugs. Other selectors out of CDs such as octyl-β-D-maltopyranoside [12], succinyl avidin (sucAVI) [13] and *N*-bezoxycarbonylglycyl-L-proline [14] were also found to be useful in the separation of bupivacaine enantiomers.

Different approaches using several proteins as chiral selectors have been recently reported in the literature [15–21]. General drawbacks of the use of proteins as chiral selectors are that they are relatively expensive and/or present a strong absorbance in the UV region thus providing high detection limits in the determinations. In order to overcome this limitation, several approaches such as the partial-filling technique were developed [22]. In this methodology, the capillary is partially filled with the chiral selector solution leaving all the rest, including the detection window, free of it. The electrophoretic buffer is composed of plain buffer with no additive. The experimental conditions are selected so that the analytes migrate through the selector plug, where they have become separated, and are detected out of it. However, in some cases chiral selector plug length applied should be very large in order to obtain complete enantioresolution of both analytes. In such cases, strategies to either, accelerate the elution of analytes or retard chiral selector plug should be applied in order to perform the analytes detection detection out of the chiral selector plug thus reducing the limits of detection (LODs) and increasing reproducibility. In this sense, cationic capillary wall coatings would be of interest for this purpouse since they would retard electro-osmotic flow (EOF) due to its interaction with the free silanol groups in the inner capillary wall. Consequently, the chiral selector plug would be slowed and capillary efficiencies may be improved due to the decrease in analyte–capillary wall interaction [23,24]. Therefore, a strict control and tunning of the interaction between buffer additives and capillary surface is needed in order to slow the EOF without sacrifying other desirable analytical features such as short analysis times and resolution power.

In the present paper, we describe a methodology for the separation of bupivacaine enantiomers by capillary electrophoresis using human serum albumin (HSA) as chiral selector. The partial filling technique was applied providing complete enantioresolution only at high HSA concentration and plug length. The addition of different cetyltrimethylammonium bromide (CTAB) concentrations to the buffer and HSA solution was evaluated in terms of bupivacaine enantiomers resolution and detection out/in HSA plug. The influence of other experimental conditions such as pH, temperature, voltage and buffer concentration were evaluated. Under the selected conditions, the method was applied to the determination of bupivacaine enantiomers in a pharmaceutical preparation. The results obtained showed that the proposed methodology is adequate in terms of speed, cost, resolution power, reproducibility and accuracy for the quality control of the enantiomeric composition of bupivacaine in pharmaceuticals.

2. Materials and methods

2.1. Chemicals and samples

All reagents were of analytical grade. Human serum albumin fraction V (HSA) was purchased from Sigma (St. Louis, MO, USA); tris(hydroxymethyl)aminomethane (Tris) was from Scharlab (Barcelona, Spain); cetyltrimethylammonium bromide was purchased from Acros Organics (Geel, Belgium); racemic bupivacaine hydrochloride standard was kindly donated by Laboratorios Inibsa (Barcelona, Spain). *S*-(−)-Bupivacaine hydrochloride (99%) was purchased from Aldrich (Milwaukee, WI, USA) and *S*-(−)-epinephrine (+)-bitartrate salt was from Sigma. Pharmaceutical preparation Svedocain 0.25% (Laboratorios Inibsa, Barcelona, Spain), is commercially available in Spain. Barnstead E-pure deionized water (Sybron, Boston, MA, USA) was used to prepared solutions. All solutions were filtered prior use through 0.45 μm pore size nylon membranes (Micron Separation, Westboro, MA, USA).

2.2. Instruments and measurements

A Hewlett-Packard HP ^{3D}CE capillary electrophoresis system (Hewlett-Packard, Waldbronn, Germany) equipped with a diode array detector (DAD) and HP ^{3D}CE Chemstation software was used throughout. A 50 μm inner diameter (i.d.) and 363 μm outer diameter (OD) fused-silica capillary with total and effective lengths of 48.5 and 40 cm, respectively was employed (Polymicro Technologies, Phoneix, AZ, USA). The other CE conditions for bupivacaine enantiomers separation were: appiled voltage 15, 20, 25 and 30 kV; detection wavelenght, 220 nm; capillary cassette temperature was set at 25, 30, 35 and 45 °C. Electrolytic solutions were degassed in an ultrasonic bath (JP Selecta, Barcelona, Spain). A Crison Micro pH 2000 pH meter from Crison Instruments (Barcelona, Spain) was employed to adjust the pH of the separation buffer.

2.3. Procedure

Buffers containing variable Tris concentrations were prepared by dissolving the appropriate amount of solid tris(hydroxymethyl)aminomethane in Barnstead E-pure water and adjusting the pH with 0.5 M HCl. CTAB solutions were prepared weekly by weighting the corresponding amount of solid and dissolving it with plain 50 mM Tris pH 8. 500 μm human serum albumin stock solution was daily

prepared by weighting the corresponding amount of protein powder and dissolving it with Tris buffer to a final volume of 1 ml. Working protein solutions were obtained by dilution of filtered HSA stock solution with Tris buffer to a final concentration ranged between 40 and 160 μM. Stock standard solution of bupivacaine hydrochloride (1 mg ml^{-1}) was prepared by weighting the corresponding amount of solid and dissolving it with 50 mM Tris buffer pH 8 containing 10% (v/v) methanol. Working solutions were obtained by dilution with Tris buffer from the corresponding stock solution. In the study of the different variables involved in the enantioseparation the experiments were conducted using 50 mM Tris buffer supplemented with 50 μM CTAB at pH 8 as electrophoretic buffer, using a 120 μM HSA plug applied at 50 mbar for 150 s as chiral selector, temperature kept at 30 °C and applying 20 kV voltage unless otherwise stated.

In order to obtain good peak shapes and reproducible migration data, the capillary was conditioned at the beginning of the day with the following sequence: (i) 2-min rinse with deionized water (ii) 2-min rinse with sodium hydroxide 1 M (iii) 2-min rinse with deionized water, and (iv) 15-min rinse with the running buffer at 1000 mbar. Between runs, the capillary was conditioned with deionized water for 1 min, 2 min 1 M NaOH, water for 1 min and running buffer for 2 min. Before sample injection, the capillary was partially filled with the chiral selector (HSA concentration ranging from 0 to 160 μM) by applying 50 mbar pressure for 0, 50, 100, 125, 150, 175 or 200 s. Injection of bupivacaine solutions were performed hydrodynamically at 50 mbar for 3 s.

2.4. Sample preparation

For analysis of injectable solutions containig racemic bupivacaine hydrochloride, an alicuot of the preparation was simply taken and diluted with Tris 50 mM pH 8 to a final volume of 25 ml. Three independent sample solutions were prepared for all the analyses.

2.5. Data treatment

From the experimental data, electrophoretic mobilities corrected for eletroosmotic flow variations were calculated according to the following equation:

$$\mu_{\text{eff}} = \frac{lL}{V}\left(\frac{1}{t_{\text{i}}} - \frac{1}{t_0}\right) \quad (1)$$

where L and l are the total capillary length and the length from the inlet to the detector respectively, V the run voltage, t_{i} the analyte migration time and t_0 is the migration time of acetone, used as non-interacting electro-osmotic flow marker [25].

Enantiomeric resolution (R_{s}) was calculated according to Eq. (2) [26]:

$$R_{\text{s}} = \frac{1.18(t_2 - t_1)}{w_1 + w_2} \quad (2)$$

where t_1 and t_2 are the migration time of each enantiomer and w_1 and w_2 represent the half-height peak widths. Microsoft Excel 2002 and Statgraphics Plus 2.1 software were used for data treatment.

3. Results and discussion

3.1. Enantiomeric separation of bupivacaine

In preliminary studies, the interaction between HSA and several compounds was investigated by affinity capillary electrophoresis. For this purpose, prior to injection of compounds, capillary was filled with HSA solution and electrophoresis was carried out using plain 50 mM Tris solutions at pH 7.4 as electrolytic solution. For racemic bupivacaine, it was observed that upon addition of a large enough HSA concentration progressive splitting of the peak occurred. This behaviour was attributed to the differential affinity of each individual enantiomer for the protein. Then, a more exhaustive study on the influence of several parameters on enantioresolution of bupivacaine using HSA as chiral selector was performed.

During these experiments a drifty baseline was observed due to the strong UV absorption of HSA. Therefore, the partial filling technique was applied. For this purpose the capillary was only partially filled with a 120 μM HSA solution leaving the detection window free from protein. However, it was observed that to achieve complete separation of bupivacaine enantiomers a relatively long HSA plug was needed. Thus, cationic surfactant CTAB was added to the electrophoretic buffer to control the mobility of the HSA plug. In order to optimize the CTAB concentration, electrophoretic buffers containing 50 mM Tris and increasing CTAB concentrations were prepared. The CTAB concentration in both the plain background electrolyte zone and the HSA was the same. Fig. 1 shows the results obtained and as can be observed increasing CTAB concentration enabled the detection of both bupivacaine enantiomers out of the protein zone. However, CTAB concentrations above 50 μM decreased resolution and increased the migration times of analytes. The increase in the migration time may be explained due to the EOF reversal at CTAB concentration over 50 μM as previously reported by Galli et al. [27]. Once the analytes peaks leave the HSA plug they may be again overlapped due to their diffusion. Therefore, a 50 μM CTAB concentration was selected.

The effect of pH on the separation of bupivacaine enantiomers was studied. The effective mobilities of both bupivacaine enantiomers decrease with pH in the range between 7 and 9 as expected taking into account that bupivacaine is a basic drug with a pK_{a} of 8.1 [11]. As can be observed in Fig. 2

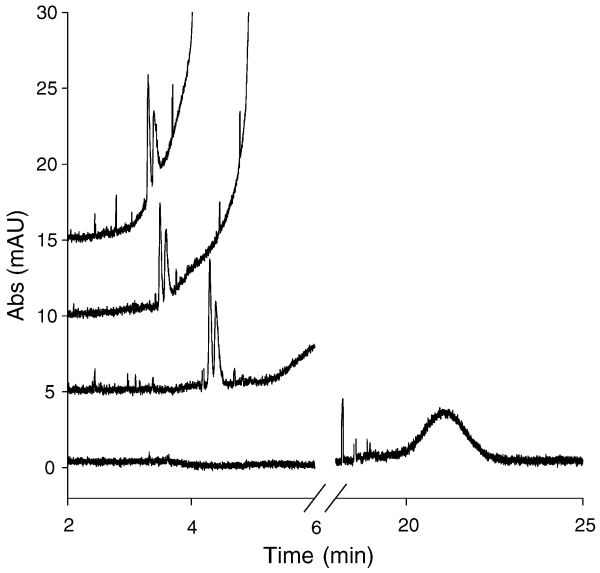


Fig. 1. Electropherograms obtained using a background electrolyte composed of 50 mM Tris pH 8 supplemented with different CTAB concentrations of 0, 25, 50 and 70 μM (from top to bottom). Electrophoresis carried out at 30 °C and 20 kV. UV detection at 220 nm.

the highest resolution was achieved at pH 8. This behaviour can be explained taking into account that the increase of pH produces an increase of residence time of analyte in the chiral selector plug while higher pH values provided analyte peaks already in the HSA plug. Therefore, pH 8 was selected for further studies.

The influence of HSA concentration was also evaluated. HSA solutions from 0 to 160 μM were prepared using the electrophoretic buffer containing and were applied at 50 mbar

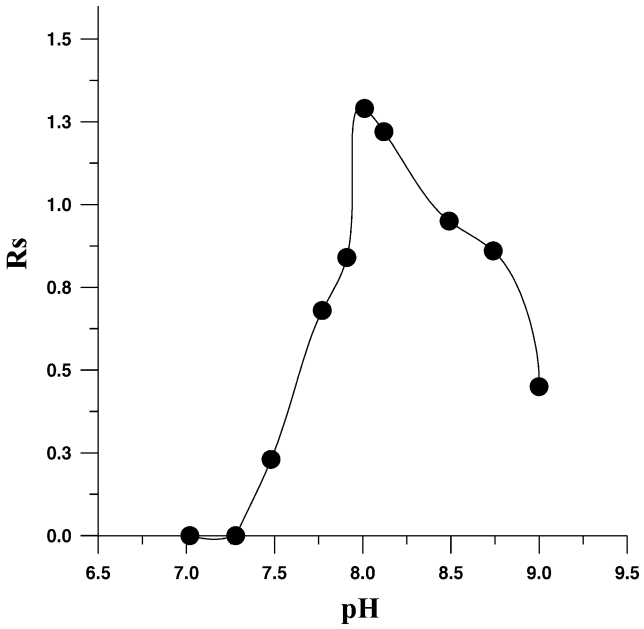
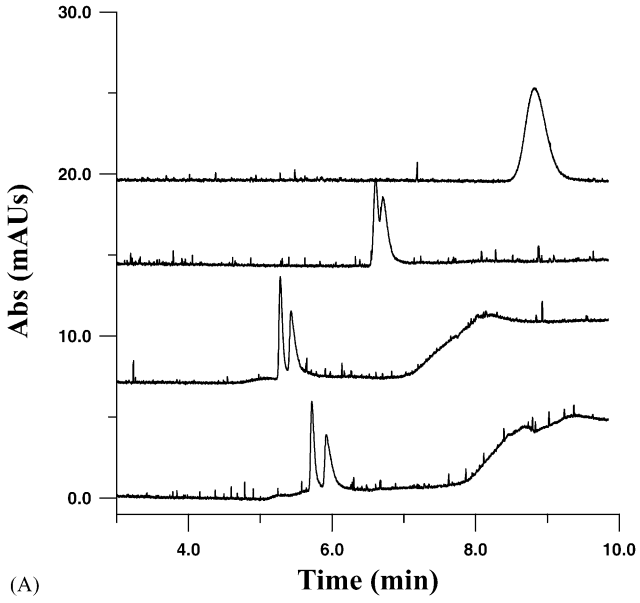


Fig. 2. Resolution of bupivacaine enantiomers at different pH values from 7 to 9. Experiments were carried out using 50 mM Tris–50 μM CTAB as electrophoretic buffer. All the rest of conditions as in Fig. 1.



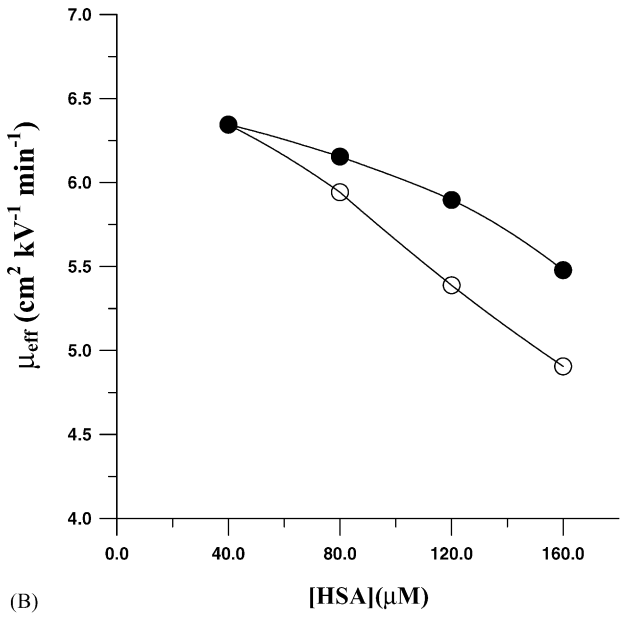


Fig. 3. (A) Electropherograms obtained for 240 mg l^{-1} of racemic bupivacaine solutions using different HSA concentrations in the chiral plug (0, 80, 120, 160 μM HSA solutions, from top to bottom), applied at 50 mbar for 150 s. Rest of condtions as in Fig. 1. (B) Effective mobility of *S*- (●) and *R*-bupivacaine (○) as a function of HSA concentration.

for 150 s just before racemic bupivacaine injection. As can be observed in Fig. 3A, partial enantioseparation was obtained using 80 μM HSA concentration while baseline resolution was found at HSA concentrations above 120 μM. In addition for the highest HSA concentrations good efficiency values were obtained for both bupivacaine enantiomers with a number of plates of aproximately 147 500 and 51 000 plates/m for the first and second migrating enantiomer, respectively. Because different affinities of the *R*- and *S*-bupivacaine for the HSA exist, the molar fraction of complex is different for each

enantiomer. Therefore, the higher the HSA concentration the stronger interaction between bupivacaine enantiomers and HSA what leads to a decrease in the mobility of both bupivacaine enantiomers as HSA concentration was increased (see Fig. 3B).

In order to study the effect of the chiral selector plug length on the bupivacaine enantiomers separation experiments using a 120 μM HSA solution applied for variable times from 0 to 200 s just before racemic bupivacaine injection was performed. Both HSA solutions and electrophoretic buffers contained 50 mM Tris and 50 μM CTAB at pH 8. The results are shown in Fig. 4 and as can be observed when the HSA solution was applied less than 50 s no chiral recognision was obtained. When the chiral selector plug was applied for longer times resolution increased but application times over 150 s provided asymmetric and broader analyte peaks at the beginning of the HSA zone. Therefore, a maximum HSA application time of 150 s was kept for further studies.

Buffer concentration may also play a significant role in the chiral separations of drugs. Being a zwiterionic buffer Tris buffer may minimize solute–wall and protein-wall interactions by shielding the capillary surface charge [28]. The higher the Tris concentration the more effective charge shielding. For this reason, a study of the Tris concentration in the electrophoretic buffer was performed with 10, 25, 50 and 75 mM Tris buffers all at pH 8 and containing 50 μM CTAB were assayed. Maximum resolution and efficiencies were observed with 50 mM Tris buffer. At higher Tris concentrations resolution drops, presumably as a result of increased current generation and Joule heating.

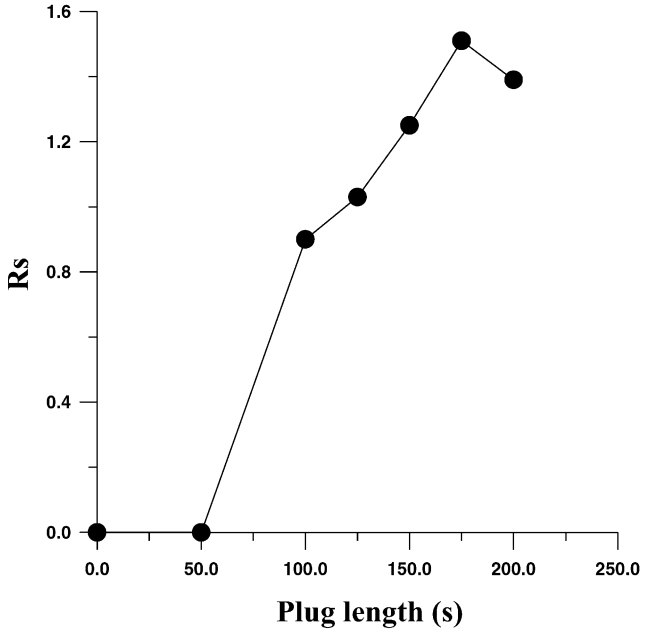


Fig. 4. Influence of the chiral selector plug length on the resolution of 240 mg l^{-1} of racemic bupivacaine. Chiral selector: 120 μM HSA solution applied at 50 mbar for different times from 0 to 200 s. All the rest of conditions as in Fig. 1.

Temperature is considered one of the key parameters in the optimization process of CE separations. Because enantioseparation is based on the different binding ability of both enantiomers to the protein, capillary temperature can affect the separation degree [29]. In order to study the effect of the temperature on the separation of bupivacaine enantiomers series of runs applying 50 mbar for 150 s with a 120 μM HSA solution were performed at 25, 30, 35 and 40 °C injecting a 230 mg l^{-1} racemic bupivacaine hydrochloride solution. Lower temperatures were avoided since it was observed that CTAB crystalized at temperatures round 15 °C. Resolution only showed a modest improvement as the temperature was raised from 25 to 30 °C (R_s of 1.30 and 1.50, respectively) while higher temperatures provided a decrease in the resolution (R_s of 1.43 and 1.30 at 35 and 40 °C, respectively).

Finally a study of the applied voltage was also performed in terms of maximizing resolution and efficiency while minimizing migration time. It is known that an increased voltage yields to shorter migration times and higher efficiencies and resolution [28,30]. But on the other hand the generation of Joule heat may limit the theoretical gain in resolution and efficiency with voltage. In the present work voltages ranged between 15 and 30 kV were assayed. Migration times became shorter and more reproducible at higher voltages while resolution slightly decreased from 1.16 to 0.94. Efficiency was not apparently affected by voltage suggesting little change in Joule heating with increased voltages. However, when the voltage was set at 30 kV analyte peaks were detected already within the HSA plug zone what made difficult their integration. Therefore a voltage of 25 kV was selected since it provided short migration times (4 min) still out of the HSA zone.

From the results of the studies carried out the following experimental conditions for the chiral separation of bupivacaine enantiomers were selected: electrophoretic buffer composed of 50 mM Tris, 50 μM CTAB at pH 8; 160 μM HSA solution applied at 50 mbar for 150 s as chiral selector; the electrophoretic runs performed at 30 °C applying 25 kV voltage. Under these experimental conditions both bupivacaine enantiomers are baseline resolved in 4 min.

In order to assign each peak to the corresponding *R*- or *S*-enantiomer, solutions of 160 mg l^{-1} of racemic bupivacaine hydrochloride spiked with different amounts of *S*-bupivacaine were injected. The results shown in Fig. 5 revealed that the the first migrating peak should be assigned to the *S*-enantiomer and therefore the second to *R*-bupivacaine.

3.2. Enantiomeric determination of bupivacaine in pharmaceutical preparations.

The proposed method was applied to determine the content of bupivacaine hydrochloride enantiomers in the pharmaceutical preparation Svedocain 0.25%. This is commercialised as injectable solutions containing racemic bupivacaine hydrochloride together with a small concentration

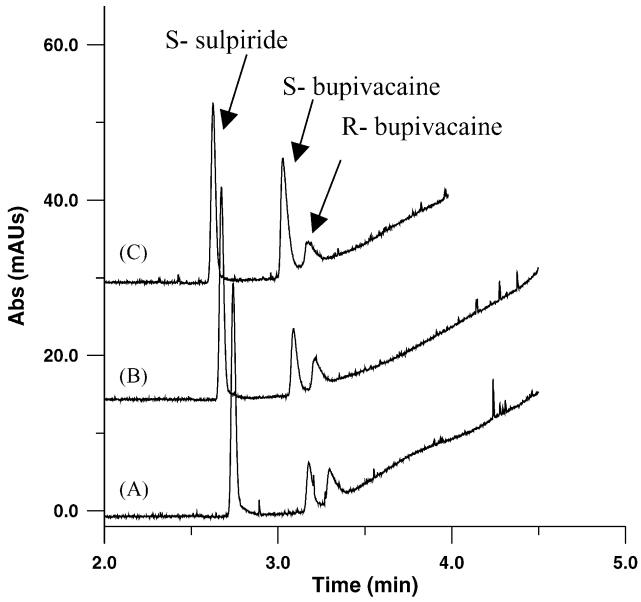


Fig. 5. (A) Electropherogram of a 160 mg l^{-1} solution of racemic bupivacaine and 80 mg l^{-1} *S*-(−) sulpiride. Same sample spiked with 80 mg l^{-1} (B) and 160 mg l^{-1} of *S*-bupivacaine (C). Experimental conditions: 50 mM Tris pH 8 with 50 μM CTAB cat pH 8 as electrophoretic buffer; Chiral selector, 160 μM HSA solution applied at 50 mbar for 150 s; electrophoretic runs carried out at 30 °C and 25 kV. UV detection at 220 nm.

of epinephrine in order to reduce the absorption of the anesthetic by the tissues prolonging the anesthetic effect.

According to the International Conference on Harmonisation (ICH) technical requirements for pharmaceuticals for human use recommendations on the validation of analytical procedures, detection and quantification limits are not critical features in these analytical methods in "assays procedures" for determining major components in pharmaceutical preparations. However, an estimation of the detection limit of the proposed methodology was performed according to the ICH criterion based on the signal-to-noise ratio [31]. It was found that concentrations close to 20 mg l^{-1} provided signal-to-noise ratios slightly over the critical value, 3. In addition, it was also checked that this concentration level provided analyte peaks that could be quantified with acceptable precision (i.e. relative standard deviation, R.S.D. < 10%).

Calibration graphs containing 20–120 mg l^{-1} of each bupivacaine hydrochloride enantiomer were obtained. Sulpiride was selected as internal standard because it does not interact with HSA and migrates close to the analytes. All the standards and samples solutions were spiked with a fixed concentration (80 mg l^{-1}) of *S*(−) sulpiride. Linear calibration graphs were obtained for both bupivacaine enantiomers using the absolute peak area and the ratio analyte/internal standard peak area as dependent variables. Table 1 summarises the statistical features of the calibration graphs. In all cases, the calibration curves showed adequate regression coefficients (>0.99).

Precision was evaluated at two concentration levels by injecting five independent solutions containing 20 and 80 mg l^{-1} of each *R*- and *S*-bupivacaine. The results are shown in Table 2 and as can be observed the relative standard deviation expressed as percentage were similar using either absolute area or the ratios analyte/internal standard. As expected the precision is slightly higher at 80 than at 20 mg l^{-1}.

The proposed method was applied to the analysis of the pharmaceutical preparation Svedocain 0.25% containing 2500 mg l^{-1} of racemic bupivacaine hydrochloride and 5 mg mg l^{-1} of epinephrine bitartrate. The results expressed as percentage of recovery relative to the content declared by the manufacturer were always ranged between 92 and 102% with both, absolute peak area and area ratios (see Table 3) which agrees with the tolerance limits for this kind of samples (±15%).

Epinephrine, another sample component was not detected in the sample due to its low concentration (only 0.4 mg l^{-1} of epinephrine bitartrate for 100 mg l^{-1} of each bupivacaine enantiomer) (see Fig. 6A). However, in order to check the absence of interference due to epinephrine, a synthetic sample containing 80 mg l^{-1} sulpiride, 160 mg l^{-1} of each bupivacaine enantiomer and 200 mg l^{-1} epinephrine bitartrate was injected. As can be observed in Fig. 6B epinephrine did not overlap with either bupivacaine enantiomer or sulpiride under the selected experimental conditions what ensures complete separation of the components in this kind of injectable samples of anesthetics.

In order to study the accuracy of the proposed method three samples solutions of the pharmaceutical preparation Svedocain 0.25% were spiked with a certain volume of stock

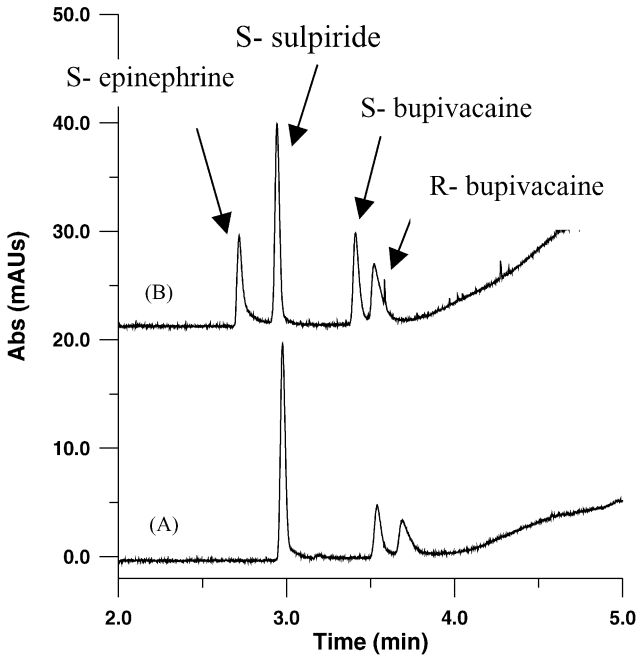


Fig. 6. (A) Electropherogram of the sample Svedocain 0.25% (after appropiate dilution) spiked with 80 mg l^{-1} *S*-(−) sulpiride. (B) Synthetic sample containing 80 mg l^{-1} *S*-(−) sulpiride, 160 mg l^{-1} of each bupivacaine enantiomer and 200 mg l^{-1} epinephrine bitartrate.

Table 1
Statistical features of the calibration graphs for the first and second migrating bupivacaine enantiomer using both absolute areas and area ratio analyte/internal standard

	Absolute area			Analyte/internal standard ratio		
	$b_0 \pm ts_{b_0}$	$b_1 \pm ts_{b_1}$	r	$b_0 \pm ts_{b_0}$	$b_1 \pm ts_{b_1}$	r
S-bupivacaine	1.1 ± 1.9	0.19 ± 0.02	0.996	0.03 ± 0.04	0.0039 ± 0.0006	0.995
R-bupivacaine	1 ± 3	0.20 ± 0.04	0.991	0.02 ± 0.05	0.0042 ± 0.0006	0.994

b_0, intercept; b_1, slope; ts, confidence interval at the 95% probability level; R.S.D., relative standard deviation.

Table 2
Precision of the proposed method using absolute peak area or analyte/internal standard area ratio at 20 and 80 mg l^{-1} concentration levels for five independent solutions

	R.S.D. (%)[a]		R.S.D. (%)[b]	
	20 mg l^{-1}	80 mg l^{-1}	20 mg l^{-1}	80 mg l^{-1}
S-bupivacaine	5	6	6	5
R-bupivacaine	8	5	9	5

[a] Relative standard deviation with absolute areas.
[b] Relative standard deviation with analyte/internal standard area ratio.

Table 3
Recoveries found for the determination of bupivacaine hydrochloride enantiomers in the pharmaceutical preparation Svedocain

	$(R \pm s_{n-1})$ (%)[a]	$(R \pm s_{n-1})$ (%)[b]
S-bupivacaine	98 ± 6	92 ± 7
R-bupivacaine	102.0 ± 1.1	95 ± 6

R: recovery with respect to the content declared by the manufacturer; s_{n-1}: standard deviation of the recoveries found for the analysis of three independent sample solution.
[a] With absolute areas.
[b] With analyte/internal standard area ratio.

racemic bupivacaine equivalent to 14.82 mg l^{-1} of each enantiomer. These samples were analysed following the proposed methodology and provided recoveries for the added bupivacaine ranged between 103 ± 6 and 106 ± 8% for *S*- and *R*-bupivacaine, respectively. These results clearly show that the accuracy of the proposed methodology is good enough for quality control of pharmaceutical containing mixtures of the enantiomers of this local anesthetic together with epinephrine in the usual levels found in this kind of samples.

An important factor of the proposed methodology is the low cost per analysis; the chiral selector solution of HSA is pumped through the capillary before applying voltage on the electrophoretic buffer. Thus, the HSA solution in the vial did not become electrolyzed in each analysis and can be used several times. The low cost of the proposed methodology together with its speed make it suitable for quality control of pharmaceutical in terms of economy and sample throughput.

4. Conclusions

This paper describes the application of the partial filling technnique to the separation and determination of bupivacaine hydrocloride enantiomers by capillary electrophoresis using HSA as chiral selector. It was found that the addition of CTAB to the background electrolyte enabled the detection of the separated enantiomers out of the protein zone yielding to good reproducibility. Several other variables involved in the separation such as pH, HSA concentration, chiral selector plug length, background electrolyte concentration, temperature and voltage were also studied. Under the selected experimental conditions the determination of bupivacaine hydrochloride enantiomers in a pharmaceutical preparation was performed, providing recoveries close to 100%.

The proposed methodology provides adequate results in terms of simplicity, cost, sample throughput, reproducibility and accuracy for quality control of bupivacaine enantiomers in pharmaceutical preparations.

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References

[1] S.C. Stinson, Chem. Eng. News 75 (1997) 38.
[2] S.C. Stinson, Chem. Eng. News 76 (1998) 83.
[3] S.C. Stinson, Chem. Eng. News 77 (1999) 101.
[4] N.M. Maier, P. Franco, W. Lindner, J. Chromatogr. A 906 (2001) 3.
[5] A. Tahraoui, D.G. Watson, G.G. Skellern, S.A. Hudson, P. Petrie, K. Faccenda, J. Pharm. Biomed. Anal. 15 (1996) 251.
[6] J. Hermansson, J. Chromatogr. 269 (1983) 71.
[7] M. Siluveru, J.T. Stewart, J. Pharm. Biomed. Anal. 15 (1996) 389.
[8] I. Abraham, J.P. Fawcett, J. Kennedy, A. Kumar, R. Ledger, J. Chromatogr. B 703 (1997) 203.
[9] C.E. Sänger-van de Griend, K. Gröningsson, D. Wester-lund, Chromatographia 42 (1996) 263.
[10] A. Amini, U. Paulsen-Sörman, Electrophoresis 18 (1997) 1019.
[11] S. Cherkaoui, J.L. Veuthey, J. Pharm. Biomed. Anal. 27 (2002) 615.
[12] Z. El Rassi, J. Chromatogr. A 875 (2000) 207.
[13] Y. Tanaka, S. Terabe, Chromatographia 49 (1999) 489.
[14] Y. Hedeland, M. Hedeland, U. Bondesson, C. Petterson, J. Chromatogr. A 984 (2003) 261.
[15] Y. Tanaka, S. Terabe, J. Biochem. Biophys. Methods 48 (2001) 103.

[16] E.D. Lorenzi, G. Massolini, M. Quaglia, C. Galbusera, G. Caccialanza, Electrophoresis 20 (1999) 2739.
[17] S. Busch, J:C. Kraak, H. Poppe, J. Chromatogr. 635 (1993) 119.
[18] D.K. Lloyd, S. Li, P. Ryan, J. Chromatogr. A 694 (1995) 285.
[19] F. Kilar, Electrophoresis 17 (1996) 1950.
[20] N. Mano, Y. Oda, Y. Ishihama, H. Katayama, N. Asakawa, J. Liq. Chromatogr. Rel. Technol. 21 (1998) 1311.
[21] M. Hedeland, R. Isaksson, C. Pettersson, J. Chromatogr. A 807 (1998) 297.
[22] L. Valtcheva, J. Mohammad, G. Petterson, S. Hjertén, J. Chromatogr. 638 (1993) 263.
[23] O. Nuñez, E. Moyano, L. Puignou, M.T. Glaceran, J. Chromatogr. A 912 (2001) 353.
[24] M. Youssouf Badal, M. Wong, N. Chiem, H. Salimi-Moosavi, D.J. Harrison, J. Chromatogr. A 947 (2002) 277.
[25] J. Yang, D.S. Hage, Anal. Chem. 66 (1994) 2719.
[26] Y. Martín-Biosca, C. García-Ruiz, M.L. Marina, Electrophoresis 22 (2001) 3216.
[27] V. Galli, C. Barbas, Anal. Chim. Acta 482 (2003) 37.
[28] D.N. Heiger, High Performance Capillary Electrophoresis–An introduction, Hewlett-Packard, France, 1992.
[29] B. Chankvetadze, Capillary Electrophoresis in Chiral Analysis, Wiley, Chichester, 1997.
[30] J.E. Melanson, N.E. Baryla, C.A. Lucy, Trends Anal. Chem. 20 (2001) 365.
[31] International Conference on Harmonization, http://www.ifpma.org.