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Validation of a capillary electrophoresis method for the enantiomeric purity testing of ropivacaine, a new local anaesthetic compound

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Abstract

A capillary electrophoresis method for the determination of the enantiomeric purity of ropivacaine, a new local anaesthetic compound developed by Astra Pain Control AB, has been validated. The method showed the required limit of quantitation of 0.1% enantiomeric impurity and proved to be robust. Good performances were also shown for specificity, linearity, system repeatability and accuracy. The same capillary electrophoresis method can also be used to simultaneously chirally resolve homologues and impurities of ropivacaine.

Keywords: Capillary electrophoresis; Enantiomer separation; Local anaesthetics; Pharmaceutical analysis; Ropivacaine; Validation

1. Introduction

With the development of capillary electrophoresis (CE) as a powerful tool in pharmaceutical analysis, a discussion about method validation has begun [1–5]. This paper contributes to this discussion by presenting the data obtained in validating the CE method for the enantiomeric purity determination of ropivacaine (*S*-(−)-1-propyl-2′,6′-pipecoloxylidide) hydrochloride monohydrate, which is the first enantiomerically pure local anaesthetic. It is chemically homologous with bupivacaine and mepivacaine, and exists as the S enantiomer. Ropivacaine is a long-acting local anaesthetic, and in preclinical studies it produced less central nervous system and cardiovascular toxicity than bupivacaine [6]. In healthy volunteers, ropivacaine was less toxic than bupivacaine with regard to the production of mild central nervous system and cardiovascular changes during intravenous infusion [7]. Clinically, ropivacaine has produced effective and well-tolerated local anaesthetic blocks [8,9].

For quality control, a sensitive, selective and robust separation method is required. Liquid chromatography (LC) using chiral stationary

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phases, e.g. α_1-acid glycoprotein, has frequently been used for the separation of local anaesthetic enantiomers of the amide type [10]. The CE method described in this paper shows selectivity and efficiency that are superior to the chiral LC method currently used in our laboratory.

2. Experimental

2.1. CE conditions

The validation was performed on a HP 3D CE instrument (Hewlett Packard, Waldbronn, Germany), comprising a diode-array detector and ChemStation software for data handling. The capillary (Hewlett Packard, Waldbronn, Germany) was 80.5 cm long (72.0 cm effective length) with a 50 μm internal diameter. The applied voltage was 30 kV, with an initial ramping of 500 V s^{-1}. The temperature was 30°C. Injection was performed at 50 mbar over 5 s (5 nl injection volume). Detection was at 206 nm. The preconditioning of the capillary was programmed for each run and consisted of a 1 min flush with water, a 4 min flush with 0.1 mol l^{-1} NaOH (sodium hydroxide solution for HPCE, Fluka BioChemika, Buchs, Switzerland), a 1 min flush with water and a 4 min flush with the run buffer.

The background electrolyte solution (BGE) was prepared by adjusting a solution of 0.1 mol l^{-1} phosphoric acid (p.a.; Merck, Darmstadt, Germany) to pH 3.0 with triethanolamine (p.a.; Merck, Darmstadt, Germany). The run buffer consisted of 133 mg heptakis (2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD; Sigma, St. Louis, MO, USA) per 10 ml of BGE, resulting in a concentration of 10 mmol l^{-1} of DM-β-CD. For the robustness test, DM-β-CD obtained from other suppliers (Tokyo Kasei, Tokyo, Japan and Pharmatec, Alachua, FL, USA) were also used.

All solutions were freshly prepared using MilliQ purified water and filtered with nylon filters, 0.45 μm pore size (Micron Separations, Westboro, MA, USA).

2.2. Test components

See Fig. 1, 2′,6′-pipecoloxylidide hydrochloride (PPX; racemate; working standard), ropivacaine hydrochloride monohydrate ((*S*)-propyl-PPX; Chemical Reference Standard, batch no. 106/93), (*R*)-propyl-PPX hydrochloride monohydrate ((*R*)-PrPPX; working standard), mepivacaine hydrochloride (MePPX; racemate; working standard), ethyl-PPX hydrochloride (EtPPX; racemate; working standard), bupivacaine hydrochloride monohydrate (BuPPX; racemate; working standard) and pentyl-PPX hydrochloride (PePPX; racemate; working standard) were obtained from Astra Pain Control AB (Södertälje, Sweden).

2.3. Purity determination

The enantiomeric purity of ropivacaine hydrochloride monohydrate was determined by analysing a 2 mg ml^{-1} solution (6 mmol l^{-1}) of the ropivacaine hydrochloride monohydrate batch in purified water. The percentage of the R enantiomer in ropivacaine hydrochloride monohydrate is calculated by internal normalisation from corrected peak areas (peak area/migration time) and according to Eq. (1)

$$\%\text{Impurity} = \left(\frac{\text{Peak area}_{(R)\text{-PrPPX}}}{\text{Migration time}_{(R)\text{-PrPPX}}} \Big/ \frac{\text{Peak area}_{(S)\text{-PrPPX}}}{\text{Migration time}_{(S)\text{-PrPPX}}} \right) \times 100 \quad (1)$$

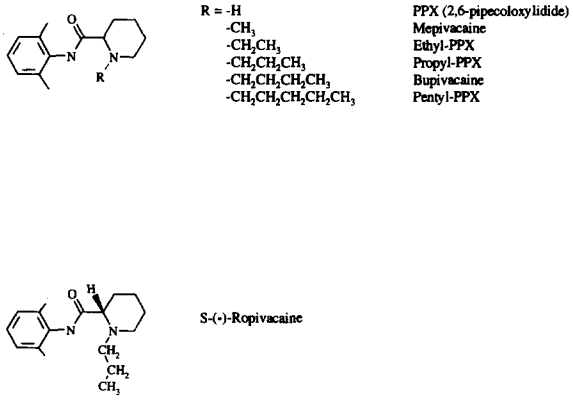

Fig. 1. Structures of *n*-alkyl PPX derivatives.

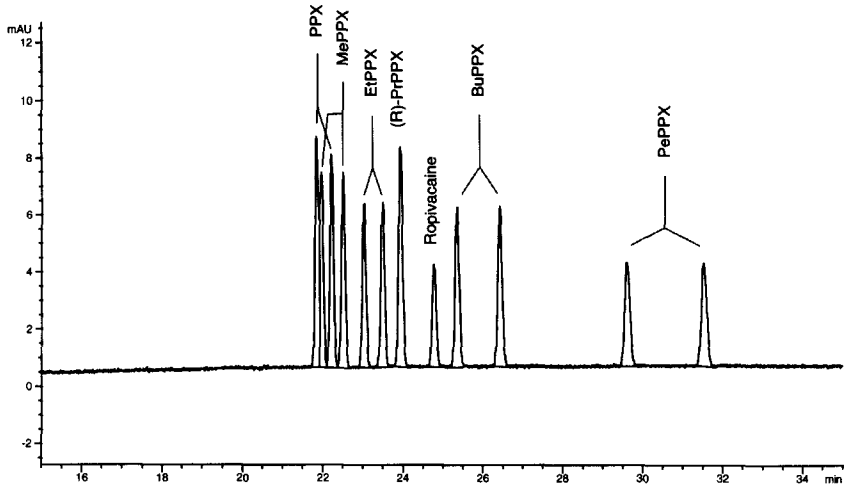

Fig. 2. Electropherogram of 44 μmol l^{-1} of ropivacaine and 100 μmol l^{-1} of its enantiomer, (*R*)-propyl-PPX, and other *n*-alkyl-PPX derivatives (90–200 μmol l^{-1} of their respective racemate). Conditions as given in the Experimental section.

2.4. Resolution

The resolution (R_s) between the two enantiomers was calculated using the following formula

$$R_s = \frac{t_2 - t_1}{w_{1/2,1} + w_{1/2,2}} \times 1.18 \quad (2)$$

where t_1 and t_2 are the migration times of the two enantiomers, and $w_{1/2,1}$ and $w_{1/2,2}$ are their respective peak widths at half the peak heights.

3. Results and discussion

The validation criteria used were similar to those applicable to the validation of an LC method at our laboratory and were derived from officially adopted guidelines for method validation [11–13].

The selectivity of the system was tested by injecting all available *n*-alkyl-PPX derivatives, including the marketed local anaesthetics mepivacaine (MePPX) and bupivacaine (BuPPX). The electropherogram, which is shown in Fig. 2, shows that the selectivity of the system is excellent and allows one to distinguish between all the analogues and their respective enantiomers. This makes the method also valuable for identification purposes. The resolution (R_s) between (*R*)-propyl-PPX and ropivacaine is as high as 4.8.

A calibration curve of ropivacaine showed good linearity in the practical concentration range 4.5–7.5 mmol l^{-1} ($y = (20.81 \pm 0.50)x + (-3.2 \pm 3.1)$; $r^2 = 0.9997$, $n = 6$), but linearity was observed even up to 30 mmol l^{-1}. A calibration curve for (*R*)-propyl-PPX was obtained in the concentration range 3–30 μmol l^{-1}, corresponding to 0.05–0.5% of the R-form in ropivacaine hydrochloride monohydrate when 6 mmol l^{-1} solutions are injected, and showed good linearity ($y = (0.024 \pm 0.001)x + (0.016 \pm 0.020)$; $r^2 = 0.9973$; $n = 6$).

Six duplicate injections of a mixture of 0.2% (*R*)-propyl-PPX in ropivacaine were carried out to test the system repeatability. The relative standard deviation was 5.6%. The corresponding figure for a mixture of 0.5% (*R*)-propyl-PPX in ropicvacaine was 3.0% (Table 1). These relative standard deviations must be considered acceptable for such low impurity concentrations. For the LC method currently used in our laboratory, the relative standard deviation at the 0.2% level is about 10%.

The limit of detection (LOD) is defined as the lowest concentration of analyte that can be detected. The LOD was determined by injecting test solutions of various concentrations of (*R*)-propyl-PPX. The limit of detection for (*R*)-propyl-PPX

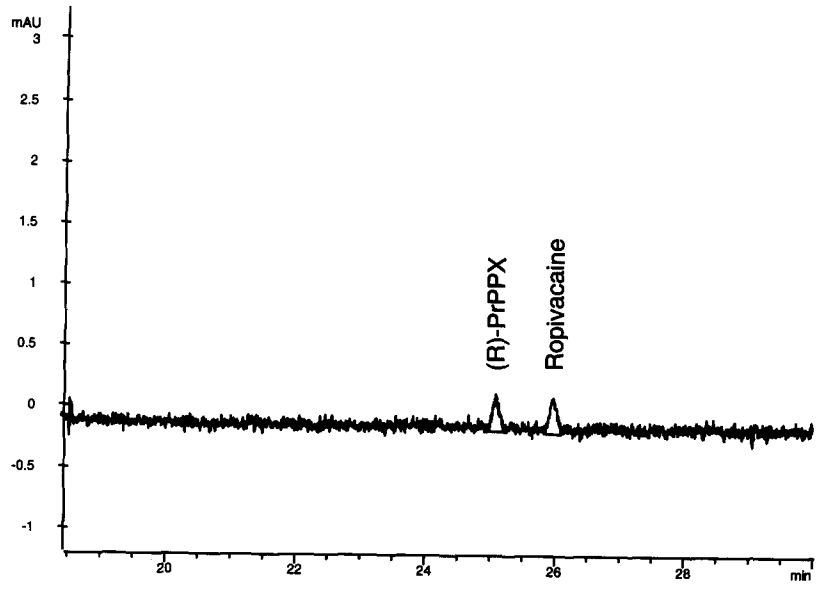

Fig. 3. Limit of detection for (*R*)-propyl-PPX. Injection of 3 μmol l^{-1} (*R*)-propyl-PPX. Conditions as described in the Experimental section.

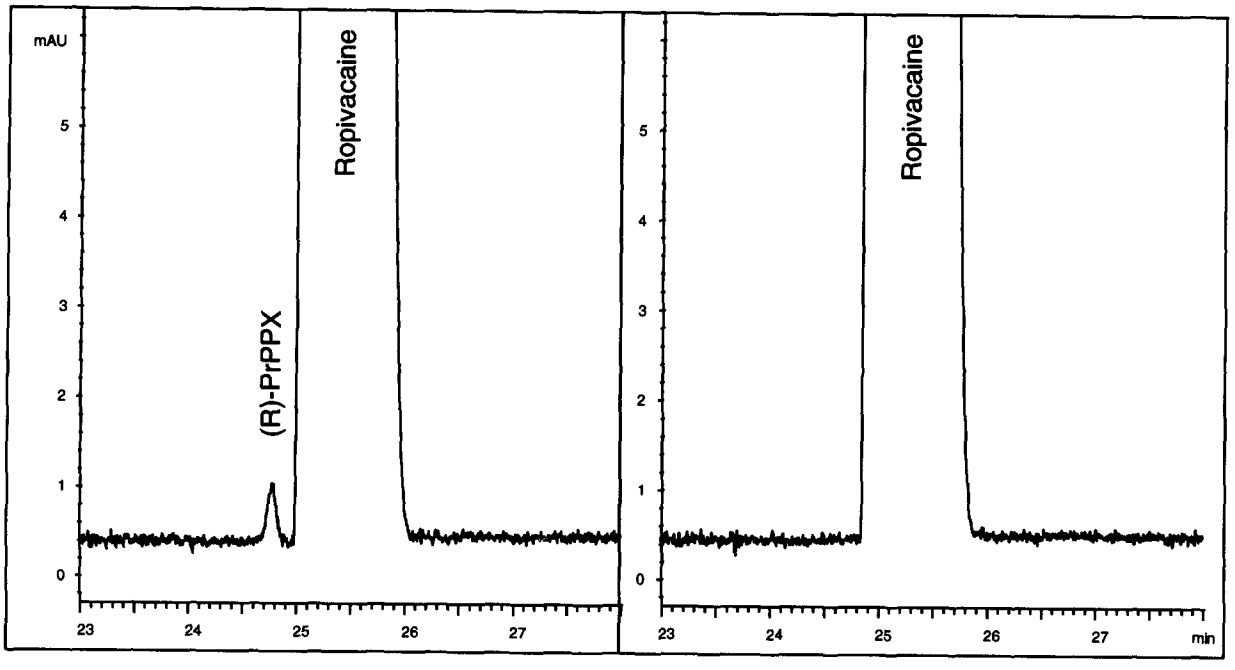

Fig. 4. Electropherograms of 0.1% (*R*)-propyl-PPX spiked in ropivacaine and of ropivacaine without (*R*)-propyl-PPX added (blank). Conditions as described in the Experimental section.

Table 1
System repeatability of 0.2 and 0.5% (*R*)-propyl-PPX in ropivacaine

Sample no.	% Impurity	Sample no.	% Impurity
1	0.21	1	0.49
2	0.21	2	0.51
3	0.21	3	0.50
4	0.19	4	0.49
5	0.20	5	0.50
6	0.18	6	0.49
Average	0.20		0.50
Standard deviation	0.011		0.015
Relative standard deviation	5.6%		3.0%

was thus determined as 3 μmol l^{-1}, i.e. corresponding to 0.05% when a 6 mmol l^{-1} solution of ropivacaine is injected. In the electropherogram (Fig. 3), a small ropivacaine peak was also observed, though not present in the sample injected. This peak resulted from carryover from a previous injection with a high concentration of ropivacaine. The size of the peak, compared to the normal peak size of ropivacaine in a purity determination, is negligible and does not interfere with the determination.

The limit of quantitation (LOQ) is defined as the lowest concentration of analyte in the sample matrix that can be determined with acceptable precision and accuracy. The LOQ of (*R*)-propyl-PPX was determined by injecting test solutions of various concentrations of (*R*)-propyl-PPX in ropivacaine and was 0.1% (Fig. 4). The relative standard deviation of six duplicate injections was 15%.

The accuracy was tested by the determination of seven samples of (*R*)-propyl-PPX added to ropivacaine in the range 0–0.5%. In Fig. 5, the theoretical concentration of impurity is plotted against the experimentally determined concentration. The slope of the plot is 0.96 ± 0.04 and the coefficient of determination, r^2 is 0.995. A small positive intercept of 0.018 ± 0.008 is observed. This is due to overestimation of the peak area by the integration software at the lower concentra-

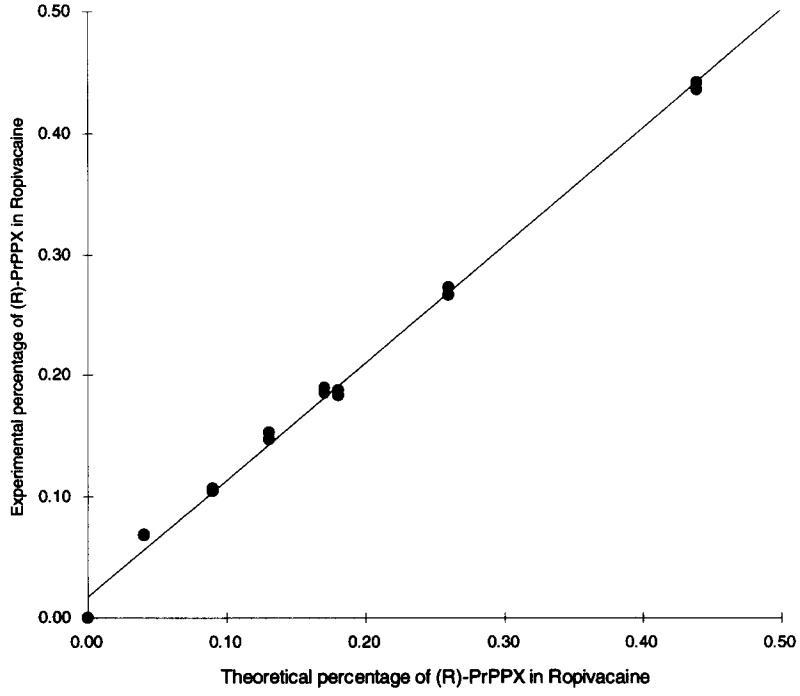

Fig. 5. Accuracy plot. The theoretical percentage of (*R*)-propyl-PPX in ropivacaine versus the experimentally determined percentage.

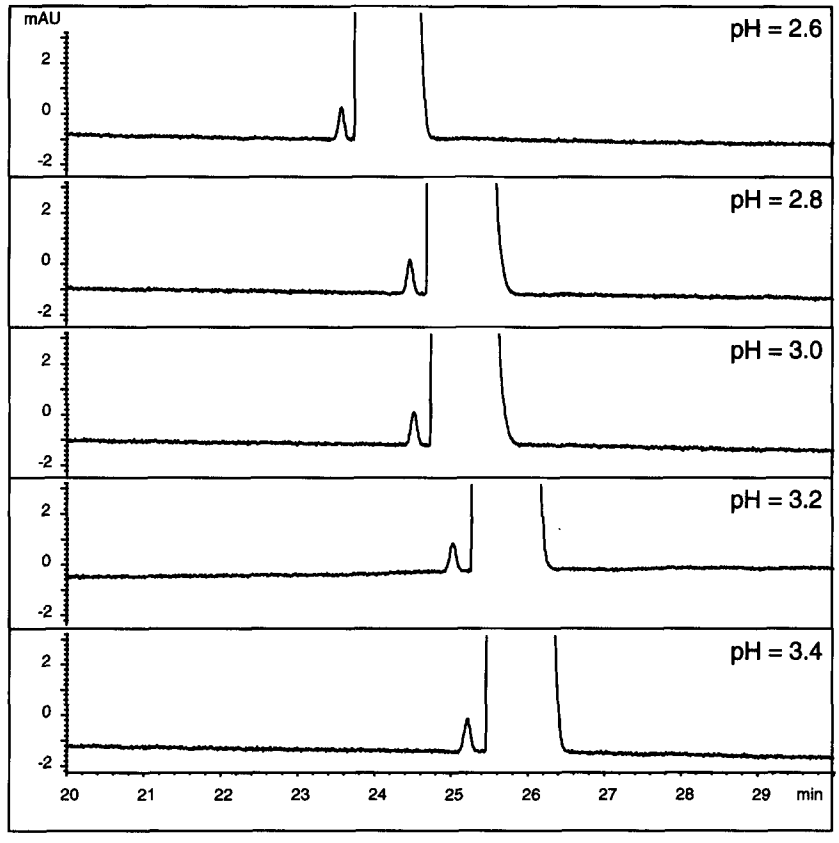

Fig. 6. Robustness test. Variation of the pH of the background electrolyte. Conditions as described in the Experimental section, except for the adjustments of the pH of the BGE solutions, which were as indicated in the figure.

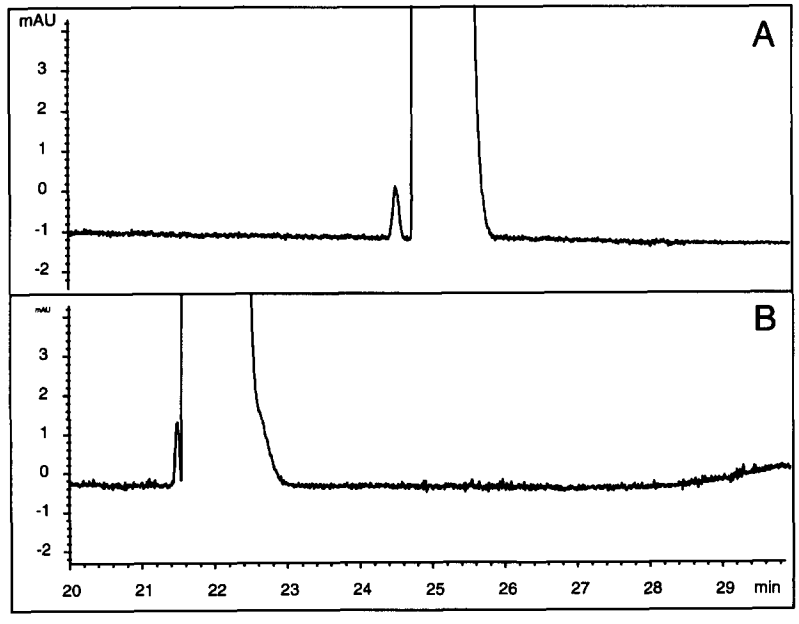

Fig. 7. Robustness test. Variation of the concentration of the background electrolyte. Conditions as described in the Experimental section, except for the BGE: (A) 0.1 mol l^{-1} phosphoric acid adjusted to pH 3.0 with triethanolamine as BGE; (B) 0.05 mol l^{-1} phosphoric acid adjusted to pH 3.0 with triethanolamine as BGE.

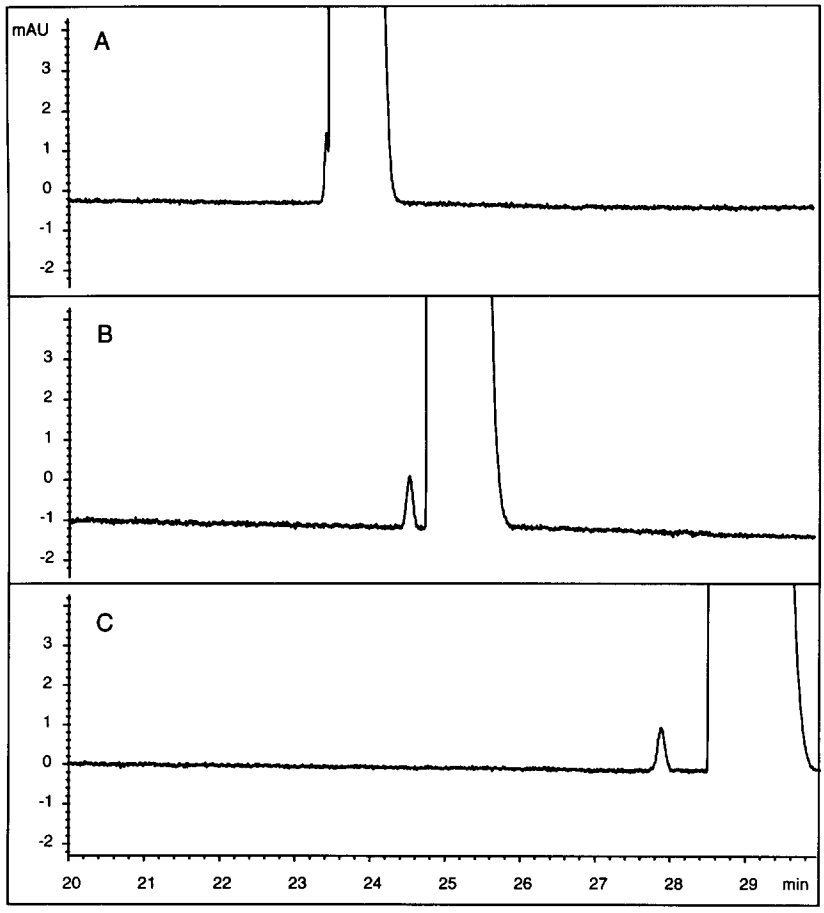

Fig. 8. Robustness test. Variation of the dimethyl-β-cyclodextrin concentration. Conditions were as described in the experimental section, except for the cyclodextrin concentration: (A) 5 mmol l^{-1} dimethyl-β-cyclodextrin; (B) 10 mmol l^{-1} dimethyl-β-cyclodextrin; (C) 20 mmol l^{-1} dimethyl-β-cyclodextrin.

tion. This effect has also been observed by other workers [14]. Nevertheless, the method proved to be sufficiently accurate.

The robustness of an analytical method is defined as a measure of its capacity to remain unaffected by small but deliberate variations in method parameters. The robustness of this CE method is tested by variations in the concentration and pH of the BGE, by variations in the concentration and origin of DM-β-CD, and by variations in the analysis temperature.

The pH of the background electrolyte was varied between 2.6 and 3.4 at 0.2 pH unit intervals. An increase in migration times with unchanged resolution between (*R*)-propyl-PPX and ropivacaine was observed by increasing the pH (Fig. 6).

A lower concentration of BGE (0.05 mol l^{-1}) was compared to 0.1 mol $^{-1}$ l of BGE, which is used in the method. At the lower concentration, the migration times and resolution were decreased (Fig. 7). The lower resolution can be explained by the lower stacking capacity of the BGE, which results in a broadening of the ropivacaine peak. The migration time decrease resulted from an increased electroosmotic flow, which was due to both the lower BGE and triethanolamine concentration.

Concentrations of 5 mmol l^{-1} and 20 mmol l^{-1} of DM-β-CD in the background electrolyte were

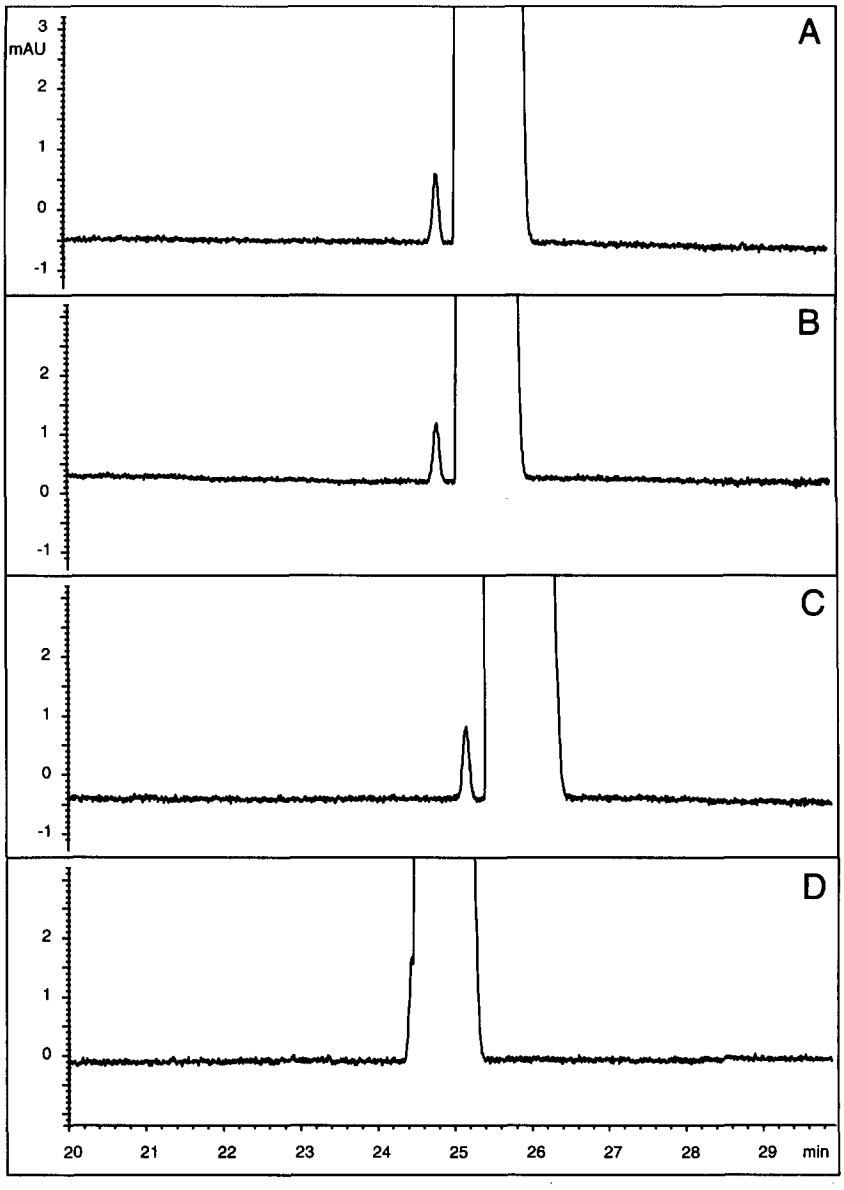

Fig. 9. Robustness test. Comparison of dimethyl-β-cyclodextrins obtained from different suppliers. (A) heptakis(2,6-di-*O*-methyl)-β-cyclodextrin obtained from Sigma, St. Louis, MA, USA; (B) dimethyl-β-cyclodextrin obtained from Tokyo Kasei, lot no. AU01; (C) dimethyl-β-cyclodextrin obtained from Pharmatec.

compared with 10 mmol l^{-1} of DM-β-CD, used in the method (Fig. 8). At 5 mmol l^{-1}, the migration times were shorter and baseline resolution was no longer obtained. However, the presence of (*R*)-propyl-PPX could still be observed. At 20 mmol l^{-1} the migration times were longer and the resolution between (*R*)-propyl-PPX and ropivacaine had increased. This cyclodextrin concentration could be used to inject higher concentrations of ropivacaine and thus achieve a gain in sensitivity expressed as percentage of the R enantiomer.

Four different lots of DM-β-CD were tested. The DM-β-CD used for the validation was

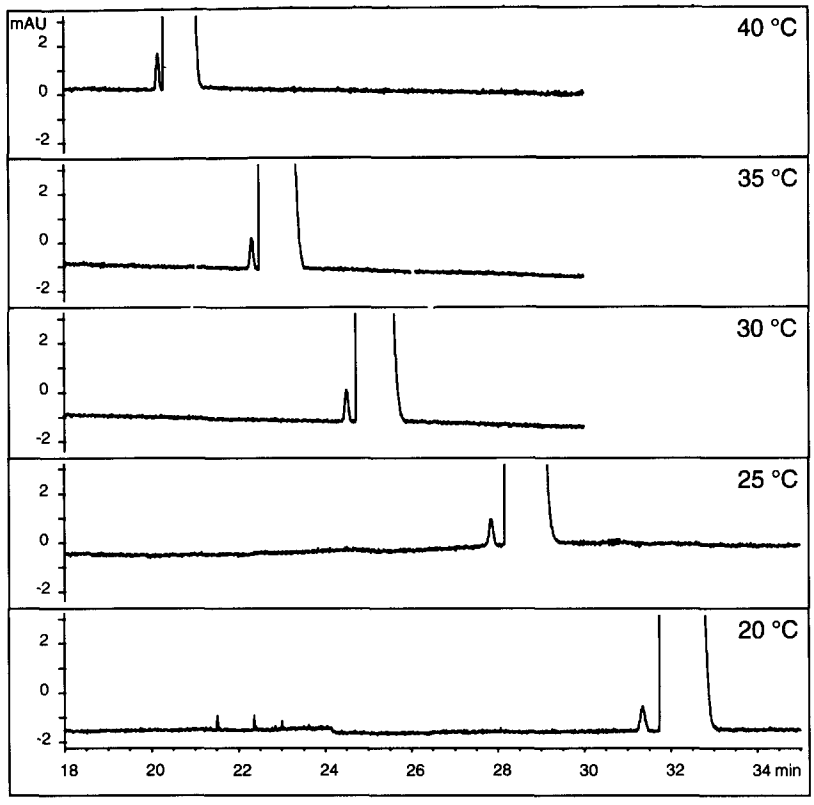

Fig. 10. Robustness test: variation of temperature. Conditions were as described in the Experimental section except for the analysis temperatures, which are indicated in the figure.

obtained from Sigma. Two different lots of DM-β-CD were obtained from Tokyo Kasei and one from Pharmatec. The lots from Sigma and Tokyo Kasei gave similar results (Fig. 9). Slight variations in migration times, but with unchanged resolutions, were observed. The lot from Pharmatec gave faster migration and decreased resolution. Preliminary time-of-flight mass spectroscopic analyses of these different lots of DM-β-CDs indicate that the degree of methylation of the DM-β-CD from Pharmatec differs from that of the others. The effect of differences in the degrees of substitution of the cyclodextrin have been discussed by Valkó [15].

The analysis temperature was varied between 20 and 40°C at 5°C intervals and was compared with 30°C, which was used in the method (Fig. 10). The increase in temperature resulted in decreased migration times and resolutions, although over the entire range 20–40°C, baseline separation was observed. A lower analysis temperature could be used to inject higher concentrations of ropivacaine when a higher sensitivity of the method is required.

4. Conclusions

The capillary electrophoresis method, comprising the use of dimethyl-β-cyclodextrin, offers very good separation of ropivacaine and its enantiomer, (*R*)-propyl-PPX. The method has been validated and shows good performance with respect to selectivity, linearity, system repeatability, accuracy, robustness and the required limits of detection and quantitation. It is a robust method, which makes it very suitable for the quality control of the enantiomeric purity of ropivacaine.

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