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A chiral capillary electrophoresis method for ropivacaine hydrochloride in pharmaceutical formulations: validation and comparison with chiral liquid chromatography

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Abstract

A capillary electrophoresis method for the determination of the enantiomeric purity of the local anaesthetic ropivacaine hydrochloride in injection solutions has been validated. The method showed the required limit of quantitation of 0.1% enantiomeric impurity. Good performances were shown for specificity, linearity, system repeatability, intermediate precision and accuracy. Robustness was tested via a full factorial design at two levels and the method proved to be robust. Comparison of the capillary electrophoresis method with the liquid chromatographic method currently used for several years at our laboratory on real samples of ropivacaine injection solutions showed that the techniques do not give significantly different results. © 1997 Elsevier Science B.V.

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

1. Introduction

Capillary electrophoresis (CE) is a powerful tool for the separation of enantiomeric drugs. In an earlier paper we presented the results of the validation and robustness testing of the enantiomeric purity determination of (*S*)-ropivacaine hydrochloride monohydrate raw material [1]. In this paper the results of the validation of the chiral CE method for the enantiomeric purity testing of ropivacaine hydrochloride injection solutions (Naropin® solutions) in polypropylene ampoules and bags (Polyamp DuoFit® and Polybag®) are presented.

The validation criteria and definitions were similar to those applicable to the validation of a chromatographic method in our laboratory and were derived from officially adopted guidelines for method validation [2–5]. Chiral CE methods that to a greater or lesser extent were validated have been reported [1,6–15].

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2. Experimental

2.1. Capillary electrophoresis

2.1.1. Conditions

The validation was performed on an 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 i.d. 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). UV detection was at 206 nm with a band width of 4 nm. Preconditioning of the capillary was programmed for each run and consisted of 1 min flush with water, 4 min flush with 0.1 mol l^{-1} of NaOH (sodium hydroxide solution for HPCE, Fluka BioChemika, Buchs, Switzerland), 1 min flush with water and 4 min flush with run buffer.

The background electrolyte solution (BGE) was made by adjusting a solution of 0.1 mol l^{-1} of phosphoric acid (AG, Merck, Darmstadt, Germany) to pH 3.0 with triethanolamine (AG, Merck, Darmstadt, Germany). The run buffer consisted of 133 mg heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD, Sigma, St. Louis, USA) per 10 ml BGE, resulting in a concentration of 10 mmol l^{-1} of DM-β-CD.

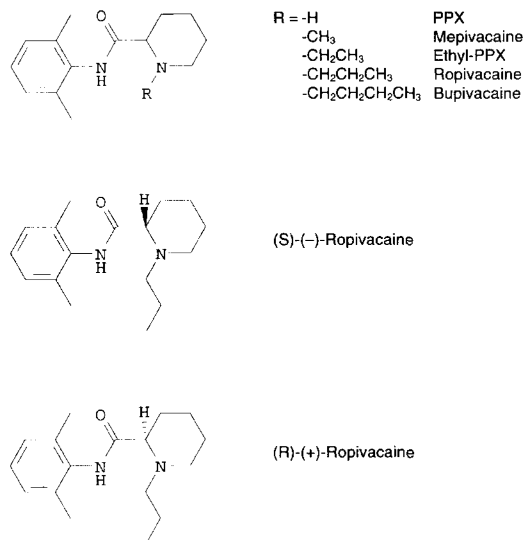

Fig. 1. Structures of the PPX analogues.

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

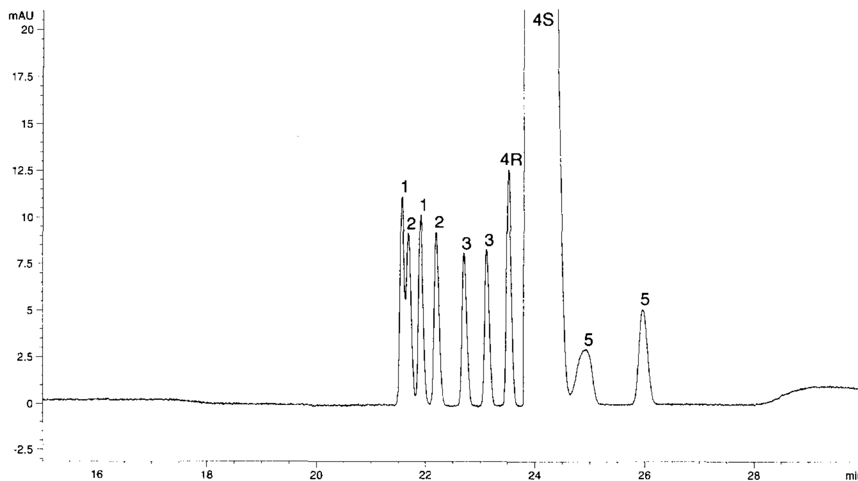

Fig. 2. Electropherogram of ropivacaine and other *n*-alkyl-PPX analogues. Conditions as given in Section 2. 1, PPX; 2, mepivacaine; 3, ethyl–PPX; 4R, (*R*)-ropivacaine; 4S, (*S*)-ropivacaine; 5, bupivacaine.

Fig. 3. LOQ for (*R*)-ropivacaine in ropivacaine hydrochloride injection solution. Conditions as described in Section 2.

2.1.2. Purity determination

The enantiomeric purity of a 2 mg ml^{-1} injection solution of ropivacaine hydrochloride was determined by direct injection. Injection solutions with a concentration of 7.5 mg ml^{-1} or 10 mg ml^{-1} were diluted in MilliQ filtered water to a concentration of 2.0 mg ml^{-1}. The percentage of the (*R*)-enantiomer is calculated by internal normalisation from corrected peak areas (peak area/migration time) and according to Eq. (1):

$$\%\text{impurity} = \frac{A_R/t_R}{A_S/t_S + A_R/t_R} \cdot 100 \quad (1)$$

where A_R and A_S are the measured peak areas and t_R and t_S the migration times for (*R*)-ropivacaine and (*S*)-ropivacaine respectively.

2.1.3. Resolution

The resolution between (*R*)-ropivacaine and (*S*)-ropivacaine was calculated according to Eq. (2):

$$R_S = 1.18 \frac{t_S - t_R}{w_{1/2,S} + w_{1/2,R}} \quad (2)$$

where $w_{1/2,S}$ and $w_{1/2,R}$ are the peak widths at half the peak height of (*S*)-ropivacaine and (*R*)-ropivacaine respectively.

For evaluation of the robustness testing, graphical software for statistical experimental design, Modde™ (Umetri, Umeå, Sweden), was used. The model was a full factorial two-level design. The factors were the phosphoric acid concentration (high 0.11 mol l^{-1}, low 0.09 mol l^{-1}), the DM-β-CD concentration (high 12 mmol l^{-1}, low 8 mmol l^{-1}), the pH (high 3.1, low 2.9) and the temperature (high 32°C, low 28°C). The response was the resolution between the *R* and the *S* form of ropivacaine.

Table 1
Limit of quantitation of the (*R*)-form

Injection number	(*R*)-ropivacaine (%)
1	0.074
2	0.075
3	0.081
4	0.078
5	0.082
6	0.090
Mean	0.08
R.S.D. (%)	7.6

Six repeated injections of a solution of 2.0 mg ml^{-1} of (*S*)-ropivacaine hydrochloride monohydrate working standard solution in 0.9% sodium chloride.

2.2. Liquid chromatography

2.2.1. Conditions

The separation was performed on a 100 mm long and 4.0 mm i.d. chiral-AGP colum (Chromtech, Middelburg, Netherlands) with 5 μm particles. A 10 mm long and 3.0 mm i.d. chiral-AGP guard column was used. The mobile phase consisted of 35.0 ml isopropanol (May and Baker, Dagenham, UK) diluted to 500.0 ml with phosphate buffer pH 7.2, $\mu = 0.05$. The flow rate was 1.0 ml min^{-1}. The injected volume was 20 μl. UV detection was performed at 220 nm.

2.2.2. Purity determination

The ropivacaine hydrochloride injection solution was diluted with the mobile phase to a concentration of 75 μg ml^{-1} of ropivacaine hydrochloride. The percentage of the R-enantiomer was calculated from the peak areas A_R and A_S by internal normalisation according to Eq. (3):

$$\%\text{impurity} = \frac{A_R}{A_S + A_R} \cdot 100 \qquad (3)$$

For the system suitability test, a solution of about 75 μg ml^{-1} of ropivacaine hydrochloride with 0.5–1.0% (R)-form is injected. There should be baseline separation between the ropivacaine enantiomers and the R.S.D. of the peak area of the (R)-form of three replicate injections should be less than 5%.

2.3. Test components

2′,6′-pipecoloxylidide hydrochloride (PPX, racemate, working standard), (S)-ropivacaine hydrochloride monohydrate ((S)-propyl-PPX, working standard, batch no. 201/94), (R)-ropivacaine hydrochloride monohydrate ((R)-propyl-PPX), mepivacaine hydrochloride (methyl-PPX, racemate, working standard), ethyl-PPX hydrochloride (racemate) and bupivacaine hydrochloride monohydrate (butyl-PPX, racemate, working standard) were obtained from Astra Pain Control, Södertälje, Sweden (Fig. 1).

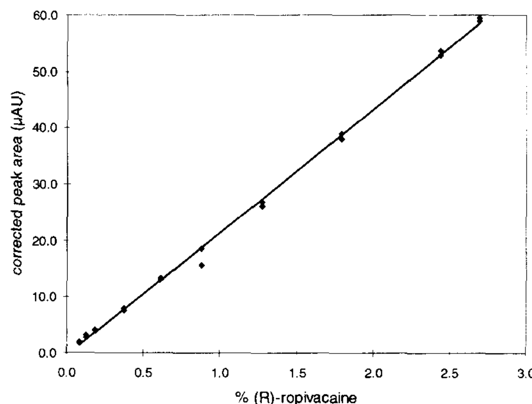

Fig. 4. Linearity of (R)-ropivacaine in ropivacaine hydrochloride injection solution. $y = (21.9 \pm 0.5)x + (-0.4 \pm 0.7)$, $r^2 = 0.9981$. Conditions as described in Section 2.

3. Results and discussion

In order to obtain robust capillary electrophoresis methods, we apply some general rules for capillary electrophoresis in our laboratory. New capillaries are washed before being used for the first time to remove possible contaminants from the manufacture of the capillary and to enable reproducible results to be obtained. The capillary is flushed with water at a pressure of approximately 1 bar for 1 min and then for 30 min with 0.1 mol l^{-1} NaOH. Capillaries that are stored are first flushed for 10 min with 0.1 mol l^{-1} NaOH and then for 10 min with water. Since each capillary has its own history, e.g. buffer additives are not always easily removed from the capillary wall, it is dedicated to a specific application.

Buffer solutions that contain chiral selectors or other additives are always freshly prepared. The solutions are filtered through filters with a pore size of 0.45 μm immediately before use to remove particles and air bubbles.

Since the injected volume is influenced by viscosity differences, it is important that the capillary temperature is constant to obtain good reproducibility of peak areas. The sample vials are allowed to attain a constant temperature on the autosampler. When possible, longer injection times, e.g. 5–10 s are employed. The pressure difference generated for hydrodynamic injection is moni-

tored during injection and if it does not accord with the set level, the injection time is adjusted automatically by the instrument. If short injection times are employed, the ability to regulate the injection is reduced. During injection the capillary end is immersed in a vial containing electrophoresis buffer at a constant level, to avoid fluctuations in injection volume due to differences in hydrodynamic forces. To minimise the effect of sample loss due to thermal expansion, an electrolyte plug can be injected after the sample plug or a voltage gradient can be applied, as was done in this method. Since variance in precision is mostly due to fluctuations in injection volume, the incorporation of an appropriate internal standard can minimise this source of error. For this method, however, calculations were done by internal normalisation, so no internal standard was needed.

In each analysis sequence, a system suitability test is performed at the beginning and end of the sequence. For the method presented in this paper, the test mixture was a solution of 2.0 mg ml^{-1} of (*S*)-ropivacaine hydrochloride and 4.0 μg ml^{-1} of (*R*)-ropivacaine hydrochloride in 0.9% sodium chloride solution, corresponding to a 2 mg ml^{-1} of ropivacaine hydrochloride injection solution containing 0.2% enantiomeric impurity ((*R*)-form).

3.1. Specificity

The specificity was tested by mixing the PPX analogues (Fig. 1) into the sample matrix, i.e. a physiological salt solution. The electropherogram in Fig. 2 shows that the specificity of the system is excellent and also separates the enantiomers of the other PPX analogues.

3.2. Detection and quantitation limits

The electropherogram in Fig. 3 shows that the limit of detection (LOD) is clearly lower than the 0.08% of (*R*)-form originally present in the (*S*)-ropivacaine hydrochloride monohydrate working standard. The working standard available at the time of validation of the method for the purity determination of the raw material had less chiral impurity. The LOD then determined corresponded to 0.05% of the (*R*)-form [1]. For the chiral LC method, a detection limit of 0.1% of the (*R*)-form has been reported [16].

The limit of quantitation (LOQ) for (*R*)-ropivacaine was determined by injecting a solution of the working standard corresponding to 2.0 mg ml^{-1} of ropivacaine hydrochloride in 0.9% NaCl, since this solution contained a quantifiable amount of (*R*)-ropivacaine (Fig. 3). The chiral impurity was 0.08% and the R.S.D. of six duplicate injections of this solution was 8% (Table 1).

3.3. Linearity

A calibration curve for the (*R*)-form was made in a concentration range of 0–3% in ropivacaine hydrochloride injection solution. Since a small amount of the (*R*)-form is present in the (*S*)-ropivacaine hydrochloride monohydrate working standard, this was taken into account. The plot of corrected peak areas versus amount of (*R*)-ropivacaine showed good linearity (Fig. 4).

3.4. Accuracy

The accuracy was tested by the determination of (*R*)-ropivacaine added to a solution of (*S*)-ropivacaine hydrochloride monohydrate working standard in a physiological salt solution. Nine

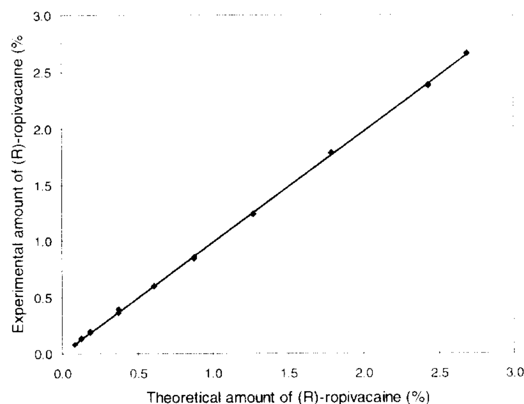

Fig. 5. Accuracy test: experimentally determined amount of (*R*)-form vs. the theoretical amount. The slope of the plot is 0.98 ± 0.01, the intercept is 0.01 ± 0.01 and the coefficient of determination, r^2, is 0.9998. Conditions as described in Section 2.

Table 2
System repeatability and intermediate precision. A, B and C are the results obtained on three different days

	Injection number	Levels of (*R*)-form (%)				
		Level 1	Level 2	Level 3	Level 4	Level 5
A	1	0.11	0.38	0.52	1.22	2.56
	2	0.12	0.38	0.53	1.25	2.60
	3	0.12	0.37	0.53	1.22	2.58
	4	0.13	0.35	0.53	1.23	2.59
	5	0.13	0.37	0.51	1.21	2.56
	6	0.13	0.37	0.53	1.18	2.58
	Mean	0.12	0.37	0.53	1.22	2.58
	R.S.D. (%)	6.6	2.6	1.4	1.8	0.6
B	1	0.13	0.36	0.53	1.22	2.58
	2	0.15	0.35	0.51	1.23	2.60
	3	0.15	0.35	0.52	1.21	2.60
	4	0.13	0.36	0.53	1.24	2.58
	5	0.13	0.35	0.52	1.22	2.57
	6	0.12	0.36	0.51	1.23	2.59
	Mean	0.14	0.36	0.52	1.23	2.58
	R.S.D. (%)	8.1	1.8	1.6	0.7	0.4
C	1	0.13	0.37	0.50	1.23	2.57
	2	0.14	0.37	0.54	1.21	2.58
	3	0.13	0.37	0.52	1.22	2.55
	4	0.13	0.36	0.51	1.19	2.58
	5	0.12	0.36	0.54	1.20	2.58
	6	0.14	0.36	0.53	1.20	2.59
	Mean	0.13	0.37	0.52	1.21	2.58
	R.S.D. (%)	4.5	1.6	2.6	1.2	0.6
	Mean of means	0.13	0.36	0.52	1.22	2.58
	Within assay R.S.D. (%)	6.6	2.0	1.9	1.3	0.5
	Between assays R.S.D. (%)	4.9	2.0	0.5	0.7	0.2

mixtures in the range 0–3% of (*R*)-form were injected. When calculating the theoretical percentages of (*R*)-ropivacaine, the amount present in the (*S*)-ropivacaine hydrochloride monohydrate working standard was taken into account. In Fig. 5 a plot of the theoretical concentration of impurity against the experimentally determined concentration shows that the method is accurate.

3.5. System repeatability and intermediate precision

To test the system repeatability, five different levels between 0.1–2.6% of (*R*)-ropivacaine in injection solution were each injected six times. The R.S.D. were 6.6–0.6% (Table 2A). The poorer precision at the lowest concentrations is related to the small peak size. For the LC method currently used in our laboratory, the R.S.D. at the 0.2% impurity level is about 10% and at the 2% impurity level 1.5% R.S.D. [16]. These figures support the use of the CE method.

The intermediate precision reflects the within-laboratory variation. In our laboratory, at the time of the validation, this meant that the same instrument was used by the same analyst but on three different days and with freshly prepared solutions. The results presented in Table 2 show that the R.S.D. are about the same as for the system repeatability.

Table 3
Full factorial two-level design robustness test

Experiment	Run order	pH	H_3PO_4 concentration (mol l^{-1})	Cyclodextrin concentration (mmol l^{-1})	Temperature (°C)	Resolution
+		3.1	0.11	12	32	
−		2.9	0.09	8	28	
N1	1	2.9	0.09	8	28	3.75
N2	18	3.1	0.09	8	28	3.82
N3	19	2.9	0.11	8	28	4.01
N4	16	3.1	0.11	8	28	3.96
N5	11	2.9	0.09	12	28	5.00
N6	12	3.1	0.09	12	28	5.13
N7	2	2.9	0.11	12	28	5.29
N8	14	3.1	0.11	12	28	5.19
N9	6	2.9	0.09	8	32	3.68
N10	7	3.1	0.09	8	32	3.70
N11	3	2.9	0.11	8	32	3.82
N12	10	3.1	0.11	8	32	3.80
N13	8	2.9	0.09	12	32	4.92
N14	17	3.1	0.09	12	32	4.94
N15	5	2.9	0.11	12	32	5.04
N16	4	3.1	0.11	12	32	5.09
N17[a]	13	3.0	0.10	10	30	4.45
N18[a]	15	3.0	0.10	10	30	4.43
N19[a]	9	3.0	0.10	10	30	4.48

[a] Centre points.

3.6. Robustness

In the robustness test the method parameters that are expected to vary between days and circumstances or those known to affect the resolution were tested. A great deal is already known about these parameters and the aim of this study was to determine whether the analytical results would be affected by small changes. The earlier robustness test for ropivacaine hydrochloride monohydrate substance was performed by varying the experimental parameters one by one [1]. The parameters were now varied according to a full factorial design at two levels. A Plackett-Burman designed test was presented by Rogan [17]. An inter-company cross-validation showed that a chiral CE method could successfully be transferred between laboratories [6].

Four experimental parameters were studied, i.e. the concentration of DM-β-CD, the concentration of phosphoric acid, the temperature and the pH. The concentration of the cyclodextrin has a strong effect on the resolution. The model developed by Wren [18,19] predicts an optimum concentration. Our previous experiments where the association equilibrium constants for the ropivacaine–cyclodextrin complex were calculated showed that the method concentration is below the optimum concentration [20]. This means that a higher concentration of DM-β-CD will result in a higher resolution.

The way the BGE is prepared is important. The concentration of phosphoric acid not only affects the stacking of the injected zone, but also the amount of triethanolamine added. Triethanolamine is used to adjust the pH and affects the electroosmotic flow (EOF) [21], which is reversed in this system [20]. The amount of triethanolamine added results in a more or less negative EOF, which in turn affects the resolution [22].

An important observation in previous work was that the quality of the cyclodextrin varied among the different suppliers and that this had an

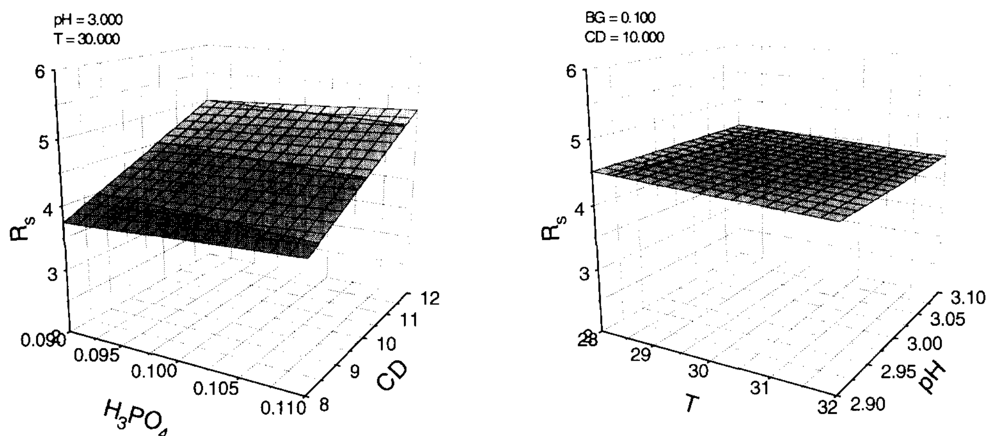

Fig. 6. Robustness test: response surface for the concentration of DM-β-CD (CD) and concentration of phosphoric acid (H_3PO_4) and for temperature (T) and pH.

important impact on the determination [1]. Other groups observed similar variations for hydroxypropyl-β-cyclodextrin or DM-β-CD [10,23–25]. We nowadays only use DM-β-CD supplied by Sigma, so the quality of DM-β-CD was not included in this test. At this moment, we have been using three different batches of DM-β-CD that were supplied by Sigma and have not found any significant differences.

The selection of four parameters suggests a 2^4 full factorial design, i.e. 16 experiments. Three centre points, i.e. the normal experimental conditions, were added. The total of 19 experiments were performed in random order and the resolution between (R)- and (S)-ropivacaine was measured. Since the peak for (S)-ropivacaine at the assay concentration is triangular and highly asymmetric (Fig. 3), calculation of the resolution on such a separation is not meaningful. To determine the effect of the parameters on the separation, a lower concentration of (S)-ropivacaine hydrochloride (15 μg ml^{-1}) was used.

The experimental set-up and the results are presented in Table 3 and Fig. 6.

The resolution varies between 3.7 and 5.3 with the concentration of the cyclodextrin having the strongest effect. Fig. 7 shows the separation of a real injection solution under the conditions that gave the lowest resolution, i.e. $T = 32°C$, $[H_3PO_4] = 0.09$ mol l^{-1}, pH = 2.9, [DM-β-CD] = 8 mmol l^{-1}. Even under these conditions an acceptable separation was obtained and the determination of the enantiomeric purity was not affected. This proves that this CE method is robust.

3.7. Comparison with liquid chromatography

At present a chiral liquid chromatographic (LC) method is used to determine the enantiomeric purity of ropivacaine hydrochloride in pharmaceutical formulations (Fig. 8). Several injection solutions were determined by LC as well as CE. The samples were injection solutions at concentrations of 2, 7.5 or 10 mg ml^{-1} of ropivacaine hydrochloride in different sizes of polypropylene ampoules or bags. They represented samples normally analysed in our laboratory and originated from stability trials i.e. they were stored for different periods of time at different temperatures and humidities. The purity determinations were performed as described in Section 2.

The results of both methods are presented in Table 4. A paired t-test was performed giving a calculated value for t of 0.62. The critical value for $|t|$ is 2.06 (25 degrees of freedom, 95% confidence interval), which means that the two different methods do not give significantly different results.

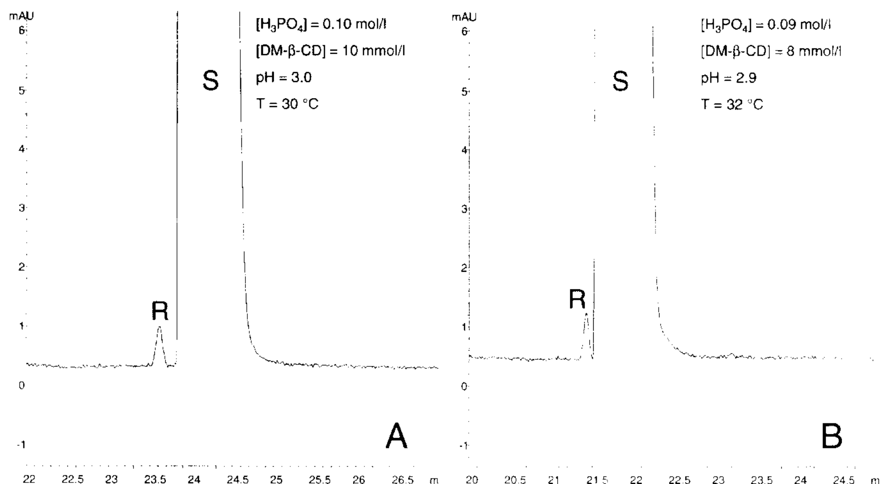

Fig. 7. Robustness test: comparison of the standard conditions (A) with the conditions that gave the lowest resolution in the robustness tests (B).

Since the LC method was developed and validated earlier, it is filed in the New Drug Application (NDA). At present, both methods are used in parallel in ongoing stability studies to gain more experience in routine analysis and to train more analysts in the CE technique.

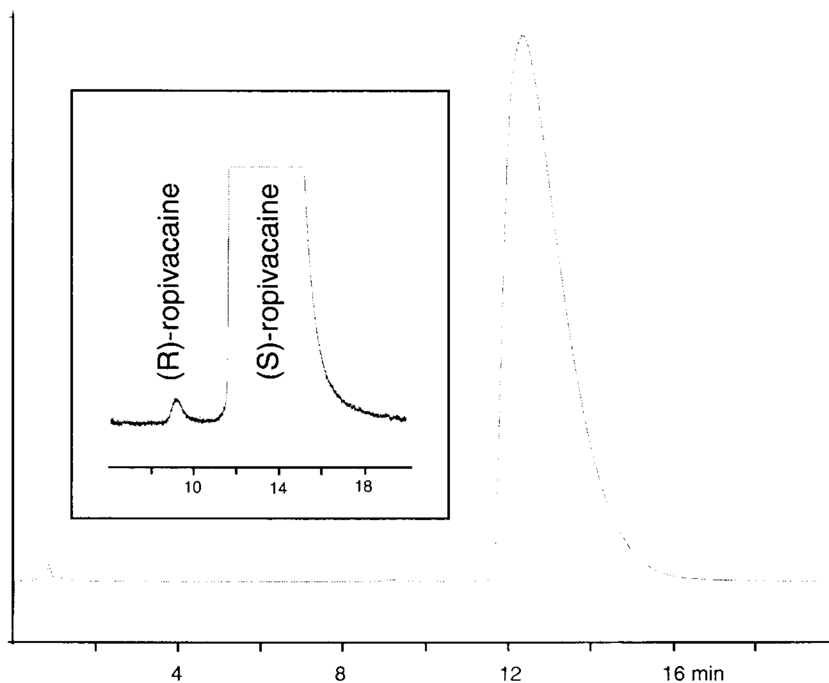

Fig. 8. Chiral LC separation of (*S*)-ropivacaine with 0.2% chiral impurity of its enantiomer (*R*)-ropivacaine. Conditions as described in Section 2.

Table 4
Comparison of chiral LC and CE for the enantiomeric purity determination of ropivacaine hydrochloride injection solutions

Sample	Concentration ropivacaine HCl	Batch	Packaging	Storage time (months)	Storage temperature (°C)	Storage humidity (%)	(*R*)-ropivacaine LC (%)	(*R*)-ropivacaine CE (%)
1	10	465-19-3	10 ml pp-amp	21	30	50	0.10	0.11
2	10	465-19-3	10 ml pp-amp	21	40	75	0.30	0.32
3	10	465-30-3	10 ml pp-amp	3	30	50	0.24	0.25
4	10	465-30-3	10 ml pp-amp	3	40	75	0.26	0.27
5	10	465-22-3	20 ml pp-amp	21	30	50	0.14	0.17
6	10	465-22-3	20 ml pp-amp	21	40	75	0.39	0.40
7	7.5	472-36-2	10 ml pp-amp	38	30	50	0.26	0.28
8	7.5	472-36-2	10 ml pp-amp	38	40	75	0.69	0.68
9	7.5	472-38/1-2	20 ml pp-amp	35	30	50	0.27	0.29
10	7.5	472-38/1-2	20 ml pp-amp	35	40	75	0.60	0.60
11	2	1202-35-7	10 ml pp-amp	3	30	50	0.23	0.24
12	2	1202-35-7	10 ml pp-amp	3	40	75	0.30	0.29
13	2	1202-62-7	10 ml pp-amp	0			0.18	0.19
14	2	1202-6/1-2	20 ml pp-amp	35	30	50	0.55	0.51
15	2	1202-6/1-2	20 ml pp-amp	35	40	75	1.55	1.47
16	2	1202-59-7	20 ml pp-amp	3	30	50	0.35	0.35
17	2	1202-59-7	20 ml pp-amp	3	40	75	0.36	0.36
18	2	1202-60-7	20 ml pp-amp	3	30	50	0.52	0.49
19	2	1202-60-7	20 ml pp-amp	3	40	75	0.57	0.55
20	2	1202-63-7	20 ml pp-amp	0			0.27	0.27
21	2	1202-33-7	200 ml polybag	8	30	50	0.43	0.42
22	2	1202-33-7	200 ml polybag	8	40	75	0.71	0.67
23	2	1202-13/1-2	100 ml polybag	33	30	50	0.43	0.44
24	2	1202-13/1-2	100 ml polybag	33	40	75	1.03	0.99
25	2	1202-12/1-2	100 ml polybag	34	30	50	1.10	1.12
26	2	1202-12/1-2	100 ml polybag	34	40	75	2.55	2.57
							Mean difference:	0.003
							S.D.:	0.025
							t-ratio:	0.62

pp-amp, Polypropylene ampoule; polybag, polypropylene bag.

4. Conclusions

The capillary electrophoresis method comprising the use of DM-β-CD offers very good enantiomeric separation of ropivacaine hydrochloride in injection solutions. The method has been validated and shows good performance with regard to selectivity, linearity, system repeatability, intermediate precision and accuracy and meets the required limit of quantitation. It is robust, which makes it very suitable for quality control of the enantiomeric purity of ropivacaine hydrochloride in pharmaceutical formulations. Comparison of the CE method with the existing chiral LC method showed that the methods do not give significantly different results for the determination of the enantiomeric purity of ropivacaine hydrochloride injection solutions.

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