Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 41 (2006) 77–83
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Method development for the enantiomeric purity determination of low concentrations of adrenaline in local anaesthetic solutions by capillary electrophoresis

Cari E. Sänger-van de Griend*, Anders G. Ek, Monica E. Widahl-Näsman, E.K. Margareta Andersson

Analytical Development, Pharmaceutical and Analytical R&D, AstraZeneca, SE-151 85 Södertälje, Sweden

Received 12 August 2005; received in revised form 11 October 2005; accepted 12 October 2005
Available online 21 November 2005

Abstract

L-Adrenaline is often included in local anaesthetic (LA) solutions for injection to improve the quality of the anaesthetic block. The concentration of the LA is between 2.5 and 20 mg/ml and the concentration of adrenaline is typically ≤0.1% of the LA concentration. In order to follow the racemization into D-adrenaline, not only is chiral separation needed but also sufficient resolution from the LA and other components of the injection solution. Furthermore, very high sensitivity is needed in order to be able to determine the D-enantiomer at very low concentrations, i.e. down to about 0.1 μg/ml. The development of a chiral capillary electrophoresis method that is able to determine the racemization of adrenaline is described, together with a limited validation. Samples are injected without pretreatment and analysed with a capillary electrophoresis buffer containing 40 mM heptakis(2,6-di-*O*-methyl)-β-cyclodextrin, 0.10 M phosphoric acid and 0.05 M triethanolamine. The amounts of D-adrenaline found in the LA products tested were typically <3% of the L-adrenaline concentration and <0.003% of the LA concentration.

Keywords: Chiral CE; Adrenaline; Epinephrine; Local anaesthetic; Bupivacaine; Lidocaine; Prilocaine; Mepivacaine

1. Introduction

Local anaesthetics (LAs) offer the possibility to anaesthetize a selective part of the body by reversibly blocking the propagation of nerve impulses. To do this, the LA solution is injected at its site of action, e.g. a peripheral nerve. A shorter or longer duration of block can be obtained by choosing a suitable type of LA. To improve the quality of the block, adrenaline (Fig. 1) is commonly included in LA solutions to produce vasoconstriction at the site of injection. This will delay the absorption of the LA, decrease its peak blood concentration and prolong the duration of block [1]. The L (levo)-form of adrenaline, which is 10 times more potent than the D (dextro)-form [2], is used in solutions for injection. The products contain adrenaline tartrate and the standard concentration of L-adrenaline (base) is 5 μg/ml, although concentrations of 2.5–12.5 μg/ml of L-adrenaline are used [1]. The LAs that are combined with L-adrenaline are lidocaine, bupivacaine, prilocaine and mepivacaine in the form of hydrochlorides (Table 1). Their concentrations as hydrochloride in solution vary between 2.5 and 20 mg/ml. Thus, the concentration of L-adrenaline is typically ≤0.1% of the concentration of the LA.

L-Adrenaline in solution may be deactivated by racemization or other degradation pathways. In order to follow the racemization of L-adrenaline into D-adrenaline, not only is chiral separation needed, but also sufficient resolution from the LA and other components of the injection solutions. Furthermore, very high sensitivity is needed to determine the D-enantiomer at very low concentrations down to 0.1 μg/ml. The ionic strength of the solutions is high as the injection solutions contain sodium chloride to make them isotonic. The pH of the injection solutions ranges between 3 and 5.

Britz-McKibbin et al. published some interesting papers on the quantitative (achiral) assay of adrenaline in dental anaesthetic solutions by capillary electrophoresis (CE) [3–5]. They made use of the composition of the adrenaline-containing LA

* Corresponding author. Tel.: +46 8 553 27733.
E-mail address: cari.sanger@astrazeneca.com (C.E. Sänger-van de Griend).

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.10.012

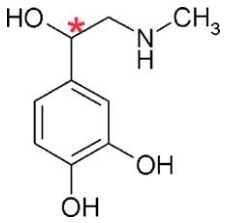
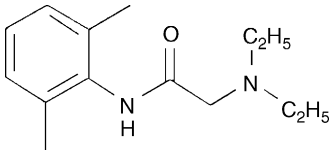
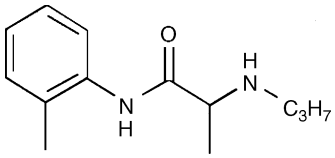
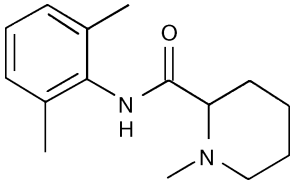
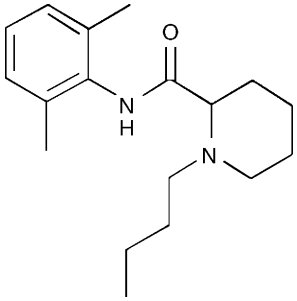


Fig. 1. Adrenaline (epinephrine), pK_a's: 8.7 and 9.9.

solutions to selectively focus adrenaline using a dynamic pH junction. Although the sample matrix had high conductivity, adrenaline was selectively stacked because of the pH difference between the sample and the electrolyte and because of the presence of borate in the electrolyte, which selectively complexes with adrenaline. Thus, they were able to apply large sample plugs and obtain a highly sensitive assay for adrenaline.

There are several papers in the literature dealing with the enantiomeric separation of adrenaline by CE [6–13]. In all of them cyclodextrins were used as chiral selector, both charged and uncharged, and at low, intermediate and high pH. Some of them involve plain adrenaline pharmaceuticals [7,8], though none in the concentration range concerned and none with the presence of high concentrations of LAs.

Table 1
Local anaesthetics

Local anaesthetic	pK_a	Structure
Lidocaine	7.8	
Prilocaine	7.9	
Mepivacaine	7.7	
Bupivacaine	8.1	

In this paper a method is presented for determining the enantiomeric purity of L-adrenaline at ppm levels in isotonic LA solutions.

2. Experimental

2.1. Chemicals

Sodium hydroxide and phosphoric acid, both of analytical grade were obtained from Merck (Darmstadt, Germany). Triethanolamine (analytical grade) was from Riedel-deHaën (Seelze, Germany). Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD, batches 063K3413, 063K3525 and 014K0734) and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-CD) were obtained from Sigma (Steinheim, Germany), while β-cyclodextrin (β-CD) came from Fluka Chemie AG (Buchs, Switzerland). Hydroxypropyl-β-cyclodextrin (HP-β-CD), hydroxypropyl-γ-cyclodextrin (HP-γ-CD) and methyl-β-cyclodextrin (M-β-CD) were obtained from Aldrich (Steinheim, Germany). Carboxymethyl-β-cyclodextrin (CM-β-CD) and carboxyethyl-β-cyclodextrin (CE-β-CD) were from Cyclolab, Budapest, Hungary. Heptakis-6-sulphato-β-cyclodextrin (HS-β-CD), heptakis-(2,3-dimethyl-6-sulphato)-β-cyclodextrin (HDMS-β-CD) and heptakis-(2,3-diacetyl-6-sulphato)-β-cyclodextrin (HDAS-β-CD) came from Regis Technologies Inc. (Morton Grove, IL, USA).

All the LA solutions were from AstraZeneca. Adrenaline hydrochloride racemate was obtained from Sigma.

The water was of MilliQ quality (Millipore, Bedford, MA, USA).

2.2. Capillary electrophoresis

The experiments were performed on an HP^{3D}CE instrument (Agilent, Waldbronn, Germany), comprising a diode array detector and ChemStation Software Version A.09.03 for data processing. The bare fused-silica capillaries (Agilent or Composite Metal Services Ltd., Hallow, UK) were of varying lengths, with a 75 or 50 μm internal diameter. The capillaries had the polyimide coating removed at the detection window, about 8.5 cm from one end, and at the capillary ends (2–4 mm). Background electrolyte (BGE) was injected after sample injection to prevent sample loss due to thermal expansion. The voltage (positive polarity) was applied with an initial ramping over 30 s. The UV detection was performed at 200 nm with a bandwidth of 4 nm. Preconditioning of the capillary was included for each run and consisted of a 1 min flush with water, 2 min with 0.1 M NaOH, 1 min with water and 4 min with the electrolyte. Before starting a new sequence, the capillary was flushed for 1 min with water, 10 min with 0.1 M NaOH and 1 min with water. At the end of each sequence the capillary was flushed with water for 1 min, 0.1 M NaOH for 3 min, water for 1 min and air for 10 min. New capillaries were first flushed for 1 min with water and then for 30 min with 0.1 M NaOH and 1 min with water. Flushing was performed at a pressure of approximately 1 bar. The electrodes and pre-punchers were cleaned between sequences.

2.3. Background electrolytes

The BGEs contained various amounts of different cyclodextrins in a phosphoric acid-triethanolamine buffer. All solutions were freshly prepared and filtered through Gelman GHP Acrodisc 13 mm syringe filters, 0.45 μm pore size (Pall, East Hills, USA). The phosphoric acid-triethanolamine buffer consisted of 0.10 M H_3PO_4 and 0.05, 0.07 or 0.09 M triethanolamine, resulting in a pH of 2.15, 2.5 or 3.0, respectively.

2.4. Experimental design

Chemometric experimental design and evaluation of the model was performed with the help of MODDE 6.0 (Umetrics, Umeå, Sweden) software.

3. Results and discussion

3.1. Cyclodextrin screening

Method development started with the screening of cyclodextrins as chiral selectors in a low-pH buffer, since adrenaline and the LAs are amines and positively charged in the products. A standard low-pH BGE in our laboratory is phosphoric acid-triethanolamine. Phosphoric acid has good buffering properties at low pH and very low UV absorption at low wavelengths. Triethanolamine forms a dynamic coating at the capillary wall so that the electroosmotic flow (EOF) is reversed and very stable [14,17,18]. From previous experience with the chiral separations of the LAs [15–18], it was known that lidocaine migrates fastest and bupivacaine slowest in cyclodextrin-modified phosphate-triethanolamine systems at low pH (uncharged CDs). Accordingly, LA products with bupivacaine and lidocaine were mainly used for method development.

Initially, 11 different cyclodextrins, both charged and uncharged, were tested using a BGE containing 0.10 M H_3PO_4 and 0.07 M triethanolamine (pH 2.5) (Table 2). The concentrations of the uncharged CDs were around 10 mM, while the concentrations of the charged CDs were around 1 mM. The other conditions were as in Table 2. Besides the resolution between the adrenaline enantiomers, the migration order of adrenaline and the LAs was observed.

Table 2
Initial screening of cyclodextrins for the enantiomeric separation of adrenaline

Cyclodextrin	Concentration (mM)	R_s adrenaline enantiomers
β-CD	5.1	0
M-β-CD	10.4	2.0
DM-β-CD	11.0	2.8
TM-β-CD	11.3	0
HP-β-CD	10.6	1.5
HP-γ-CD	10.0	0
CM-β-CD	10.3	4.2
CE-β-CD	10.7	1.1
HS-β-CD	1.1	0.7
HDMS-β-CD	1.2	0
HDAS-β-CD	0.8	7.5

Sample: 50 μg/ml of racemic adrenaline HCl. Conditions: BGE: 0.10 M phosphoric acid, 0.07 M triethanolamine (pH 2.5) and cyclodextrin as in the table, 75 μm i.d. fused-silica capillary 64.5 cm total length, 56.0 cm to detector, temperature 25 °C, sample injection 30 mbar × 3 s, BGE injection 30 mbar × 3 s, applied voltage +24 kV (initial ramping over 30 s). Detection at 200 nm (4 nm bandwidth).

The highest resolution between the adrenaline enantiomers was obtained when using HDAS-β-CD. However, adrenaline migrated last in this system and there was no baseline resolution between bupivacaine and one of the adrenaline enantiomers. The CM-β-CD system also gave good chiral resolution, although the adrenaline enantiomers migrated between lidocaine and bupivacaine. Bearing in mind the peak shapes, a system where adrenaline migrates first was preferred to prevent adrenaline peak distortion in the presence of high amounts of LA. Of the systems where the adrenaline enantiomers migrated first, the system with DM-β-CD gave the best resolution (see Fig. 2). In total, the screen with the 11 CDs showed that this system was the most promising for our application.

3.2. Two-level multifactorial design

Further development of the DM-β-CD system was performed using a full factor two-level multifactorial design. The factors selected were the capillary length, the DM-β-CD concentration and the concentration of triethanolamine.

The cyclodextrin concentration was varied between 5 and 40 mM. The upper limit was chosen for practical reasons: the viscosity of the solution increases with the cyclodextrin concentration and previous (unpublished) experiments have shown that it is very difficult to obtain a stable and reproducible baseline at room temperature at concentrations above 40–50 mM.

The concentration of triethanolamine affects the pH, the ionic strength of the BGE and the amount of dynamic coating of the capillary, and thus the EOF. The lower limit was 50 mM triethanolamine, i.e. half the concentration of phosphoric acid (thus the $pH = pK_a$), giving very good buffering capacity. Moreover, at this concentration the EOF is reversed. The upper limit was 90 mM triethanolamine, resulting in a pH of 3.0. Although the buffering capacity is reduced, there is a very stable EOF because of the wall interactions of the triethanolamine [17,18].

The capillary length was varied between 48.5 and 81.5 cm (40.0 and 72.0 cm effective length, respectively), while the applied field strength remained constant (37.2 kV/m).

Other factors, such as pH, ionic strength, injected plug length and applied voltage was indirectly varied through the above-mentioned factors. The temperature was thought to have a minor effect on the chiral resolution and was excluded from the experimental design. Since resolution has a non-linear relationship with the cyclodextrin concentration, curvature was expected in the experimental model, so axial points were added to the design. In total, 17 experiments were performed, of which three were centre points.

The samples used were lidocaine hydrochloride with adrenaline (lidocaine adrenaline) and bupivacaine hydrochloride with adrenaline (bupivacaine adrenaline) solutions for injection, spiked with adrenaline racemate. The responses were the

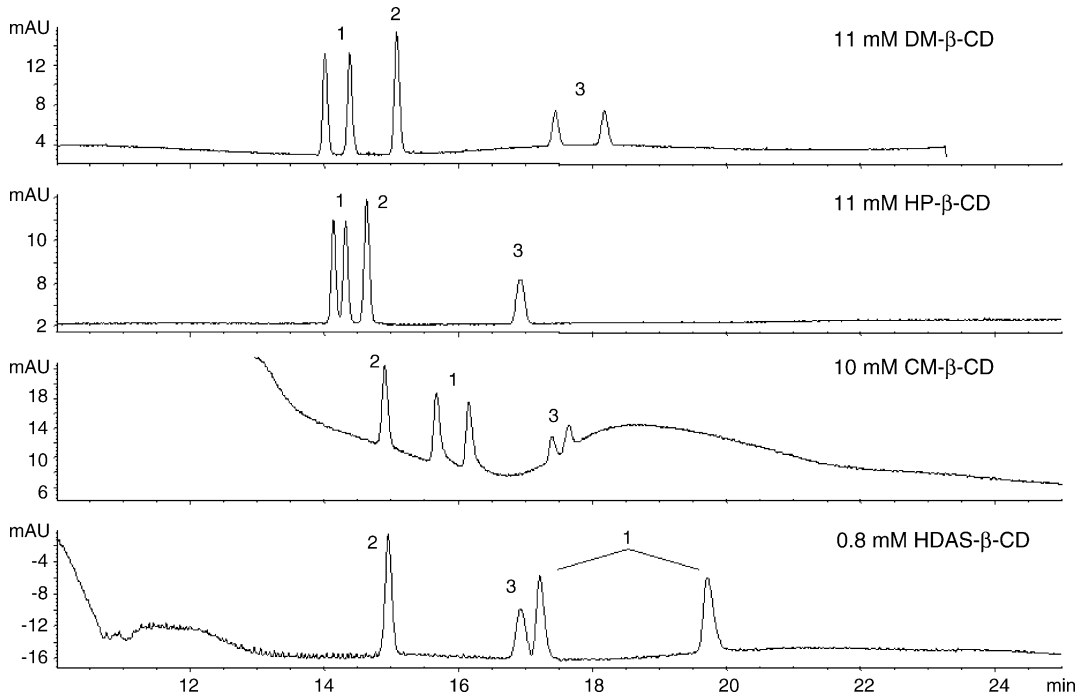


Fig. 2. Selection of cyclodextrin for the enantiomeric separation of adrenaline in local anaesthetic solutions for injection. Sample: (1) 17 μg/ml adrenaline HCl, (2) 18 μg/ml lidocaine and (3) 15 μg/ml bupivacaine HCl. Conditions: BGE: 0.10 M phosphoric acid, 0.07 M triethanolamine (pH 2.5), cyclodextrin as in figure, 75 μm i.d. × 64.5 cm (56.0 cm to detector) fused-silica capillary, temperature 25 °C, sample injection 30 mbar × 3 s, BGE injection 30 mbar × 3 s, applied voltage +24 kV (initial ramping over 30 s). Detection at 200 nm (4 nm bandwidth).

enantiomeric resolution of adrenaline (measured from the bupivacaine adrenaline sample) and the resolution of D-adrenaline from lidocaine.

Summarizing, the resolution of the adrenaline enantiomers was the highest using a long capillary and high concentration of cyclodextrin. Even a high concentration of triethanolamine had a positive effect, though one that was less significant. The resolution of D-adrenaline from lidocaine was best using a long capillary, a low concentration of cyclodextrin and an intermediate concentration of triethanolamine. Thus, when the chiral separation was high, no sufficient separation from lidocaine was obtained. When the resolution between D-adrenaline and lidocaine was maximized, there was only approximately baseline resolution between the adrenaline enantiomers (Fig. 3). The separation of the adrenaline enantiomers from bupivacaine was sufficient for all the conditions tested. Overall, high currents (90–100 μA) were measured, sometimes resulting in baseline distortions.

3.3. Bupivacaine adrenaline

Some bupivacaine adrenaline solutions for injection were analysed with a method derived from the above-mentioned experimental design, with some adjustments. A 50 μm capillary was used instead of a 75 μm capillary to reduce the current and the radial temperature gradient. The smaller diameter capillary resulted in less heat generation and less band distortion. In fact, the resolution between D-adrenaline and bupivacaine was much improved using a smaller diameter capillary, so much so that sensitivity was increased instead of decreased. An intermediate concentration of cyclodextrin was selected, since the chiral separation was sufficient at these concentrations, the run buffer was less viscous and so better baseline stability was obtained. With the resulting system it was possible to increase the injection volume (by increasing the injection time). The products were analysed using 50 mbar × 40 s injections. An example of a bupivacaine adrenaline analysis is shown in Fig. 4.

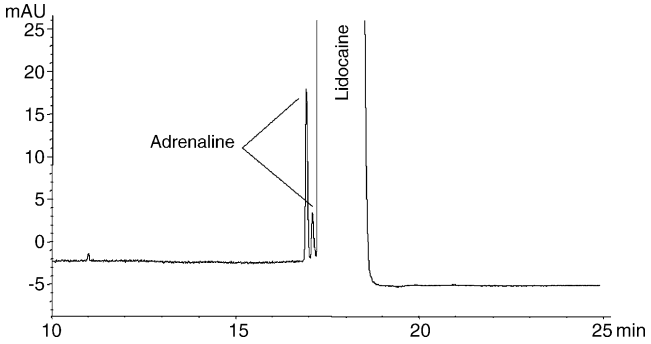


Fig. 3. Electropherogram at the condition from the first experimental design with the best resolution between the D-adrenaline and lidocaine. Sample: lidocaine HCl + adrenaline, 20 mg/ml + 12.5 μg/ml, spiked with adrenaline HCl, total concentration L-adrenaline 15 μg/ml, D-adrenaline 4 μg/ml. Calculated resolution (ChemStation Software) between adrenaline enantiomers: 1.5; between D-adrenaline and lidocaine: 0.22. Conditions: BGE: 0.10 M phosphoric acid, 0.07 M triethanolamine, 5 mM DM-β-CD, 75 μm i.d. × 80.5 cm (72.0 cm to detector) fused-silica capillary, temperature 25 °C, sample injection 30 mbar × 3 s, BGE injection 30 mbar × 3 s, applied voltage +30 kV (initial ramping over 30 s). Detection at 200 nm (4 nm bandwidth).

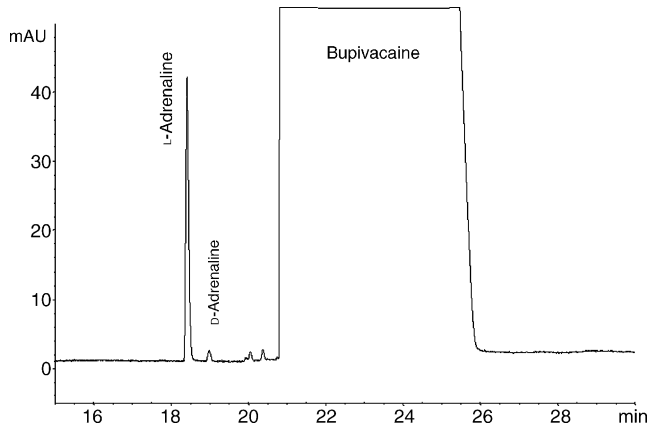


Fig. 4. Enantiomeric separation of adrenaline in bupivacaine HCl + adrenaline, 5 mg/ml + 5 μg/ml, solution for injection. Conditions: BGE: 0.10 M phosphoric acid, 0.07 M triethanolamine, 20 mM DM-β-CD, 50 μm i.d. × 80.5 cm (72.0 cm to detector) fused-silica capillary, sample injection 40 s × 50 mbar, BGE injection 10 s × 50 mbar, temperature 35 °C, applied voltage +30 kV (initial ramping over 30 s). Detection at 200 nm (4 nm bandwidth).

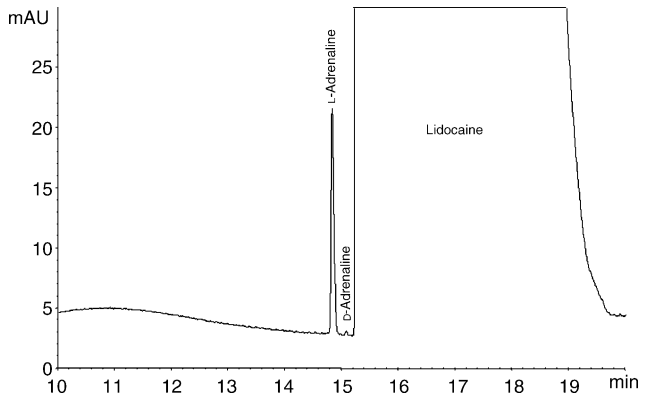


Fig. 5. Enantiomeric separation of adrenaline in lidocaine HCl + adrenaline, 20 mg/ml + 12.5 μg/ml, solution for injection. The calculated resolution (ChemStation Software) between the adrenaline enantiomers is 3.0; between D-adrenaline and lidocaine 0.09. Conditions: BGE: 0.10 M phosphoric acid, 0.05 M triethanolamine and 40 mM DM-β-CD, 50 μm i.d. × 80.5 cm (72.0 cm to detector) fused-silica capillary, injection 50 mbar × 15 s, BGE injection 10 s × 50 mbar, applied voltage 30 kV (initial ramping over 30 s), temperature 45 °C. Detection at 200 nm (4 nm bandwidth).

3.4. Improving the resolution between D-adrenaline and lidocaine

Although products containing the later migrating LAs, such as bupivacaine, could be analysed with the method described above, the resolution between D-adrenaline and the faster migrating LAs, such as lidocaine needed improvement.

Further modifications of the DM-β-CD system were tested such as sample dilution with water (to reduce the ionic strength) and varying the run temperature. The most positive effect was

Table 3
Multifactorial optimisation of the resolution between D-adrenaline and lidocaine in lidocaine HCl + adrenaline, 20 mg/ml + 12.5 μg/ml, solutions for injection spiked with adrenaline HCl, total concentration L-adrenaline 24 μg/ml, D-adrenaline 12 μg/ml

Temperature (°C)	Triethanolamine (mM)	DM-β-CD (mM)	R_s D-adrenaline–lidocaine undiluted sample	R_s D-adrenaline–lidocaine diluted sample
25	50	20	0	0
25	50	30	0	0
25	50	40	0	0.07
25	70	20	0	0
25	70	30	0	0.08
25	70	40	0	0
25	90	20	0	0
25	90	30	0	0.09
25	90	40	0	0
35	50	20	0.08	0.34
35	50	30	0.19	0.63
35	50	40	0.57	1.10
35	70	20	0	0.20
35	70	30	0.32	0.80
35	70	40	0.44	1.22
35	90	20	0	0.36
35	90	30	0.31	0.79
35	90	40	0.46	1.08
45	50	20	0.43	0.94
45	50	30	0.72	1.29
45	50	40	1.01	1.91
45	70	20	0.39	1.01
45	70	30	1.02	1.60
45	70	40	0.84	1.69
45	90	20	0.49	1.42
45	90	30	1.03	1.62
45	90	40	0.92	1.89

Other conditions: BGE: 0.10 M phosphoric acid, triethanolamine and DM-β-CD according to design, 50 μm i.d. fused-silica capillary, sample injection 50 mbar × 5 s, BGE injection 50 mbar × 10 s, applied voltage 30 kV (initial ramping over 30 s). Detection at 200 nm (4 nm bandwidth).

from the temperature: a rise of temperature to 35 °C improved the resolution between adrenaline and lidocaine and did not decrease the chiral resolution. Since the temperature was not a factor included in the original factorial design and since the results from the 75 μm capillary could not be extrapolated to a 50 μm capillary (due to the radial temperature gradient), an additional multifactorial experiment was performed with a diluted and undiluted lidocaine adrenaline product. The factors were the temperature, the triethanolamine concentration and the DM-β-CD concentration. As response, the resolution between D-adrenaline and lidocaine was measured, as the chiral resolution of adrenaline was not a critical factor under these conditions. The resolutions were calculated by the ChemStation Software according to the pharmacopoeia definitions, which are based on Gaussian peaks. Since the LA peak is triangular in shape, this means that even peaks with calculated resolutions less than 1.5 can be baseline-resolved (see, for example, Fig. 5). In this design, D-adrenaline and lidocaine were baseline-resolved at a ChemStation calculated resolution of about 0.3.

Table 3 shows that the best resolution between the D-adrenaline and lidocaine was obtained at a high DM-β-CD concentration and a high temperature. The concentration of triethanolamine was less significant. Accordingly, the lowest concentration was selected to give the best buffering capacity of the BGE (pH = pK_a). Further experiments were performed to increase the sensitivity by increasing the injection volume and testing sample dilution. As expected, the injection time (and therefore volume) could be increased if the concentration of lidocaine in the sample was decreased. A lidocaine HCl + adrenaline, 5 mg/ml + 5 μg/ml, solution could be injected for 60 s × 50 mbar, while lidocaine HCl + adrenaline, 20 mg/ml + 5 μg/ml, solutions were limited to 15 s × 50 mbar injections. A two-fold dilution of a lidocaine adrenaline solution enabled a two-fold increase in injection time, which did not result in improved sensitivity.

Fig. 5 shows an electropherogram of a lidocaine adrenaline product analysed with the final method. The method parameters are summarized in Table 4. This final method also worked well for the other adrenaline-containing LA products with bupivacaine, prilocaine or mepivacaine.

3.5. Method performance

To test specificity, electropherograms of plain LA products and LA adrenaline products were compared. All products had been stored past their shelf lives so as to include possible degradation products. The concentration of L-adrenaline is ≤0.1% of the concentration of the LA, and the concentration of D-adrenaline found was typically less than 0.003% of the LA concentration. That means that even very minor, insignificant impurities from the LA are in the same concentration range as adrenaline. No peaks were interfering with the adrenaline peaks.

Table 4
Method parameters for the capillary electrophoretic enantiomeric separation of adrenaline in local anaesthetic solutions for injection

Parameter	Settings
Capillary	80.5 cm (72.0 cm to detector) × 50 μm i.d. fused silica
BGE	40 mM DM-β-CD, 0.10 M phosphoric acid, 0.05 M triethanolamine
Temperature	45 °C
Instrumental preparation	Clean electrodes and pre-punchers
Conditioning of a new capillary	1 min flush with water 30 min flush with 0.1 M NaOH 1 min flush with water
Conditioning of the capillary at the beginning of a sequence	1 min flush with water 10 min flush with 0.1 M NaOH 1 min flush with water
Preconditioning	1 min flush with water 2 min flush with 0.1 M NaOH 1 min flush with water 4 min flush with BGE
Sample pretreatment	None, the samples were injected undiluted
Injection	15 s × 50 mbar sample 10 s × 50 mbar BGE
Applied voltage	+30 kV, initial ramping over 30 s
Detection	200 nm, 4 nm bandwidth, 1.0 s response time
Calculation	Internal normalization of corrected adrenaline peak areas
Shutdown procedure	1 min flush with water 3 min flush with 0.1 M NaOH 1 min flush with water 10 min flush with air

Table 5
Repeatability of the enantiomeric purity determination of adrenaline in adrenaline containing local anaesthetic solutions (conditions as in Table 4)

Product	D-Adrenaline	
	(% of total)	(R.S.D. %, n = 6)
Lidocaine HCl + adrenaline, 5 mg/ml + 5 μg/ml	2	13
Lidocaine HCl + adrenaline, 20 mg/ml + 5 μg/ml	3	14
Bupivacaine HCl, 5 mg/ml spiked with adrenaline 5 μg/ml	5	12
Bupivacaine HCl, 5 mg/ml spiked with adrenaline 5 μg/ml	7	8
Bupivacaine HCl, 5 mg/ml spiked with adrenaline 5 μg/ml	9	3
Bupivacaine HCl, 5 mg/ml spiked with adrenaline 5 μg/ml	23	2
Bupivacaine HCl, 5 mg/ml spiked with adrenaline 5 μg/ml	29	1

The linearity was tested by spiking racemic adrenaline into plain lidocaine HCl 20 mg/ml solutions, i.e. a "worst case scenario" with the highest concentration of the local anaesthetic migrating closest to the adrenaline enantiomers. Linearity proved to be satisfactory for both L- and D-adrenaline up to 40 μg/ml. The correlation coefficients (r) were 0.99 for both enantiomers, without the use of an internal standard for correction of the injection volume variability.

The repeatability of the method was determined by replicate injections of LA adrenaline products and of plain LA products spiked with L- and D-adrenaline in different ratios. The electropherograms were integrated by the ChemStation Software, but corrected manually when necessary. The relative standard deviations (R.S.D.s) at the lower levels of D-adrenaline, 2–3% of the total adrenaline content, were 10–15% (Table 5). The quantification limit (LOQ) was estimated to be 2% D-form, as a R.S.D. of 20% is considered acceptable at this concentration level. This LOQ of 2% was obtained by highly experienced scientists and the acceptance of manual corrections of the integration baseline. If manually corrected integration is not acceptable, the LOQ will be significantly higher.

The accuracy was assessed by injecting racemic solutions spiked into plain lidocaine 20 mg/ml solutions, i.e. "worst case" situation with the strongest influence from the LA band on the adrenaline bands. The average response was 49.5% of L-adrenaline and 50.5% of D-adrenaline with R.S.D.s of 1.4% for concentrations up to 90 μg/ml.

The multifactorial approach during method development additionally gave information about the robustness of the method. The BGE was designed for robustness and the method was as robust as can reasonably be required for determinations at such low levels in the actual matrix. A normal consideration for this type of chiral method is the quality of the chiral selector. New batches of DM-β-CD should always be tested. Three different batches from the same supplier were used without significant problems.

Summarizing, the method was suitable for its intended use, enantiomeric purity investigations of adrenaline in LA solutions.

4. Concluding remarks

A chiral CE method for the determination of racemization of adrenaline in LA injection solutions is described. In order to analyse all the various local anaesthetic products with one chiral CE method, the method development was focused on the chiral separation as well as on preventing interference from the highest concentration of the LA migrating closest to adrenaline. If higher sensitivities are needed for the solutions with lower concentrations of the LA, or of solutions with late migrating LAs, the injection volume can be increased.

Bupivacaine adrenaline and lidocaine adrenaline products of different ages stored at 25 °C/60%RH were analysed. The amounts of D-adrenaline found were low, ≤3% of the L-enantiomer, corresponding to ≤0.2 μg/ml (ppm), even in injection solutions that had passed their shelf life by up to 6 months.

The chiral CE method is sufficiently sensitive and robust for enantiomeric purity investigations of adrenaline in LA solutions.

Acknowledgements

We are grateful to Mats Svensson for support and Dr. Valérie Harang for assistance with the MODDE software.

References

[1] D. Selander, http://www.anaesthesia-az.com.
[2] B.B. Hoffman, R.J. Lefkowitz, Catecholamines, sympathomimetic drugs, and adrenergic receptor antagonists, in: J.G. Hardman, L.E. Limbird, P.B. Molinoff, R.W. Ruddon (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics, ninth ed., McGraw-Hill Companies, Hightstown, 1996.
[3] P. Britz-McKibbin, A.R. Kranack, A. Paprica, D.D.Y. Chen, Analyst 123 (1998) 1461–1463.
[4] P. Britz-McKibbin, J. Wong, D.D.Y. Chen, J. Chromatogr. A 853 (1999) 535–540.
[5] P. Britz-McKibbin, D.D.Y. Chen, Anal. Chem. 72 (2000) 1242–1252.
[6] S. Fanali, J Chromatogr. 474 (1989) 441–446.
[7] S. Fanali, P. Boček, Electrophoresis 11 (1990) 757–790.
[8] T.E. Peterson, D. Trowbridge, J. Chromatogr. 603 (1992) 298–301.
[9] C. Quang, M.G. Khaledi, J. Chromatogr. A 692 (1995) 253–265.
[10] S. Fanali, S. Furlanetto, Z. Aturki, S. Pinzauti, Chromatographia 48 (1998) 395–401.
[11] W. Wei, G. Luo, C. Yan, Z. Sun, J. High Resolut. Chromatogr. 21 (1998) 440–444.
[12] W.C. Yang, A.M. Yu, X.D. Yu, H.Y. Chen, Electrophoresis 22 (2001) 2025–2031.
[13] W. Maruszak, M. Trojanowicz, M. Margasinska, H. Engelhardt, J. Chromatogr. A 926 (2001) 327–336.
[14] I. Bechet, Ph. Paques, M. Fillet, Ph. Hubert, J. Crommen, Electrophoresis 15 (1994) 818–823.
[15] C.E. Sänger-van de Griend, K. Gröningsson, J. Pharm. Belg. 50 (1995) 391.
[16] C.E. Sänger-van de Griend, K. Gröningsson, D. Westerlund, Chromatographia 42 (1996) 263–268.
[17] C.E. Sänger-van de Griend, K. Gröningsson, J. Pharm. Biomed. Anal. 14 (1996) 295–304.
[18] C.E. Sänger-van de Griend, H. Wahlström, K. Gröningsson, M. Widahl-Näsman, J. Pharm. Biomed. Anal. 15 (1997) 1051–1061.