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Short communication

Enantiomeric resolution of bupivacaine by high-performance liquid chromatography and chiroptical detection

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Abstract

This work reports a new analytical procedure for the separation and determination of the enantiomers of bupivacaine and the determination of the enantiomeric purity. The isomers were separated using a Chirex 3020 (250 mm × 4.6 mm) with a mobile phase of *n*-hexane:dichloroethane:ethanol (82:9:9, v/v/v) at a flow-rate of 1 ml min^{-1} and UV, polarimetric and circular dichroism (CD) detection. Obtained retention times were 5.93 and 7.53 min (*R* and *S*) with a resolution of $R_s = 2.36$. Precisions (RSD) were 1.83 and 2.02% (CD detection) and 3.07 and 1.26% (UV detection) for *R*- and *S*-enantiomers, respectively (at 10 μg level). Detection limits were 0.5 and 0.5 μg (*R* and *S*) with CD detection, and 0.9 and 0.3 μg with UV detection. Polarimetric detection was inadequate to perform a quantitative method at similar concentration ranges as UV and CD because of poor sensitivity. A procedure for determination of enantiomeric purity using a conventional chromatographic column (RP18, Luna) coupled to a CD detector and anisotropy factor (CD/UV) as analytical signal was also developed. Obtained results show that RSDs of 6.7–1.6% were obtained in the range of 0–100% enantiomeric purity.

Keywords: Bupivacaine enantiomers; Chiral chromatography; Chiroptical detectors; Anisotropy factor

1. Introduction

Bupivacaine is a local anaesthetic amide type used as infiltration, nervous blocking and epidural and spinal anaesthetic. Is synthesized and frequently used as racemic mixture of their enantiomers, although these have different pharmacological activity [1–5]. *R*-(+)-Bupivacaine is more toxic for the central nervous and cardiovascular systems than *S*-(−)-bupivacaine. These enantiomers have the same physicochemical properties but different pharmacokinetics, pharmacodynamics and chiroptical properties. The present study was made because the different pharmacologic behaviour of the bupivacaine enantiomers and it was based on the different response against the polarized light [6,7]. Several chiral liquid chromatographic methods for the separation of the enantiomers are available [8–14]. In addition both enantiomers respond in a different manner when exposed to a chiral environment of electromagnetic nature (polarized light). When enantiomeric separation is achieved using a chiral chromatographic column, detection can proceed via conventional methods depending on the properties of the analyte (UV, fluorescence, electrochemical) or by chiroptical detection (polarimetry or circular dichroism). In the last detection approach the selective and bimodal response obtained gives additional information related to the order of elution and configuration of enantiomers. Sensitivity is highly dependent of specific optical rotation and elipticity of the compound of interest, which in turn is affected by the composition of the mobile phase. In addition, when complete separation cannot be achieved chiroptical detectors is the alternative to obtain useful quantitative and qualitative information [15–17]. With this alternative approach can be used an achiral stationary phase coupled with a CD detector and records simultaneously both CD signal ($\Delta\varepsilon$) and UV signal (ε). The anisotropy factor (g-factor = $\Delta\varepsilon/\varepsilon$) computed on line was found to be independent of concentration but proportional to the enantiomeric composition [18]. Bertucci et al. [19] and some other authors [20–23] have already proved that this methodology was suitable.

Here we describe an analytical procedure for the determination of the enantiomeric composition of bupivacaine. The procedure is based on HPLC chiral separation (column Chirex

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3020, Phenomenex) and quantitation by CD and UV detection. By using as analytical signal the anisotropy factor by dual CD/UV spectroscopy a non-chiral chromatographic method was developed to determine enantiomeric excess (e.e.).

2. Experimental

2.1. Instrumentation

The measurements were performed with a Jasco (Tokyo, Japan) liquid chromatograph equipped with a Degassy Populaire DP4003, a Jasco intelligent HPLC pump model PU-1580, a Jasco L-G-1580-04 quaternary gradient unit, a Jasco intelligent auto sampler model AS-2055 Plus with a 100 μl sample loop, a Jasco interface modulated model LC-Net II/ADC, chiral detector Jasco CD-2095 equipped with a Hg–Xe lamp (150 W), a Gland–Taylor polarizer prism, a standard tapered flow cell of 25 mm path length, a Monk–Gillieson mounting monochromator and a Jasco OR-2090 chiral detector. Data acquisition, transformation and instrument parameters were accomplished by Jasco–Borwin 1.5 software, integration was carried out with a personal computer, the calculations of area (negative and positive peaks), the peaks heights and retention timers were performed with Jasco–Borwin 1.5 software, ChromConver 1.0 and Microsoft Origin. Ultrasonic water bath model Ultrasons (50 kHz) from Selecta (Spain).

2.2. Chemicals and reagents

In enantiomeric separation the mobile phase composition was *n*-hexane–dichloroethane–ethanol (82:9:9, v/v/v). All solvents were gradient-grade LiChrosolv UV–vis (Merck, Darmstadt, Germany). A Chirex 3020 column was utilized (250 mm × 4.60 mm; Phenomenex, Torrance, CA, USA).

Without enantiomeric separation the mobile phase composition was acetonitrile–phosphate-buffered saline (PBS) pH 6.8 (65:35, v/v), both from Merck. A Phenomenex Luna 5 μ silica column was utilized (25 cm × 4.6 mm, 5 μm particle size).

The bupivacaine studied in the present work is a racemic mixture of its optical isomers of 99% purity from Sigma (St. Louis, MO, USA). The bupivacaine structure has a benzenic lypophilic ring together with a hydrophilic tertiary amine linked by hydrocarbon and an amide connection. It contains an asymmetric carbon or chiral group and therefore two stereoisomers that are specular images *R* (*rectus* or straight) and *S* (*to sinister* or left). *R*- and *S*-bupivacaine enantiomers >99.5% pure have been kindly provided by AstraZeneca (Wilmington, DE, USA).

2.3. Chromatographic conditions

A 230 nm wavelength was used in all measurements (CD and UV). A mobile phase flow-rate of 1 ml min^{-1} was used in the chiral chromatographic conditions. The injection volume was 20 μl.

A mobile phase flow-rate of 1.2 ml min^{-1} was used in the non-chiral chromatographic conditions. The amount of sample introduced in the system was 10 μg.

2.4. Mobile phase and sample preparation

For the preparation of the mobile phases, the solvents were filtered through 0.2 μm nylon membrane filters and degassed during, at least, 1 h.

Standard bupivacaine racemic solution was prepared by dissolving 50 mg of commercial product in 50 ml ethanol, stored at 4 °C and in absence of light. Working solutions were prepared by dilution with ethanol.

Standard bupivacaine pure enantiomers were prepared by dissolving 50 mg in 50 ml ethanol, stored at 4 °C and in absence of light. From them, working solutions were prepared mixing aliquot of the same ones, so that the dissolutions have the same total concentration but different enantiomeric proportions (0*S*/100*R*, 20*S*/80*R*, 40*S*/60*R*, 50*S*/50*R*, 80*S*/20*R*, 100*S*/0*R*).

3. Results and discussion

3.1. Chromatographic separation

Enantiomeric separation was accomplished using a chiral chromatographic column (Chirex 3020, Phenomenex) in normal phase conditions. Several percentages of tertiary mixture were assayed and R_s values calculated from chromatographic data. The obtained data are shown in Table 1, from which it can be seen that a percentage of 82% *n*-hexane, 9% dichloroethane and 9% ethanol, gives good R_s values (2.365). Retention times were 5.93 and 7.53 min (*R*- and *S*-enantiomers), which are considered useful to a chromatographic method because good speed and resolution.

The analytical signals were measured with three different detection methods: UV–vis, CD and polarimetric. In Fig. 2 the chromatographic profiles obtained with the various detection methods are depicted. As can be observed, UV–vis detection (b) shows two separated peaks at 5.93 and 7.53 min corresponding to *R*- and *S*-enantiomers. In addition, area and height of peaks are not exactly the same as will corresponds to a racemic mixture. This is because the commercial sample is not racemic as demonstrated in the next paragraphs.

The chromatographic profiles shown in Fig. 1a and c, corresponding to CD and polarimetric signals, have a bimodal response at the same retention times (they have a lesser drift because the in series position of the detectors). The UV–vis and CD detectors would respond linearly to the injected mass in the

Table 1
Mobile phase effect and resolution

%*n*-Hexane	%Dichloroethane	%Ethanol	R_s
72	25	3	0.613
70	25	5	0.561
65	25	10	0.498
68	23	9	0.451
71	20	9	0.494
74	17	9	0.497
79	12	9	0.495
82	9	9	2.365
80	9	11	2.113