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Enantioseparation of chiral vasodilator drug isoxsuprine in high-performance liquid chromatography and capillary electrophoresis

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Abstract

Two independent methods using high-performance liquid chromatography (HPLC) on polysaccharide type chiral stationary phase (CSP) and capillary electrophoresis (CE) with native and derivatised cyclodextrins (CD) have been proposed for the enantioseparation of chiral vasodilator drug isoxsuprine (ISP). The methods have been compared from the viewpoint of separation characteristics (efficiency, sensitivity, analysis time, costs, etc.).

Keywords: Enantioseparations; Chiral HPLC; Chiral CE; Isoxsuprine; Cyclodextrins; Polysaccharide derivatives

1. Introduction

Isoxsuprine [ISP, (±)-4-Hydroxy-α-[1-(1-methyl-2-phenoxyethylamino)ethyl]benzylalkohol] is a vasodilator drug which also stimulates beta-adrenergic receptors. ISP also produces positive inotropic and chronotropic effects. ISP (Fig. 1) is a chiral molecule containing three centres of chirality. Thus, theoretically eight stereoisomers may exist in the product of common synthesis in which no optically pure intermediates are used. However, in the synthesis of commercially available ISP either optically pure intermediates or diastereomeric crystallisation are used. The most common way for the synthesis of ISP is *N*-alkylation of racemic 4-hydroxynorephedrine with racemic 1-phenoxy-2-propylbromide [1]. As the result of this reaction ISP is obtained in the form of two diastereomeric enantiomer pairs, 1RS, 2SR/2′SR and 1RS,2SR/2′RS. The isolation of the racemic form 1RS, 2SR/2′SR which is used as

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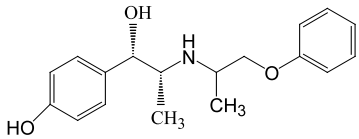

Fig. 1. Structure of (±)-ISP.

a drug may be performed by crystallisation as described in [2]. Therefore, the pharmaceutical formulations contain only two enantiomeric forms in which the chiral carbon atom in the position 1 of 1-methyl-2-phenoxyethylamino moiety is present in both configurations.

Enantioseparation of ISP has been described previously using randomly substituted sulfated β-cyclodextrins (CD) (SU-β-CD) in capillary electrophoresis (CE) [3]. SU-β-CD is not well characterised multicomponent mixtures and therefore less suitable for the validated method developments. In addition, no chromatographic method has been described for the enantioseparation of this drug at least to the best of our knowledge. The subject of the present study was to develop two alternative methods for enantioseparation of ISP using high-performance liquid chromatography (HPLC) and CE. In CE separations, main emphasis was done on the use of single component native and derivatised (neutral and charged) CDs.

2. Experimental

2.1. Chemicals

(±)-ISP, heptakis-(2,6-*O*-dimethyl)-β-CD (DM-β-CD) and heptakis-(2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD) were supplied from Sigma Chem-

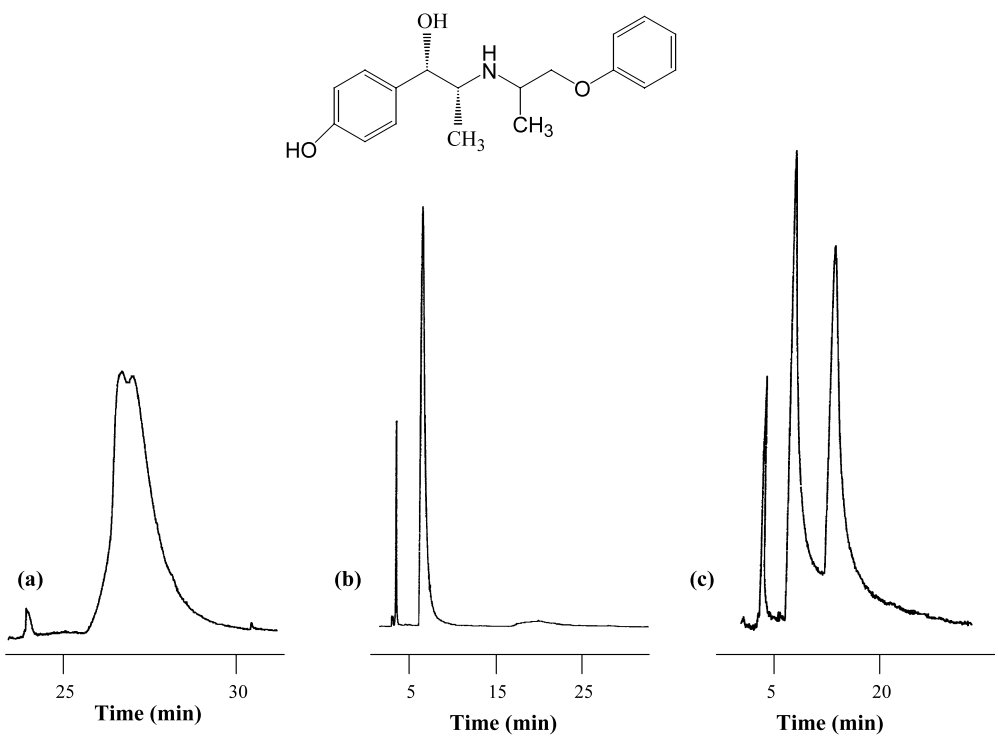

Fig. 2. Chromatograms of (±)-ISP on Chiralcel OJ (a), Chiralcel OD (b) and Chiralpak AD (c) columns. Mobile phase, n-hexane/2-propanol 80/20 (v/v); flow-rate 1 ml/min.

ical Co. (St. Louis, MO, USA). The pure enantiomers of ISP were obtained using HPLC method described below (see Section 2.2) based on the analytical scale chiral HPLC column Chiralpak AD from Daicel Chemical Ind. (Tokyo, Japan). The optical rotation sign of the enantiomers were determined by off-line polarimetric analysis. Native α, β and γ-CD and carboxymethyl β-CD (CM-β-CD) was a gift from Wacker Chemie (Wacker, Munich, Germany). Sulfobutyl-β-CD (SBE-β-CD) was from Cydex (Overland Park, KS, USA) and SU-β-CD was from Aldrich (Aldrich Chemie, Steinheim, Germany). Single isomer β-CD sulfates, heptakis-(6-sulfo)-β-CD (HS-β-CD), heptakis-(6-sulfo-2,3-diacetyl)-β-CD (HDAS-β-CD) and heptakis-(6-sulfo-2,3-dimethyl)-β-CD (HDMS-β-CD) were kind gift from Professor Gy. Vigh (Texas A & M University, College Station, TX, USA). Analytical grade phosphoric acid and triethanolamine as well as the chromatographic grade n-hexane and 2-propanol were from Merck (Merck, Darmstadt, Germany).

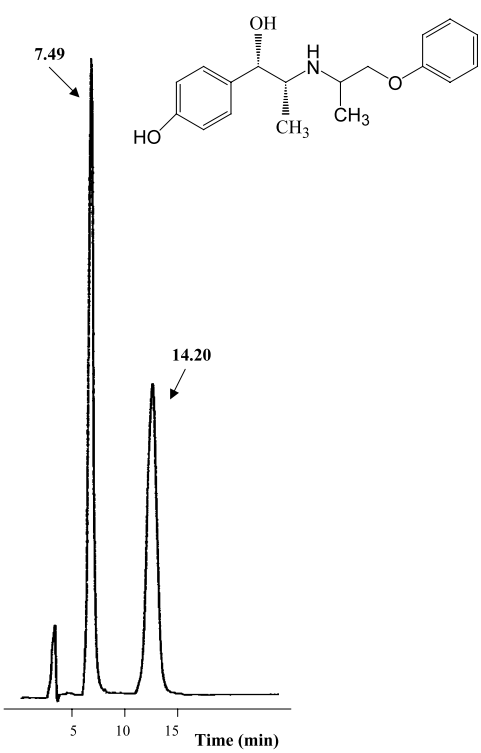

Fig. 3. HPLC enantioseparation of (±)-ISP on Chiralpak AD column using n-hexane/2-propanol/triethylamine 80/20/0.1 (v/v/v) as a mobile phase. Flow rate 1 ml/min.

2.2. Chromatographic conditions

HPLC enantioseparation was performed on the system consisted of isocratic Knauer HPLC pump (Knauer, Berlin, Germany), Merck Hitachi 655A variable wavelength UV monitor and Merck Hitachi D-2500 chromato-integrator (Merck, Darmstadt, Germany). The 250 × 4.6 mm stainless steel column packed with cellulose tris(4-methylbenzoate) (Chiralcel OJ), cellulose tris(3,5-dimethylphenylcarbamate) (Chiralcel OD) and amylose tris(3,5-dimethylphenylcarbamate) (Chiralpak AD) were from Daicel Chemical Ind. The mobile phases were n-hexane/2-propanol (80/20, v/v) or the same eluent containing 0.1% diethylamine with a flow-rate 1 ml/min. The eluent fractions corresponding to each chromatographic peaks were collected and evaporated in order to obtain enantiomerically enriched fractions of (+) and (−)-ISP. The optical rotation sign was determined using a polarimeter 341 (Perkin–Elmer, Ueberlingen, Germany).

2.3. Electrophoretic conditions

Enantioseparations in CE were performed using Beckman MDQ capillary electrophoretic system equipped with variable wavelength UV detector (Beckman, Fullerton, CA, USA). The fused-silica separation capillary was from Polymicro Technologies (Phoenix, AZ, USA) of 50 μm I.D. and 50/60 cm effective and total lengths, respectively. The separation buffer was 100 mM triethanolamine phosphate at pH 3.0. Applied voltage was 25 kV and a detection was performed at 214 nm.

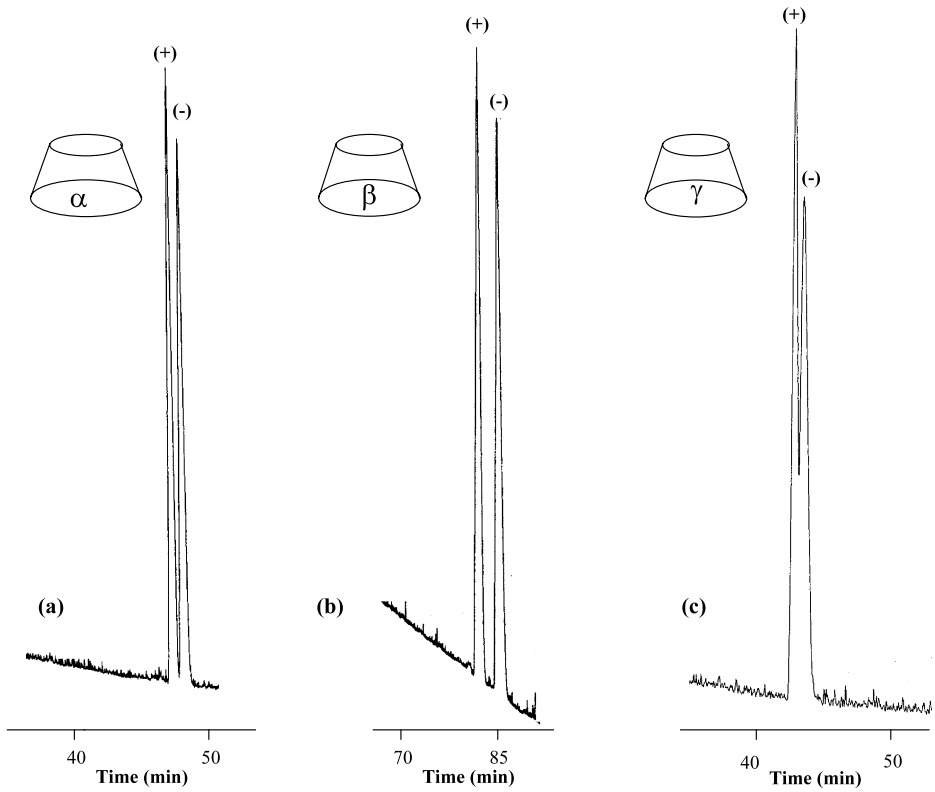

Fig. 4. CE enantioseparation of (±)-ISP using native α- (a), β- (b) and γ-CD (c) as chiral selectors. CD concentration was 60 mg/ml (a), 18 mg/ml (b) and 100 mg/ml (c) in 100 mM triethanolamine phosphate buffer at pH 3.0. Separation capillary and the applied voltage were as described in Section 2.

3. Results and discussion

3.1. HPLC enantioseparation of (±)-ISP

HPLC enantioseparation of (±)-ISP was studied on three different commercially available polysaccharide type chiral stationary phases (CSP) in particular, Chiralcel OJ, Chiralcel OD and Chiralpak AD. Both of cellulose based CSPs, Chiralcel OJ and Chiralcel OD did not result in the acceptable enantioseparation (Fig. 2a and b). However, almost baseline enantioseparation of (±)-ISP was obtained on amylose-based CSP, Chiralpak AD (Fig. 2c). The enantioseparation factor was rather high ($\alpha = 2.2$). However, the peak forms were not ideal (strong tailing) in n-hexane/2-propanol as a mobile phase. The peak forms were significantly improved with addition of 0.1% triethylamine to the mobile phase (Fig. 3). The method with n-hexane/2-propanol without the additives of triethylamine has been used for micropreparative collection of (+) and (−)-ISP. The enantiomer elution order was determined by analysing of the collected HPLC fractions using polarimeter.

3.2. CE enantioseparation of (±)-ISP

CE as a miniaturised and flexible technique offers some potential advantages compared with HPLC for enantioseparations [4–8]. Therefore, the enantioseparation of (±)-ISP was studied using various native and derivatised CDs as chi-

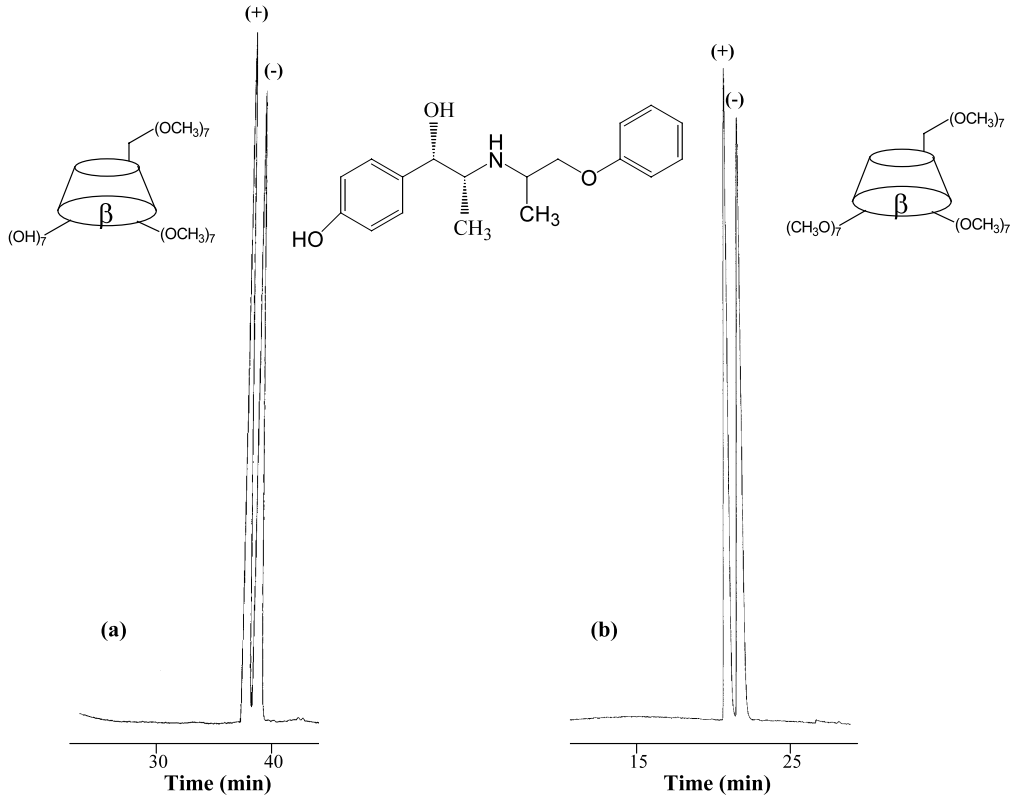

Fig. 5. CE enantioseparation of (±)-ISP using 50 mg/ml DM-β-CD (a) and 70 mg/ml TM-β-CD (b). Other conditions were as in the experiment shown in Fig. 4.

ral selectors in CE. As these studies prevailed (±)-ISP belongs to the group of chiral analytes which may be enantioselectively recognised by all three most commonly used native CDs, α-, β- and γ-CD (Fig. 4) [9,10]. β-CD exhibited the highest enantioseparation ability from native CDs and γ-CD the lowest one. The enantiomer migration order was the same with all native CDs. (+)-ISP migrated as the first peak.

Single component neutral β-CD derivatives such as DM-β-CD and TM-β-CD also exhibited significant enantioseparation ability towards the enantiomers of ISP. Especially good enantioseparation was obtained with TM-β-CD (Fig. 5b). As it can be seen from rather short migration times (Table 1 and Fig. 5b) the enantiomers of ISP exhibit very low affinity towards TM-β-CD. However, the interactions between (±)-ISP and TM-β-CD seem to be much more enantioselective compared with (±)-ISP and other CDs.

In contrast to the neutral CDs, anionic single-isomer and randomly substituted CDs were effective at low concentrations in the range of 0.5–2.0 mg/ml. Interestingly, the enantiomer migration order was the same with the most CDs (Table 1). However, in the case of randomly substituted SU-β-CD the enantiomer migration order was opposite compared with all other CDs. Thus, the mechanism of the opposite

Table 1
Enantioseparation of (±)-ISP with various CDs

Chiral selector	Concentration of chiral selector (mg/ml)	t_1 (min)	t_2 (min)	t_2/t_1	Optical rotation sign of the first migrated enantiomer
Without chiral selector	1	18.717	18.717	1.00	
α-CD	20	33.783	34.408	1.02	(+)
	40	39.421	40.463	1.03	(+)
	60	47.183	48.733	1.03	(+)
β-CD	15	40.708	42.458	1.04	(+)
	18	78.724	85.319	1.09	(+)
γ-CD	20	29.129	29.308	1.01	(+)
	40	31.013	31.342	1.01	(+)
	100	43.225	43.917	1.02	(+)
	150	40.042	40.629	1.02	(+)
	200	34.646	35.063	1.01	(+)
DM-β-CD	50	38.404	39.775	1.04	(+)
	60	48.679	50.808	1.04	(+)
	70	54.263	56.892	1.05	(+)
TM-β-CD	50	20.842	21.367	1.03	(+)
	70	21.846	22.442	1.03	(+)
	125	34.0721	35.975	1.04	(+)
	150	36.862	38.308	1.04	(+)
	180	45.017	47.442	1.05	(+)
HDAS-β-CD	0.5	25.612	30.484	1.19	(+)
	1.0	32.166	41.569	1.29	(+)
HDMS-β-CD	5	20.104	20.104	1.00	(+)
	50	25.967	26.837	1.03	(+)
HS-β-CD	2	34.296	43.924	1.28	(+)
CM-β-CD	0.25	21.421	21.421	1.00	
	1	25.538	25.538	1.00	
	2	58.895	58.895	1.00	
SU-β-CD	0.5	22.471	23.104	1.03	(−)
	1	33.221	35.427	1.07	(−)
	1.5	39.974	42.345	1.06	(−)

enantiomer migration order in the case of regioselectively substituted single-component HS-β-CD and randomly substituted multicomponent SU-β-CD seems very interesting (Fig. 6).

From the viewpoints of analysis times HPLC method developed in this study offers certain advantages. However, the low costs, less environmental problems and high flexibility of CE may be attractive in some applications.

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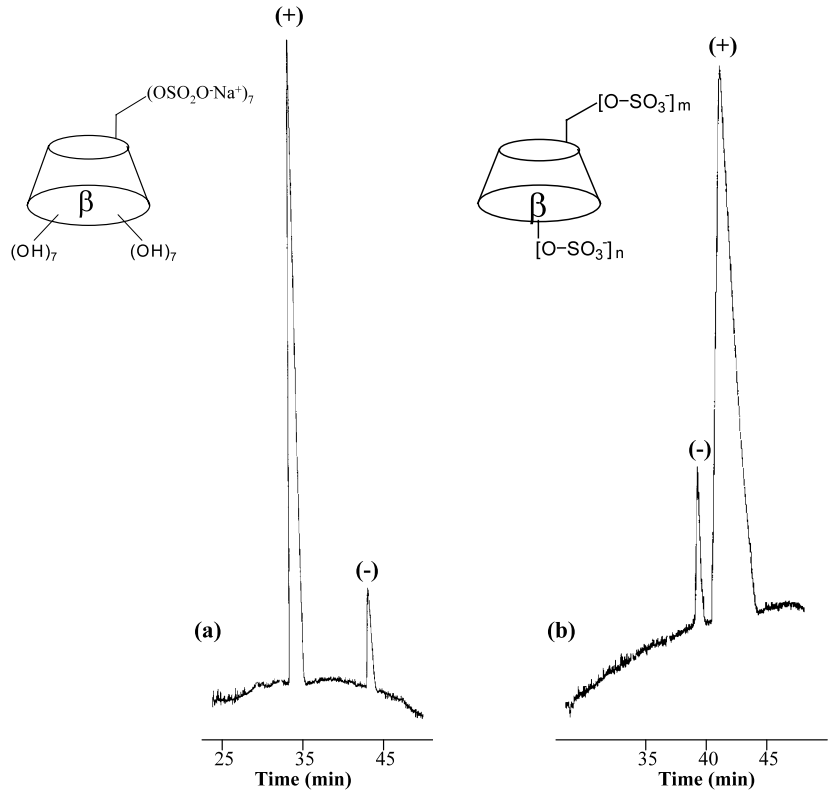

Fig. 6. CE enantioseparation of (±)-ISP spiked with (+)-ISP using 2 mg/ml HS-β-CD (a) and 1.5 mg/ml SU-β-CD (b). Other conditions were as in the experiment shown in Fig. 4.

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