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Chiral separation of methoxamine and lobeline in capillary zone electrophoresis using ethylbenzene-deactivated fused-silica capillary columns and cyclodextrins as buffer additives

M.V. Russo *

Facoltà di Agraria (DISTAAM), Università del Molise, Via de Sanctis, 86100 Campobasso, Italy

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Abstract

The complete chiral separation of methoxamine and lobeline was achieved by capillary zone electrophoresis on an ethylbenzene-deactivated fused-silica capillary column and with cyclodextrins (CDs) as buffer additives. Among the CDs investigated in this study, i.e. α-CD, β-CD, dimethyl-β-CD, hydroxypropyl-β-CD and γ-CD, all the three β-type CDs showed chiral recognition on the two drugs investigated. Under the investigated conditions, the baseline chiral separation of methoxamine can be achieved with 90 mM Tris–H_3PO_4 (pH 2.5) containing 11.5 mM of the three β-type CDs, with dimethyl-β-CD giving the best resolution, whereas the baseline chiral separation of lobeline can be realized by using 90 mM Tris–H_3PO_4 buffer (pH 2.5) containing 5.8 mM dimethyl-β-CD or 29.5 mM hydroxypropyl-β-CD. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Methoxamine; Lobeline; Deactivated column; CZE; Cyclodextrins; Racemic separation

1. Introduction

The optical isomer separation in chiral drugs is important in analytical chemistry, especially in pharmaceutical analysis, because the optical isomers of a chiral drug often present different pharmacological effects. Chromatographic techniques, such as high performance liquid chromatography (HPLC) and gas chromatography (GC), have been widely used for this purpose. Recently, a relatively new and fast growing separation technique, capillary electrophoresis (CE), has also been involved chiral separation [1–5]. Several advantages exist in CE, especially in capillary zone electrophoresis (CZE) in comparison with HPLC and GC. CE requires small volumes of sample and buffer and it can avoid the expensive chiral columns often required by HPLC and GC. In addition, chiral selectors can be easily changed in the CE operation, making the choice of chiral selectors easier, faster, and not too expensive for a specific separation. At present, cyclodextrins (CDs) are the most commonly used chiral selec-

* Fax: +39-874-404652
E-mail address: mvrusso@unimol.it (M.V. Russo).

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tors in chiral CE because of their commercial availability and their transparency in UV region, although the use of various other chiral selectors, such as bile salts, crown ethers, maltodextrin, proteins and chiral agent has also been reported [6–15]. The chiral separation of the drug methoxamine has been reported by Hohne [16] using CE, which was based on host-guest complexation with a chiral crown ethers (18)-crown-6-tetracarboxylic acid. In 1996 Wang et al. [17] reported a chiral separation of methoxamine and lobeline in 12 and 21 min, respectively, using CDs as buffer additives in CZE. This method was also performed to achieve the chiral separation of the lobeline analogs [18]. In this paper is shown the chiral separation of methoxamine and lobeline by CZE with CDs as chiral selector and using an ethylbenzene-deactivated fused-silica capillary column.

2. Experimental

2.1. Materials and reagents

α-, β-, γ-CD, heptakis(2,6-di-*O*-methyl)-β-CD (DM-β-CD) and hydroxypropyl-β-CD (HP-β-CD) were obtained from Sigma-Aldrich (Milano, Italy). Tris(hydroxymethyl)aminomethane (Tris) was from Carlo Erba (Milan, Italy). 2-Bromoethylbenzene was purchased from Fluka (Buchs, Switzerland). Racemic methoxamine ($ArCHOHC^*HNH_2CH_3$) and DL-lobeline were gifts from Istituto Superiore della Sanità (Rome, Italy). Their structures are shown in Fig. 1. All other chemicals (Carlo Erba) were of analytical grade. Ultrapure water for the preparation of buffer solutions, as well as for rinsing capillaries, was obtained from a Milli-Q water system (Millipore, Milford, MA). Fused-silica capillary tube (10 m × 50 μm ID × 180 μm OD) was obtained from SGE (Melbourne, Australia).

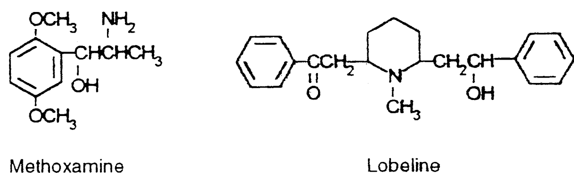

Fig. 1. Molecular structures of methoxamine and lobeline.

2.2. Apparatus and operational conditions

All the CE experiments were performed with a BioFocus 3000 CE system (Bio-Rad, Richmond, CA) equipped with a multiwavelength UV–Vis detector and controlled by a HP Vectra 4/50 computer (Hewlett-Packard, Rome, Italy) with BioFocus and Spectra v.5.00 software (Bio-Rad). Separations were carried out on an ethylbenzene-deactivated fused-silica capillary column, prepared according to the procedure described by Russo and co-workers [19,20]; total column length was 30 cm (25 cm to the detector). No particular conditioning steps were employed for the capillary column coated with ethylbenzene: it was simply rinsed with methyl alcohol (2 ml), water (2 ml) and buffer, pH 2.5, (2 ml). After the electrosmotic flow (EOF) was measured (2×10^{-5} $cm^2\ s^{-1}\ V^{-1}$) the capillary column was ready for analysis. The same procedure was applied every day before starting measurements. An alcohol benzyl solution was employed as flow marker. During separations the high voltage power supply was set to 12 kV with a resulting current of 19 μA. Samples were injected at the anodic end by pressurization (hydrodynamic mode): 34 474 Pa for 0.5 s; direct UV cathodic detection was carried out at λ of 210 nm. After every run the following washing stop was always performed: water for 1 min and buffer for 2 min. Tris–H_3PO_4 buffer (0.1 M, pH 2.5) was prepared by dissolving 6.05 g of Tris in ultrapure water, adjusting to pH 2.5 with phosphoric acid and controlling by a pH meter basic 20 (Crison Instruments, Alella, Barcelona, Spain), and diluting the solution with ultrapure water up to 500 ml in a volumetric flask. CDs were dissolved in the above buffer.

The sample solutions were prepared by dissolving each solute in Tris–H_3PO_4 buffer at concentrations of 0.02–0.2 mg ml^{-1}. Buffers and sample solutions were filtered with a 0.20-μm filter (Alltech, Deerfield, IL) and degassed by sonification prior to use.

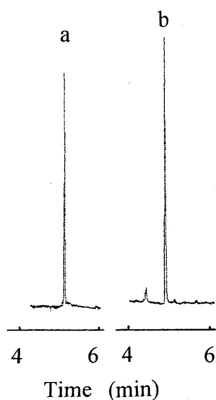

Fig. 2. Electropherograms of the (a) racemic methoxamine and (b) DL-lobeline. Conditions: background electrolyte (BGE), 90 mM Tris–H_3PO_4 (pH 2.5); separation tube (ethylbenzene-deactivated fused-silica capillary column), 30 cm (25 cm to detector) × 50 μm ID × 180 μm OD; running voltage 12 kV, detection, 210 nm (0.005 AUFS); temperature, 25 °C.

3. Results and discussion

Buffer pH plays an important role in CZE operation because the change in pH can affect the solute charge and change EOF, so influencing resolution. It is shown in Refs. [21–23] that successful chiral separation of basic drugs can be obtained only under acidic buffer conditions or more precisely at a pH value where the analyte will be positively charged. Under acidic conditions, basic drugs and their CD complexes will migrate toward the cathode and free CDs are uncharged and will move with the velocity of the EOF, which also moves from anode to cathode. At pH 2.5, the basic racemic drugs will be more positively charged and the EOF will be much smaller than at higher pH, thus providing analytes with longer time for interaction with CD moieties as they migrate through the capillary. Moreover, at pH 2.5, which is near the pK_{a1} value of H_3PO_4, the buffer has a high buffer capacity to resist the pH changes caused by electrolysis effects in CZE process [5]. Fig. 2 shows the electropherogram of the methoxamine and lobeline using only 90 mM of Tris–H_3PO_4 buffer (pH 2.5) with no chiral selector being added: no chiral separation has been obtained. Therefore, chiral separation of two investigated drugs was first examined by using 90 mM Tris–H_3PO_4 buffer solutions (pH 2.5) containing 11.5 mM α-, β-, DM-β-, HP-β- and γ-CD respectively. Fig. 3 indicates that when α-CD was used as chiral selector, we obtain two different behaviours for the two racemic compounds: for the methoxamine ones the chiral separation beings to achieve whereas chiral separation was not achieved for lobeline; neither methoxamine nor lobeline could be chirally separated with γ-CD.

Fig. 4 shows the electropherograms of the chiral separation of methoxamine and lobeline when using three β-CDs as chiral selector at a 11.5 mM each one. When β-CD was used, methoxamine was separated into two peaks and lobeline was only partially separated. When DM-β-CD was used the chiral separation of both methoxamine and lobeline was improved, but lobeline still could not be baseline separated. When HP-β-CD was used, the baseline chiral separation of lobeline were achieved. CD concentration and the addition of organic modifiers to the running buffer can influence the degree of chiral separation [21]. In order to improve the chiral separation of lobeline, the concentration of DM-β-CD and HP-β-CD was investigated in the range 2–50 mM in this work. The optimum concentration for the separation was at 5.8 mM with DM-β-CD and 29.5 mM with HP-β-CD. In both cases, the complete chiral

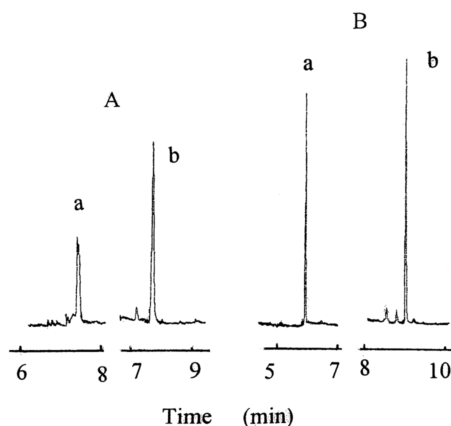

Fig. 3. Electropherograms of the (a) racemic methoxamine and (b) DL-lobeline. (A): BGE, 90 mM Tris–H_3PO_4 containing 11.5 mM α-CD (pH 2.5). (B): BGE, 99 mM Tris–H_3PO_4 containing 11.5 mM γ-CD (pH 2.5). Other conditions as in Fig. 2.

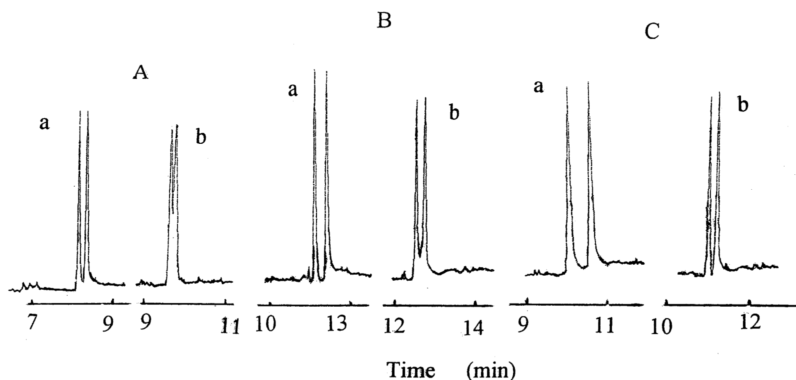

Fig. 4. Electropherograms of the chiral separation of (a) racemic methoxamine and (b) DL-lobeline. (A): BGE, 90 mM Tris–H_3PO_4 containing 11.5 mM β-CD (pH 2.5). (B): BGE, 90 mM Tris–H_3PO_4 containing 11.5 mM DM-β-CD (pH 2.5). (C): BGE, 90 mM Tris–H_3PO_4 containing 11.5 mM HP-β-CD (pH 2.5). Other conditions as in Fig. 2.

separation for lobeline was achieved (Fig. 5). In order to evaluate the separation reliability with the optimum conditions described the lobeline shows a mean M_{t1} and M_{t2} (migration time for racemic mixture) of 10.46 and 10.85 min (Fig. 5), respectively. Those M_t are highly repeatable for run-to-run, day-to-day, and sample-to-sample. Five consecutive runs were carried out and relative standard deviation (RSDs) of 0.06 and 0.07 were obtained (Table 1). In day-to-day measurements RSDs of 0.08 and 0.21% ($n = 15$ runs), and sample-to-sample measurements ($n = 5$) RSDs of 2.0% for 0.02 mg ml^{-1} and 1.2% for 0.2 mg ml^{-1}, respectively, were obtained. However, peak area repeatability were poorer with a mean RSD of 0.15 and 0.12% and 0.68 and 0.65%, respectively (Table 1).

Table 1
Repeatability of a standard solution containing lobeline (five consecutive runs)

Injection	DL-lobeline			
	M_{t1}	A_1	M_{t2}	A_2
1	10.457	6591	10.845	6585
2	10.461	6580	10.853	6595
3	10.448	6570	10.838	6590
4	10.451	6575	10.840	6580
5	10.463	6568	10.855	6575
Mean	10.456	6577	10.846	6585
SD	0.006	9.734	0.008	7.906
RSD(%)	0.061	0.148	0.070	0.120
Day-to-day (3 day)				
[a]Mean (1st day)	10.456	6577	10.848	6585
[a]Mean (2nd day)	10.422	6495	10.802	6502
[a]Mean (3rd day)	10.441	6510	10.820	6535
SD	0.008	43.60	0.023	42.36
RSD(%)	0.082	0.668	0.212	0.648

M_t (Migration time in min); A (Peak area in counts).
[a] Mean of five measurements.

4. Conclusions

The complete chiral separation of methoxamine and lobeline was achieved by CZE with CD-con-

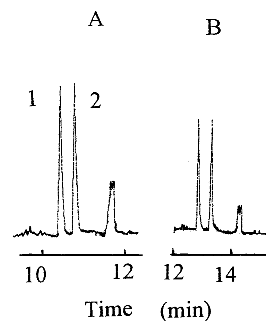

Fig. 5. Electropherograms of chiral separation of DL-lobeline. (A): BGE, 90 mM Tris–H_3PO_4 containing 5.8 mM DM-β-CD (pH 2.5). (B): BGE, 90 mM Tris–H_3PO_4 containing 29.5 mM HP-β-CD (pH 2.5). Other condition as in Fig. 2.

taining buffers and using an ethylbenzene-deactivated fused-silica capillary column. Among the CDs investigated in this work, i.e. α-, β-, DM-β-, HP-β-, and γ-CD, all the three β-type CDs showed chiral recognition on two drugs investigated. Under the investigated conditions, baseline chiral separation of methoxamine can be achieved with 90 mM Tris–H_3PO_4 (pH 2.5) containing 11.5 mM of either of the three β-type CDs, with DM-β-CD giving the best resolution, and baseline chiral separation of lobeline can be realized by using 90 mM Tris–H_3PO_4 buffer (pH 2.5) containing 5.8 mM DM-β-CD or 29.5 mM HP-β-CD. Using EB-coated columns allows to separate at low running voltage (12 kV) obtaining analysis times shorter than those reported in Ref. [17] where the running voltage is 22 kV. This separation method can be conveniently performed and routinely used in the pharmaceutical formulations quality control.

References

[1] J. Snopek, I. Jelinek, E. Smolkova-Keulemansova, J. Chromatogr. 609 (1992) 1.
[2] M. Novotny, H. Soni, M. Stefansson, Anal. Chem. 66 (1994) 646A.
[3] R. Kuhn, S. Hoffstetter-Kuhn, Chromatographia 34 (1992) 505.
[4] S. Terabe, K. Otsuka, H. Nishi, J. Chromatogr. 666 (1994) 295.
[5] H. Nishi, S. Terabe, J. Chromatogr. A 694 (1995) 245.
[6] R. Kuhn, F. Erni, T. Bereuter, J. Hausler, Anal. Chem. 64 (1992) 2815.
[7] A. D'Hulst, N. Verbeke, J. Chromatogr. 608 (1992) 275.
[8] S. Busch, J. Kraak, H. Poppe, J. Chromatogr. 635 (1993) 119.
[9] P. Sun, N. Wu, G. Barker, R. Hartwick, J. Chromatogr. 648 (1993) 475.
[10] S.A.C. Wren, R.C. Rowe, R.S. Payne, Electrophoresis 15 (1994) 804.
[11] D.W. Armostrong, K.L. Rundlett, J.R. Chen, Chirality 6 (1994) 496.
[12] S.G. Penn, E.T. Bergstrom, D.M. Goodall, J.S. Loran, Anal. Chem. 66 (1994) 2866.
[13] Y.Y. Rawjee, G. Vigh, Anal. Chem. 66 (1994) 619.
[14] Y.Y. Rawjee, R.L. Williams, G. Vigh, J. Chromatogr. 680 (1994) 559.
[15] F. Lelievre, P. Gareil, A. Jardy, Anal. Chem. 69 (1997) 385.
[16] E. Hohne, G.J. Krauss, G. Gubitz, J. High Resolu. Chromatogr. 15 (1992) 698.
[17] Z. Wang, A. Huang, Y. Sun, Z. Sun, J. High Resolu. Chromatogr. 19 (1996) 478.
[18] C.R. McCurdy, T.G. Venkateshwaran, J.W. Beach, J.T. Stewart, Electrophoresis 20 (1999) 212.
[19] M.V. Russo, G. Goretti, J. Chromatogr. A 871 (2000) 279.
[20] M.V. Russo, Chromatographia, submitted for publication.
[21] S. Fanali, J. Chromatogr. 545 (1991) 437.
[22] S.A.C. Wren, R.C. Rowe, J.Chromatogr. 603 (1992) 235.
[23] S.A.C. Wren, R.C. Rowe, J. Chromatogr. 609 (1992) 363.