ELSEVIER

Journal of Chromatography A, 684 (1994) 23–27

JOURNAL OF CHROMATOGRAPHY A

Chiral ion-pair chromatographic separation of two dihydropyridines with camphorsulfonic acids on porous graphitic carbon

Martin Josefsson*, Björn Carlsson, Björn Norlander

Department of Clinical Pharmacology, Faculty of Health Sciences, Linköping University, S-581 85 Linköping, Sweden

First received 14 March 1994; revised manuscript received 13 June 1994

Abstract

The direct enantiomeric separation of the two racemic dihydropyridines amlodipine (AML) and UK52.829 (UK) with (1*S*)-(+)-10-camphorsulfonic acid [(+)-CSA] as a chiral counter-ion, on porous graphitic carbon Hypercarb-S, is described. The enantiomers of AML and UK were separated in a mobile phase system consisting of 5 m*M* (+)-CSA in dichloromethane–methanol (25:75, v/v). When the enantiomeric separation of AML and UK was studied in a mobile phase system consisting of 5 m*M* (1*S*)-(+)-3-bromo-10-camphorsulfonic acid [Br-(+)-CSA] in dichloromethane–methanol (25:75, v/v) the capacity factor, k', was markedly increased while the separation factor, α, was slightly decreased compared to the mobile phase with (+)-CSA as chiral counter-ion. No enantiomeric separation of AML or UK was seen in a chromatographic system with acetonitrile substituted for methanol as mobile phase solvent, neither with (+)-CSA nor Br-(+)-CSA as chiral counter-ion.

1. Introduction

Recently a number of methods for chromatographic resolution of drug enantiomers have been developed. One of the main reasons is the increasing interest in finding robust systems for evaluation of differences and similarities in pharmacodynamics and pharmacokinetics of the enantiomers [1].

In high-performance liquid chromatography (HPLC) there are several possibilities for enantiomeric separation on conventional non-chiral columns. Enantiomers can be separated indirectly as diastereomeric derivatives generated by derivatization with a homochiral reagent, or directly by using a chiral additive in the mobile phase (e.g., cyclodextrin, crown ether, metal complex) that promotes enantiomeric separation [2]. Furthermore, a chiral counter-ion dissolved in the mobile phase can be used to separate enantiomers of acids and amines [3]. The enantiomers of some β-amino alcohols, for instance, have been separated with (+)-10-camphorsulfonate as chiral counter-ion in a normal-phase chromatographic system on a LiChrosorb-DIOL column [4].

Porous graphitic carbon (PGC) is a non-polar chromatographic adsorbent that allows special stereoselectivity. It has been considered a "pure" reversed-phase adsorbent with an extremely uniform surface [5,6]. The PGC Hypercarb-S has successfully been used in resolu-

* Corresponding author.

0021-9673/94/$07.00
SSDI 0021-9673(94)00560-V

tion of enantiomeric amines as diastereomeric ion pairs with N-carbobenzyloxyglycyl-L-proline (L-ZPG) or (−) - 2,3:4,6 - di - O - isopropylidene - 2 - keto - L - gulonic acid [(−)-DIKGA] as chiral counter-ion [7,8]. The enantiomers of acids like 10-camphorsulfonic acid (CSA) and N-carbobenzyloxyglycylproline (ZPG), have been separated with quinine as chiral counter-ion [8].

In a previous paper we showed a fast chromatographic separation of the (−)-menthyl chloroformate derivatives of some drug enantiomers (e.g., dihydropyridines, β-amino alcohols) [9]. In this paper we have further investigated chiral separations on PGC, of the dihydropyridines amlodipine (AML), UK52.829 (UK) and felodipine (FEL) with (1*S*)-(+)-10-camphorsulfonic acid [(+)-CSA] or (1*S*)-(+)-3-bromo-10-camphorsulfonic acid [Br-(+)-CSA] as counter-ion.

2. Experimental

2.1. Chemicals

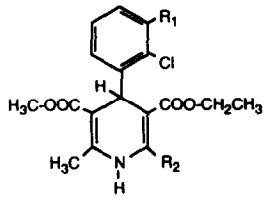
Acetonitrile (far UV), dichloromethane and methanol, all HPLC grade, were purchased from LabScan Analytical Sciences (Dublin, Ireland).

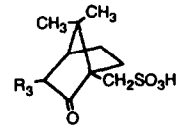
Racemic AML and UK, and *R*-(+)-amlodipine were obtained from Pfizer (New York, USA) and racemic FEL from Astra Hässle (Mölndal, Sweden). (+)-CSA was purchased from Merck (Darmstadt, Germany) and Br-(+)-CSA from Fluka (Buchs, Switzerland). The structures of the chiral substances are shown in Fig. 1.

2.2. Instrumentation

The HPLC system consisted of a Varian 2510 pump (Walnut Creek, CA, USA) a Waters 486 UV detector (Milford, MA, USA), a Spectra-Physics 4270 integrator (San Jose, CA, USA), a Jones Chromatography column heater (Hengoed, Mid Glamorgan, UK) a Rheodyne 7125 injector with a 20-μl loop and a Rheodyne 0.5-μm column inlet filter (Cotati, CA, USA).

Chromatography was carried out on a PGC Hypercarb-S column, 100 × 4.6 mm I.D. (Shandon, Runcorn, England), with well degassed HPLC-grade solvents. The PGC column was thermostated to 30°C and the mobile phase was recirculated for at least 12 h before use. In studies with only minor variations in temperature or counter-ion concentration the mobile phase was recirculated for at least 2 h before use. Mobile phase flow-rate was 1.5 ml/min. Samples containing 2 μg of the free drug were manually injected into a 20-μl loop. The ion pairs were all detected at 250 nm and the elution order of the enantiomers of amlodipine was determined with





	R_1	R_2		R_3
Amlodipine	H	CH_2-O-CH_2-CH_2-NH_2	(1S)-(+)-10-camphorsulfonic acid	H
UK52.829	Cl	CH_2-O-CH_2-CH_2-NH_2	(1S)-(+)-3-bromo-10-camphorsulfonic acid	Br
Felodipine	Cl	CH_3		

Fig. 1. Molecular structures.

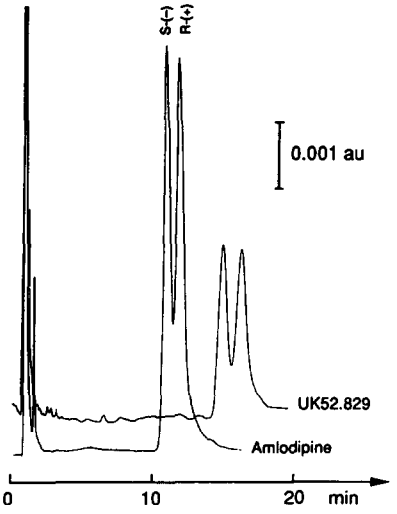


Fig. 2. Separation of racemic amlodipine and UK52.829 on Hypercarb-S. Column temperature: 30°C. Mobile phase: 5 m*M* (1S)-(+)-10-camphorsulfonic acid in dichloromethane–methanol (25:75, v/v). Flow-rate: 1.5 ml/min.

help of the enantiomerically pure *R*-(+)-amlodipine.

2.3. Solutions

Stock solutions of racemic AML, UK and FEL (1 mg/ml) were prepared with 10 mg of the free base in 10 ml methanol. Working solutions (0.1 μg/μl) for all the dihydropyridines were prepared in methanol and acetonitrile.

3. Results and discussion

Enantiomeric separation of the two dihydropyridines AML and UK was achieved in a mobile phase system consisted of 5 m*M* (+)-CSA in dichloromethane–methanol (25:75, v/v) (Fig. 2). The capacity factor, k', and the separation factor, α, of the chiral ion-pairs are presented in Table 1.

The dihydropyridine structure does not act as either a proton acceptor or a proton donor, and dihydropyridines that do not contain other groups with such properties are neutral over the whole pH range [10]. However, the primary amino group on the side chain of AML and UK gives these dihydropyridines basic properties. To verify that the primary amino group was responsible for the electrostatic interaction with the sulfonic acid in (+)-CSA, felodipine (FEL) that lacks this substituent (Fig. 1), was studied in the same chromatographic system.

The optimum (+)-CSA concentration for enantiomeric separation, with acceptable k' values, of AML and UK was determined to be

Table 1
Capacity and separation factors of the enantiomers of three dihydropyridines on Hypercarb-S

Solute	Solvent X: methanol						Solvent X: acetonitrile					
	Y: (+)-CSA		Y: Br-(+)-CSA		No Y		Y: (+)-CSA		Y: Br-(+)-CSA		No Y	
	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α
Amlodipine	22.1	1.08	39.1	1.05	[a]	–	77.6	1	18.5	1	[a]	–
UK52.829	30.9	1.08	53.1	1.05	[a]	–	129	1	30.3	1	[a]	–
Felodipine	58.6	1	55.6	1	60.0	1	71.6	1	59.4	1	55.3	1

Column temperature: 30°C. Mobile phase: 5 m*M* counter-ion Y in dichloromethane–solvent X (25:75, v/v). Flow-rate: 1.5 ml/min.

[a] No peaks visible within 90 min.

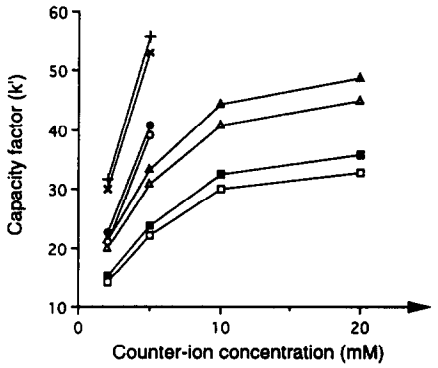


Fig. 3. Capacity factors of the enantiomers of amlodipine (AML) and UK52.829 (UK) at different counter-ion concentrations in a mobile phase system based on dichloromethane–methanol (25:75, v/v). Column: Hypercarb-S. Column temperature: 30°C. Counter-ion: (1*S*)-(+)-10-camphorsulfonic acid [(+)-CSA] or (1*S*)-(+)-3-bromo-10-camphorsulfonic acid [Br-(+)-CSA]. Flow-rate: 1.5 ml/min. □ = AML/(+)-CSA (1); ■ = AML/(+)-CSA (2); △ = UK/(+)-CSA (1); ▲ = UK/(+)-CSA (2); ○ = AML/Br-(+)-CSA (1); ● = AML/Br-(+)-CSA (2); × = UK/Br-(+)-CSA (1); + = UK/Br-(+)-CSA (2).

about 5 m*M* (Fig. 3). At (+)-CSA concentrations above 10 m*M* no further increase in the separation factor, α (k_2'/k_1') was achieved (Fig. 3). The halogenated counter-ion Br-(+)-CSA was compared with (+)-CSA in the same chromatographic system. A marked increase in k' for AML and UK but no rise in α compared to (+)-CSA, was seen (Fig. 3). When the dihydropyridines were studied in a chromatographic system without counter-ion (i.e., dichloromethane–methanol, 25:75) no peaks of AML or UK were visible, but FEL was eluted within 50 min. Migration of the enantiomers of AML and UK was restored when 1 m*M* (+)-CSA was added to the mobile phase.

When the aprotic solvent acetonitrile was substituted for the protic methanol forming a mobile phase system of 5 m*M* (+)-CSA in dichloromethane–acetonitrile (25:75, v/v), no enantiomeric separation of AML and UK was visible. The dihydropyridines, however, both were markedly more retained in this slightly less polar chromatographic system (Table 1). Furthermore, when the halogenated camphorsulfonic acid was studied as counter-ion in the chromatographic system based on acetonitrile no enantiomeric separation was visible while AML and UK surprisingly were much less retained both compared to (+)-CSA in this chromatographic system as well as Br-(+)-CSA in the mobile phase system based on methanol (Table 1).

In chiral ion-pair chromatography the electrostatic interaction alone between the two moieties may not be sufficient for enantiomeric separation [3]. A two-point attractive interaction between the selector (counter-ion) and the enantiomers might be necessary to give different solvations in the mobile phase or different interactions with the stationary phase, which can resolve the diastereomeric ion pairs. The second attachment could be another electrostatic interaction or a hydrogen bond. Beside the ion pairing of AML and UK with (+)-CSA or Br-(+)-CSA as counter-ion there is a possible hydrogen bonding. The keto group in the CSAs might form a bond with the hydrogen atom attached to the nitrogen in the dihydropyridine structure. X-Ray studies on the (−)-(1*S*)-camphanic acid derivatives of AML shows that this is quite possible [11].

Our results indicate that the primary amino group of AML and UK is responsible for a strong interaction with the PGC surface. In the mobile phase system without counter-ion, described above, no peaks of AML or UK were observed while FEL was eluted within 50 min. Charge transfer between unshared electron pairs of the primary amine in AML and UK and the delocalized band of electrons on PGC, might be the explanation [9,12]. Bassler et al. [12] showed that the k' values of some substituted aromates increased linearly, on PGC, with increasing electron donor ability. The migration observed when (+)-CSA successively was added to the mobile phase indicates increasing grade of ion pairing. Further on, we show that the choice of organic solvents play an important role for the retention and selectivity of the diastereomeric ion pairs of AML and UK. Surprisingly no separation was visible in a solvent system based on acetonitrile neither with (+)-CSA nor Br-(+)-CSA as chiral counter-ion. The ability to form stable intermolecular bonds with the coun-

ter-ion ought to be stronger in acetonitrile than in the protic methanol that is able to compete in hydrogen bonding. In a previous paper [9] we showed that an organic solvent with a high content of oxygen lone pairs (e.g., acetic acid or formic acid) in the mobile phase markedly decreased the retention of the (−)-menthyl chloroformate derivatives of AML and UK compared to solvents like acetonitrile or alcohols, on the PGC Hypercarb-S. However, in that chromatographic system the enantioselectivity was higher with acetonitrile in the mobile phase than with alcohol.

The enantiomers of dihydropyridines are generally hard to separate. Vandenbosch et al. [13] showed in their evaluation of six stationary phases, for direct separation of enantiomers, that the dihydropyridines in most systems gave α-values around 1.1. The best results were obtained on protein columns (e.g., Chiral α-glycoprotein, Ovomucoid) with α values, for some of the dihydropyridines, above 1.1.

4. Conclusions

Our results show that the dihydropyridines AML, FEL and UK are highly retained on the PGC. With addition of a chiral counter-ion enantioselective migration of AML and UK is achieved. However, since ion pairs of the solutes, counter-ions and mobile phase solvents seem to competitively interact with the PGC surface the retention mechanisms and selectivity are hard to predict. The strong retention of planar non-polar solutes on PGC are a drawback since chromatography requires high contents of organic solvents, but the stereoselectivity of the material makes it still an interesting complement as a chromatographic tool in chiral separations.

References

[1] A.R. Fassihi, *Int. J. Pharm.*, 92 (1993) 1.
[2] S. Allenmark, *Chromatographic Enantioseparation, Methods and Applications*, Ellis Horwood, Chichester, 2nd ed. 1991, pp. 60, 160.
[3] C. Pettersson, in A.M. Krstulovic (Editor), *Chiral Separations by HPLC*, Ellis Horwood, Chichester, 1991, p. 124.
[4] C. Pettersson and G. Schill, *J. Chromatogr.*, 204 (1981) 179.
[5] J.H. Knox and B. Kaur, *J. Chromatogr.*, 352 (1986) 3.
[6] B. Kaur, *The Use of Porous Graphitic Carbon in HPLC*, Shandon Scientific, Runcorn, 1991.
[7] A. Karlsson and C. Pettersson, *Chirality*, 4 (1992) 323.
[8] C. Pettersson and C. Gioeli, *Chirality*, 5 (1993) 241.
[9] M. Josefsson, B. Carlsson and B. Norlander, *Chromatographia*, 37 (1993) 129.
[10] M. Ahnoff and B.-A. Persson, *J. Chromatogr.*, 531 (1990) 181.
[11] S. Goldmann, J. Stoltefuss and L. Born, *J. Med. Chem.*, 35 (1992) 3341.
[12] B.J. Bassler, R. Kaliszan and R.A. Hartwick, *J. Chromatogr.*, 461 (1989) 139.
[13] C. Vandenbosch, D. Massart and W. Lindner, *J. Pharm. Biomed. Anal.*, 10 (1992) 895.