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Short communication

Enantioselective separation of racemic secondary amines on a chiral crown ether-based liquid chromatography stationary phase

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Abstract

The first general enantioselective separation of racemic secondary amines on a crown ether-based liquid chromatography chiral stationary phase (CSP) is presented. The CSP is based on (+)- or (−)-(18-crown-6)-2,3,11,12-tetracarboxylic acid covalently bonded to silica gel. A mobile phase containing methanol, acetonitrile, triethylamine and acetic acid was employed in these separations of secondary amines with crown ether CSPs. The separation mechanism is believed to be the secondary amine forming a complex which includes crown ether coordination and electrostatic interaction of the positively charged amine with a carboxylate anion of the immobilized crown ether.

Keywords: Enantiomer separation; Chiral stationary phases, LC; Amines

1. Introduction

Chiral stationary phases (CSPs) based on chiral crown ethers have been used extensively for the enantioselective separation of primary amines by liquid chromatography. In the late 1970s, Cram and co-workers immobilized bis-(1,1′-binaphthyl)-22-crown-6 on polystyrene or silica gel to obtain CSPs that resolved the enantiomers of α-amino acids and their derivatives [1,2]. Shinbo and co-workers dynamically coated chiral crown ethers based on disubstituted 1,1′-binaphthyl-20-crown-6 on octadecyl silica gel [3,4]. Dynamically coated CROWNPAK CR columns from Daicel Chemical Industries have been extensively used to enantioselectively separate primary amine compounds [5–7].

Recently Machida and co-workers applied covalently bonded CSPs based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid to the enantioselective separation of primary amines [8–10]. Hyun and co-workers have also applied covalently bonded CSPs based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (Fig. 1) to the enantioselective separation of primary amines [10–12]. The difference in the two phases is in the way the crown ether is attached to the silica surface.

In the above cases, only primary amines were enantioselectively separated by these crown ether-based CSPs. Machida et al. [13] chemically modified (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid with *S*-1-(1-naphthyl)ethylamine as a π-donor. A CSP based on this material was used to enantioselectively separate the secondary amine, *N*-3,5-dinitrobenzoyl-1-(α-naphthyl)ethylamine. The separation was explained by π–π interaction between the 3,5-dinitrobenzoyl function of the analyte (π-acceptor) and the *S*-1-(1-naphthyl)ethylamine moiety (π-donor), since a CSP based on (+)-(18-crown-6)-

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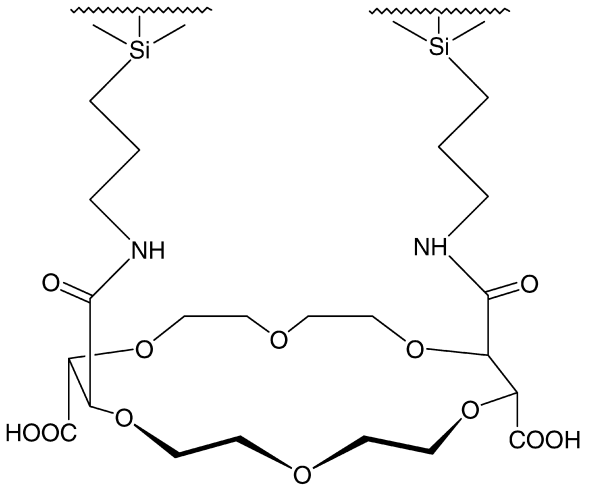

Fig. 1. Chiral crown ether stationary phase.

2,3,11,12-tetracarboxylic acid without the added π-donor could not resolve the secondary amine.

In this study we present the enantioselective separation of secondary amines using Opticrown (USmac Corporation, Thousand Oaks, CA, USA, www.opticrown.com) liquid chromatography columns based on the (+)- and (−)-(18-crown-6)-2,3,11,12-tetracarboxylic acid CSPs developed by Hyun and co-workers [10–12]. The method employs both the ability of crown ethers to complex with amine groups and the steric and electrostatic interactions afforded by the carboxylate functionalities. To the best of our knowledge, this is the first general application of crown ether CSPs to the separation of secondary amine enantiomers.

2. Experimental

2.1. HPLC equipment

The HPLC system (Hewlett-Packard, Palo Alto, CA, USA) consisted of Hewlett-Packard Series 1100 diode array detector, mass spec detector with electrospray, autosampler, quaternary pump with column heater, and degasser. The Opticrown RCA(+) and SCA(−) columns were purchased from USmac Corporation (www.opticrown.com). Columns were 150 mm×4.6 mm with 5 μm particle diameter. The mobile phase consisted of 0.1/0.1/50/50 acetic acid–triethylamine–methanol–acetonitrile (v/v). The flow-rate was 1 ml/min and with detection wavelength 260 nm. Column temperature was held at 25 °C.

2.2. Reagents and materials

All analytes were obtained from Sigma–Aldrich (St. Louis, MO, USA) as racemates, except for atenolol, which was purchased as the individual isomers. HPLC grade methanol was purchased from Burdick & Jackson (Muskegon, MI, USA). HPLC grade acetonitrile and glacial acetic acid were purchased from EM Science (Gibbstown, NJ, USA). Triethylamine was purchased from Aldrich (Milwaukee, WI, USA).

3. Results and discussion

HPLC columns based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid have been applied by Machida and co-workers to the enantioselective separation of amino acids, amino alcohols, drugs containing primary amines and other primary amine compounds [8,9,13]. Hyun and co-workers applied HPLC columns based on the (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid bonded phase (Fig. 1) to amino acids, amino alcohols and other primary amines [10–12]. In this study we expand the range of compounds that can be enantioselectively resolved by the (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid bonded phase (Fig. 1) to secondary amines.

Fig. 2 shows the enantioselective separations we obtained for some drugs with secondary amine functionality, and Table 1 shows retention factors, selectivity, and resolution data. Resolution varies from 1.16 for atenolol to 2.38 for propranolol. Baseline separation was obtained for most compounds. To confirm enantioselectivity, all separations were also run with positive mode electrospray LC/MS to confirm the masses of the separated compounds. The correct masses were obtained in all cases. Also atenolol was spiked with additional (+) isomer and only the second peak showed an increase in peak height, which both confirmed regioselectivity for atenolol and that the (+) isomer elutes second on

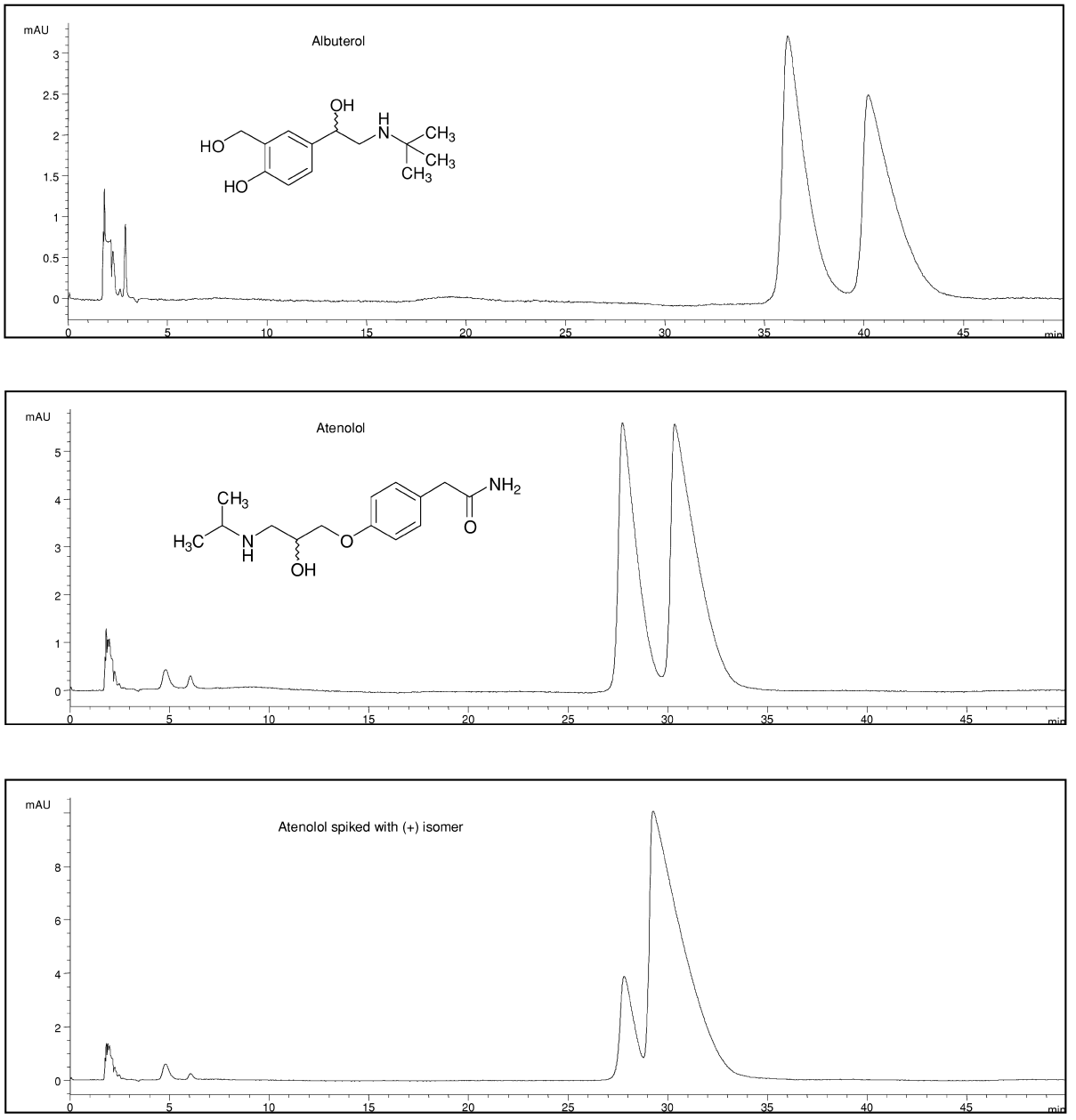

Fig. 2. Chromatograms and structures. All chromatograms were run at 260 nm with 1 ml/min of 0.1/0.1/50/50 acetic acid–triethylamine–methanol–acetonitrile (v/v) on the RCA column.

the (+) column (Fig. 2). Switching to the (−) column reversed the peak order for atenolol.

Both an amine modifier and acid modifier are required in the mobile phase. With acetic acid alone, there is little or no analyte retention and no separation. With triethylamine alone the analytes do not elute. It is interesting to note that the macrocyclic glycopeptide bonded phases based on vancomycin, teicoplanin, and ristocetin A (Advanced Separation Technologies, Whippany, NJ, USA) also use tri-

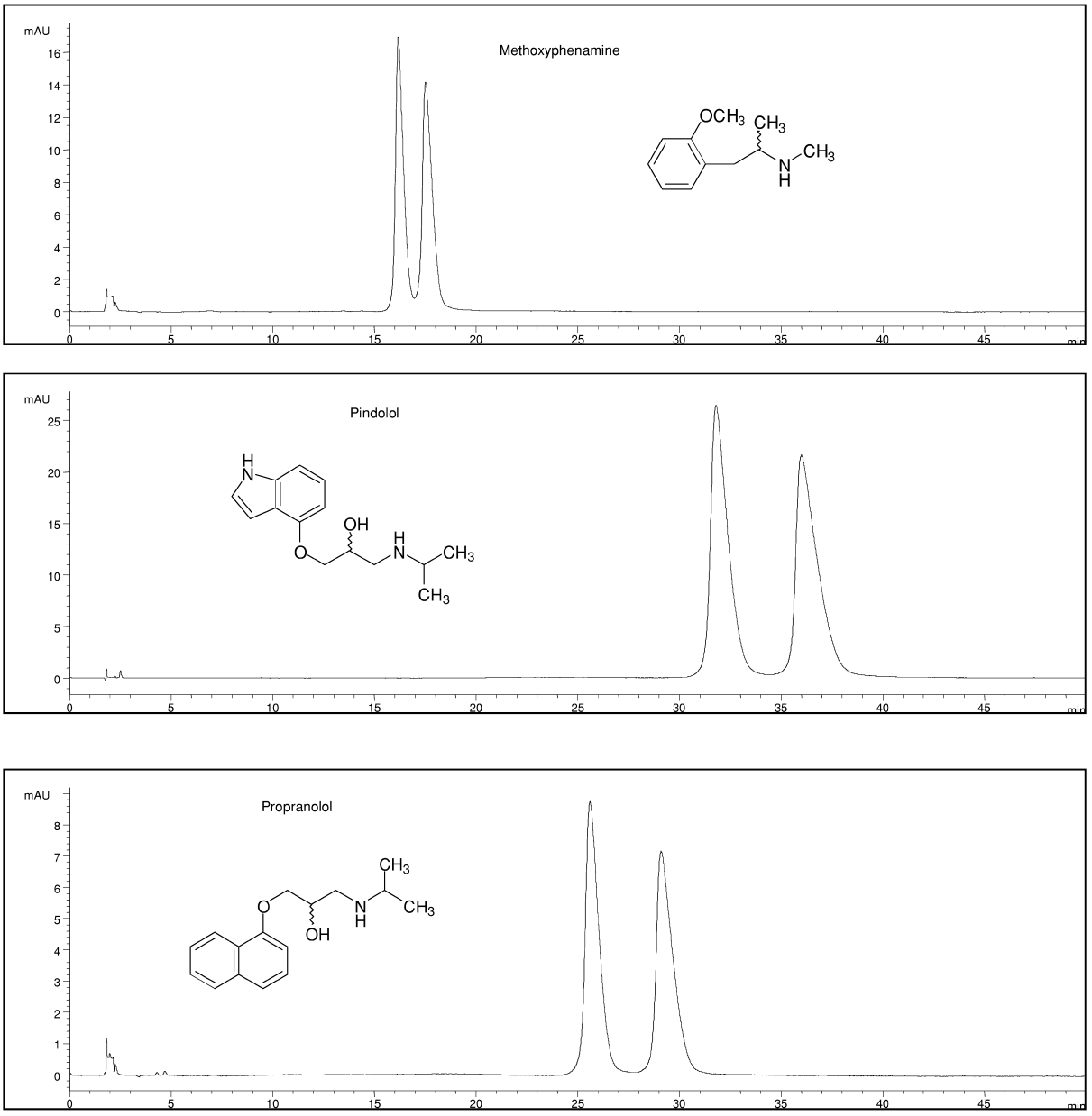

Fig. 2. (*continued*)

ethylamine and acetic acid in the nonaqueous polar organic mode of chiral separation [14].

The method presented in this work demonstrates the separation of secondary amines with crown ether CSPs, but due to long equilibration times detailed mobile phase studies were not undertaken. We believe that the methanol present in the mobile phase is partially esterifying the carboxylic acid groups of the crown ether. We were able to generate columns with stable retention times by flushing the columns with 10 m*M* sulfuric acid in water at 1 ml/min for 4 h, followed by 0.1% triethylamine in water at 1

Table 1
Capacity, selectivity, and resolution data

	Capacity peak 1	Capacity peak 2	Selectivity	Resolution
Albuterol	19.09	21.34	1.12	1.44
Atenolol	14.40	15.86	1.10	1.16
Methoxyphenamine	7.977	8.727	1.09	1.55
Pindolol	16.66	18.99	1.14	2.29
Propranolol	13.22	15.16	1.15	2.38

ml/min for 9 h to remove sulfuric acid from the column, followed by equilibration with 2.5 l of mobile phase at 1 ml/min. During mobile phase equilibration, enantioselective separations were obtained for all analytes, but retention slowly decreased until stable retention times were obtained. The separations presented in Fig. 2 were obtained after this flush and equilibration procedure.

The above observations point to the carboxylates providing electrostatic interactions in addition to possible steric interactions in the enantioselective separation of secondary amines. Crown ethers are not known to show strong binding with secondary amines alone, and in their early work on dynamically coated crown ether stationary phases [3] Shinbo et al. were unable to resolve proline, the only naturally occurring amino acid with a secondary rather than primary amine. However, Machida et al. performed crystallographic studies on complexes of the primary amine, 1-(1-naphthyl)ethylamine, with (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid and showed that the interatomic N–H···O hydrogen bond distance was shorter to the carboxylate oxygen than to the three crown oxygens [15]. In the case of secondary amines, we believe that two N–H···O hydrogen bonds are formed with the crown oxygens in conjunction with one ionic interaction with a carboxylate ion of the crown ether.

In summary, this work is the first general method for the enantioselective separation of secondary amines with a chiral crown ether-based CSP, demonstrated on compounds of pharmaceutical interest.

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