Iranian Journal of Pharmaceutical Research (2008), 7 (4): 275-281
Received: May 2008
Accepted: July 2008

Original Article

Chiral Separation of Basic and Zwitterionic Drugs by Highly Sulfated Cyclodextrins Using Short-End Injection Capillary Electrophoresis

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Abstract

Due to high resolution power of sulfated cyclodextrins (HS-CDs), utilization of these selectors for chiral resolution of 7 basic and 2 zwitterionic drugs have been examined. Experiments were performed on a HP^{3D}CE instrument equipped with an on-column diode array UV absorbance detector. Fused silica capillaries with an inner diameter of 50 μm, an outer diameter of 365 μm, and a total length of 45.5 cm (8.5 cm to the detector) were used. Capillaries were rinsed with PEO once daily. To reduce analysis time, short-end injection technique was applied. The electrophoretic conditions for the stereoselective analysis of drugs were in the carrier mode with 25 mM sodium phosphate buffer containing 1.25% w/v of each HS-CD at pH 2.5 with an applied voltage of +15 kV. At these conditions, rapid enantioresolution(≤3.9 min) of all drugs were achieved.

Keywords: Chiral; Capillary electrophoresis; Highly sulfated cyclodextrins; Short-end injection; Basic drugs; Zwitterionic drugs.

Introduction

About 40% of drugs in use are chiral (1) and in most cases two enantiomers of chiral drugs exhibit different pharmacological, toxicological or pharmacokinetic properties (2). Therefore, development of analytical methods for enantiomer separation for controlling synthesis, enantiomer purity check, and for pharmacodynamic studies is attracting area of research (3). Thus, different analytical techniques have been applied such as high-performance liquid chromatography (HPLC) (4, 5), thin-layer chromatography (TLC) (6), gas chromatography (GC) (7), supercritical fluid chromatography (SFC) (8) and recently capillary electrophoresis (CE) (9, 10).

Due to its high separation efficiency and flexibility of capillary electrophoresis (CE), this method experienced rapid growth in the field of chiral separations (11-15). In CE-based chiral analyses various chiral selectors are simply added to the separation buffer and act as a pseudostationary phase. Chiral selectors can stereoselectively distinguish both enantiomers of the analyte through different binding constants.

Cyclodextrins (CD) are cyclic oligosaccharides consisting of several D (+)-glucopyranose units joined via α-1,4 linkages (9) Common CDs consist of six, seven, or eight glycopyranose units, and are referred to as α-, β-, and γ-CDs respectively. The three-dimensional conformation of CDs displays an open cavity. The outside of this cavity is hydrophilic, making the CDs soluble in aqueous solution. The inside of the cavity is less hydrophilic in nature than the surrounding water. Chiral selectivity results from inclusion of a hydrophobic portion of the

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solute into this cavity and also from the hydrogen bonding to the stereospecific polar functionalities along the rim of the CD.

The hydroxyls on the rims of the CDs can be chemically modified to yield derivatives that differ in degree of substitution, charge and polarity. Current derivatives include: uncharged methylated-, hydroxylated-ethylated-, hydroxypropylated-, and acetylated-CDs; and charged CDs such as methylamino-, sulphobutylether-, carboxymethylated-, sulphated-, and phosphated-CDs, etc. Anionic CDs are the most popular selector for resolution of chiral compounds in CE (9). Among all the anionic CDs, sulfobutyl ether and sulfated cyclodextrins in particular appear to be the most effective charged chiral selectors. However, these anionic CDs are relatively heterogeneous and may not provide reproducible results as chiral reagents (15, 16).Highly sulfated cycldextrins (HS-CDs) are obtained as consistent products with reproducible performance as chiral selectors for CE, and contain a substantially higher degree of sulfation and narrower heterogeneity. These selectors are used for enantioseparation of neutral, acidic, and basic compounds (17, 18).

The high selectivity of these chiral selectors (19) enabled the short-end injection technique (20) to be used. In this technique, the sample is injected at the end of the capillary which is the closest to the detection window. This reduces the separation length, hence the migration times and analysis time. In this work HS-CDs are explored for CE enantioseparation of of 7 basic and 2 zwitterionic drugs.

Experimental

Reagents and chemicals

All solutions were prepared in nanopure 18 M Ω water (Barnstead, Chicago, IL). HS-α, β, and γ CDs with an average degree of substitution of 11, 12, and 13, respectively were obtained from Beckman Coulter, Inc. (Fullerton, CA) as 20% w/v solutions in water. Running buffers containing 1.25% w/v HS-CDs were prepared by diluting the appropriate HS-CD stock solution (20% w/v) with phosphate buffer. Phosphate buffers were prepared by dissolving sodium phosphate monobasic monohydrate (Merck, Darmstadt, Germany) to obtain a final concentration of 25 mM. pH adjusted to 2.5 with 1 M orthophosphoric acid (Merck, Darmstadt, Germany). All capillaries were rinsed with conditioning solution containing 0.4% w/v polyethylene oxide (PEO, MW 300,000) as received from Beckman Coulter, Inc. Atropine sulphate, chloroquin maleate, citalopram HBr, metoprolol tartrate, propranolol HCl, tramadol HCl, verapamil HCl, baclofen, and ofloxcacin was provided by Minoo Co. (Tehran, Iran). Standards of 250 ppm of drugs were prepared in nanopure water.

Apparatus

Experiments were performed on a HP3DCE capillary electrophoresis system (Hewlett-Packard, Palo Alto, and CA. USA) equipped with a diode array detector. CE Chemstation software (Version A.06.01; Hewlett-Packard) was used for control and data acquisition. The data acquisition rate was 5 Hz, and the rise time was 0.1 s. Diode array detector was set at 200 nm. Untreated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ) with a total length of 45.5 cm (8.5 cm to the detector), an inner diameter of 50 μm, and an outer diameter of 360 μm were used. Samples were injected at the end of the capillary nearest the detector by applying a pressure of -10 mbar for 5 s. Separations were performed at +15 kV which was experimentally determined to be within the linear portion of the Ohm's plot.

Under high pressure (950 mbar), each new capillary was pretreated by rinsing with 0.1 M NaOH for 10 min, water for 5 min, PEO solution for1 min, and with running buffer for 5 min, sequentially. Between runs, the capillary was flushed with the running buffer for 2 min.

Throughout the investigation to yield reproducible results, the capillaries were rinsed with PEO once daily. The pH was measured using a digital pH meter (Metrohm, Herisau, Switzerland) calibrated with standards immediately prior to use.

Results and Discussion

Resolution of basic drugs

Resolution of 7 basic chiral drugs (Figure 1)

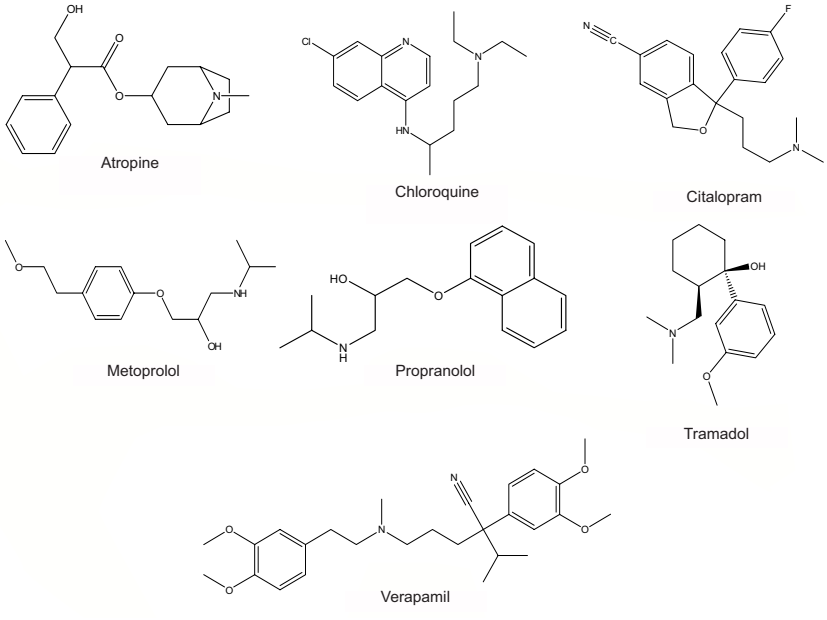


Figure 1. Structure of the basic drugs.

including atropine sulphate, chloroquin maleate, citalopram HBr, metoprolol tartrate, propranolol HCl, tramadol HCl and verapamil HCl by using HS-CDs were investigated. In all of drugs at least one aromatic ring form hydrophobic interaction with HS-CD and one amine group for electrostatic interaction with selector (21) exist. All analysis performed in selector-free buffers. Merging of two enantiomers peaks to one peak in selector-free buffers could confirm chiral resolution of drugs in selector containing buffers.

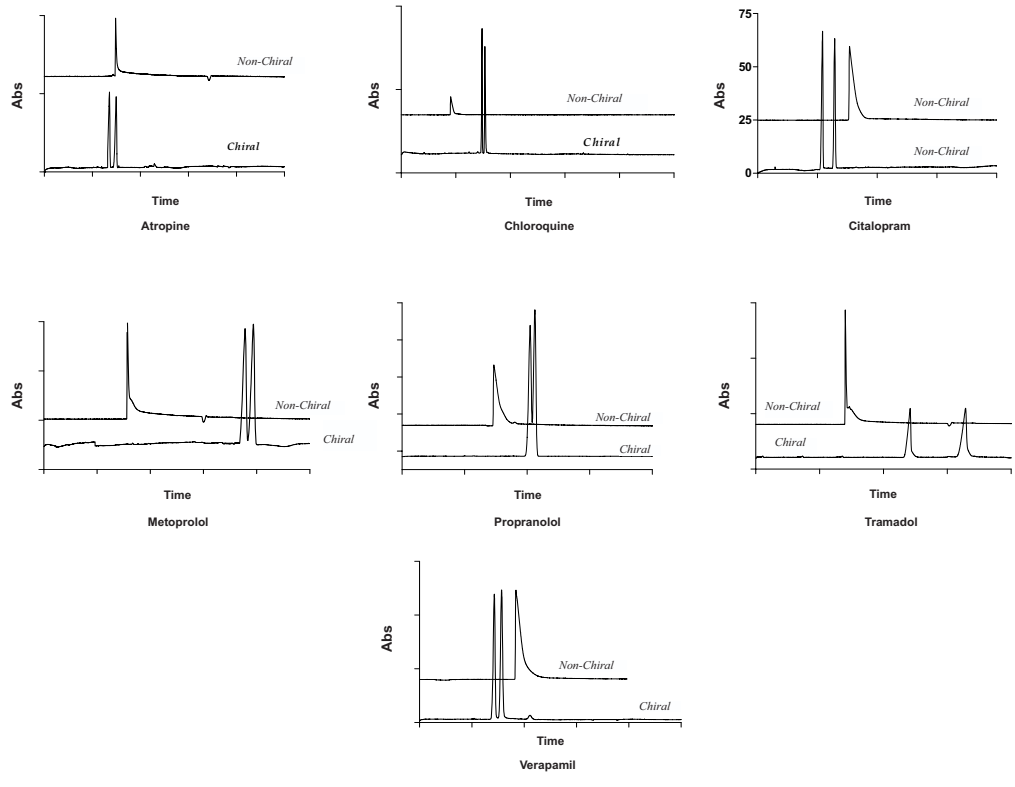
Separations were achieved using a pH 2.5 running buffer containing 25 mM sodium phosphate containing 1.25% of each HS-CD, and an applied voltage of +15 kV. These conditions previously optimized by this group for chiral resolution of basic drug, amlodipine (22).

Figure 2 shown non-chiral and chiral resolution of basic drugs. Under acidic conditions, basic drugs are positively charged and thus, intrinsically migrate toward the cathode. On the other hand, HS-CDs are polynegatively charged and migrate towards the anode. These opposite inherent mobilities enhance the effect of differences in enantiomer binding, making HS-CDs much more capable of resolving the enantiomers of these drugs. The resolving power of HS-CDs could also be a result of the electrostatic interaction between the HS-CDs and the oppositely charged solutes (15).

Typically, separations using HS-CDs are performed under conditions of suppressed EOF (17, 23-27). Among several methods that have been developed to suppress EOF, the use of low pH buffers was widely applied for analysis of basic drugs (28, 29). However, even below pH 2.0 EOF still exists. Further, bare silica capillaries produce rather irreproducible EOF (>5% RSD) under acidic conditions (pH 2.0-3.0) (30). Therefore, the migration time repeatability can be adversely affected by variable residual low level EOF. In addition, highly basic drugs can interact with residual silanols, which result in peak tailing.

Another common approach to suppress EOF has been to coat the capillary wall (30, 31). Coatings can be broadly categorized as covalently linked polymeric coatings, physically adsorbed polymer coatings, or small-molecule additives. In this work, capillaries were coated semi-permanently with PEO to suppress the EOF and to decrease irreproducible EOF and analyte adsorption.

Due to strong interaction of HS-CDs to



Non-chiral conditions: buffer, 25 mM sodium phosphate (pH 2.5); voltage, -15 kV; sample, 250 ppm.
Chiral conditions: 25 mM sodium phosphate (pH 2.5) containing 1.25% HS αCD (for metoprolol, propranolol, and verapamil) or 1.25% HS- βCD (for atropine and chloroquine) or 1.25% HS γCD (for tramadol and citalopram) ; voltage applied, +15 kV; sample concentration, 250 ppm for each drug.

Figure 2. Chiral and non-chiral resolution of citalopram.

basic drugs, these analytes dragged toward anode due the "carrier effect" (11, 32) of HS-CDs. In the carrier mode a chiral selector is not only responsible for the enantioselectivity in separation system but also transports the resolved analytes towards the detector. In carrier mode the analyte migrates toward the detector only when associated with a chiral selector (11). Thus, in short-injection mode it was necessary to apply positive polarity for detection of basic enantiomers.

The results of basic drugs resolutions with different HS-CDs are summarized in Table 1.

In all investigated basic drugs, chiral resolution achieved at least by one selector. In native cyclodextrins analyte must fit the CD cavity in order to form inclusion-complexes (21). This rule does not generally apply to the separations made by charged cyclodextrins such as HS-CDs (27). For example, in the case of tramadol with single aromatic ring superior resolution achieved with HS-γCD and not the HS-αCD. Thus, previous understanding gained from native CDs may not be directly applicable to selecting the optimal sulfated CDs prior to the separation and screening step with different HS-CDs is a requisite.

As shown in Table 1, tramadol and citalopram revealed better resolution (Rs≥4.5). Distinct character of these two molecules is location of chiral center on a ring system. Presence of chiral center on rigid ring restricts rotation of hydrophilic groups and hence enhances difference between complex constants of enantiomers.

Resolution of zwitterionic drugs

Zwitterionic compounds are neutral molecules carries formal positive and negative

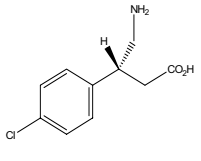
Table 1. Chiral resolution of basic drugs.

Name	Chiral selector	1st Peak migration time	2nd Peak migration time	1st Peak efficiency	2nd Peak efficiency	Resolution
	HS-αCD	1.89	…	40000	…	….
Atropine	HS-βCD	1.34	1.48	11000	8000	2.3
	HS-γCD	1.65	…	4000	…	…
	HS-αCD	1.20	…	25000	…	…
Chloroquin	HS-βCD	1.47	1.53	53000	58000	2.3
	HS-γCD	1.17	…	130000	…	…
	HS-αCD	1.70	1.81	14000	21000	2.0
Citalopram	HS-βCD	1.05	1.16	6000	7000	2.0
	HS-γCD	1.06	1.28	9000	10000	4.5
	HS-αCD	3.72	3.90	12000	14000	1.4
Metoprolol	HS-βCD	1.59	....	4000	...	.....
	HS-γCD	2.32	2.41	8000	9000	0.9
	HS-αCD	2.02	2.10	9000	16000	1.0
Propranolol	HS-βCD	1.29	....	8000	....	....
	HS-γCD	1.19	…	5000	…	…
	HS-αCD	1.81	1.96	11000	1100	2.0
Tramadol	HS-βCD	1.45	1.56	12000	15000	2.1
	HS-γCD	2.41	3.28	9000	12000	7.8
	HS-αCD	1.42	1.58	7000	9000	2.4
Verapamil	HS-βCD	1.65	1.78	16000	18000	2.3
	HS-γCD	1.56	1.64	17000	13000	1.4

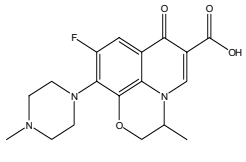
Experimental conditions: 25 mM sodium phosphate (pH 2.5) containing 1.25% of each HS-CD; voltage applied, +15 kV; sample concentration, 250 ppm for each drug.

charges on different atoms . Chiral resolution of two zwitterionic drugs including baclofen and ofloxacin (Figure 3) were investigated. Same as basic drugs under acidic conditions zwitterionic drugs acquire positive charge and migrate toward anode. Therefore, for detection of these analytes positive polarity was employed.

Same as tramadol and citalopram, in ofloxacin, chiral center located on a ring system and high resolution was obtained (Table 2 and Figure 4).



Baclofen



Ofloxacin

Figure 3. Structure of the zwitterionic drugs.

Conclution

This work demonstrates the potential of HS-CDs for rapid chiral separation of 7 basic and 2 zwitterionic drug using short-end injection method in a capillary dynamically coated with PEO to suppress the EOF. The general conditions for resolution of all drugs were with 25 mM sodium phosphate buffer containing 1.25% w/v of HS-CDs at pH 2.5 with an applied voltage of +15 kV. For each drug, resolution with every HS-α, β, and γ CDs was examined. In all investigated drugs,

Table 2. Chiral resolution of zwitterionic drugs.

Name	Chiral selector	1st Peak migration time	2nd Peak migration time	1st Peak efficiency	2nd Peak efficiency	Resolution
Baclofen	HS-αCD	1.12	…	2800	…	…
	HS-βCD	2.55	2.66	8000	7000	1.0
	HS-γCD	2.47	…	10000	…	…
Ofloxcacin	HS-αCD	2.04	…	17000	…	…
	HS-βCD	2.73	3.25	16000	7000	4.8
	HS-γCD	1.95	2.15	14000	10000	2.6

Experimental conditions: same as Table 1.

rapid chiral resolution (≤3.9 min), with at least one HS-CD was attained. Results illustrate that presence of chiral center on a ring system enhances chiral resolution of basic and zwitterionic drugs.

Acknowledgment

We wish to thank The Vice Chancellor for Research Affairs of Shaheed Beheshti University of Medical Sciences (SBMU) for the financial support of this work. The authors appreciate Minoo Company for kind gift of investigated drugs.

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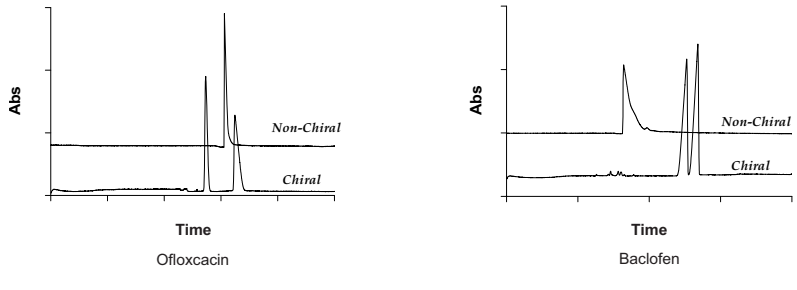

Figure 4. Chiral and non chiral resolution of zwitterionic drugs by 1.25% HS-βCD.
Experimental conditions: same as Figure 2.

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