Quantitative Chiral Analysis of Carbinoxamine, Doxylamine, and Orphenadrine by Capillary Zone Electrophoresis

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Abstract: A simple capillary zone electrophoresis method is described for the simultaneous separation and quantitation of chiral carbinoxamine maleate, doxylamine succinate, and orphenadrine citrate using achiral diphenhydramine · HCl as an internal standard. The chiral analysis of these drugs was performed in a Tris buffer (100 mM; pH 4.60) with sulfated β-cyclodextrin (15 mg/mL) as a chiral selector. Several parameters affecting the separation were studied, including the pH of the buffer and the concentrations of buffer and chiral selector. Quantitation of the individual enantiomer (prepared from the related racemate) is attainable at 25–125 μM for carbinoxamine maleate, doxylamine succinate, or orphenadrine citrate. The migration order of the separated enantiomers is compared to that of a structurally related dexchlorpheniramine, an *S*-enantiomer of chlorpheniramine.
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Key words: *carbinoxamine; doxylamine; orphenadrine; enantiomeric quantitations; capillary zone electrophoresis*

INTRODUCTION

Carbinoxamine maleate (CA), doxylamine succinate (DO) and orphenadrine citrate (OR) are antihistamines (or muscle relaxants) of substituted aminoalkyl ether (ethanolamine series) [1]. They are widely used for the treatment of various allergies. Each drug has a chiral center (Figure 1) and can exist as an *R*- or *S*-enantiomer. In a chiral environment such as a human biosystem, the *R*- and *S*-enantiomers of chiral drugs may behave differently in absorption, distribution, biotransformation and excretion. For example, the more active enantiomer of CA is reported to have an *S*-configuration [2]. Due to the general difficulties in the preparation and analysis of enantiomers that are required for a pharmacological study, many chiral drugs developed in the past were marketed as racemates. Therefore, economic approaches for the analysis and preparation of enantiomers are instrumental in the investigation of enantiomers for their possible pharmacological differences.

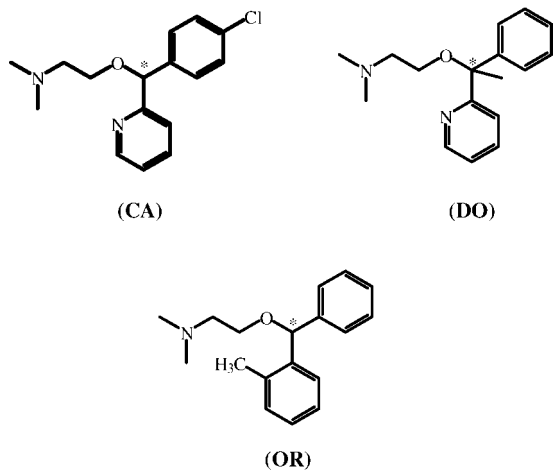

Figure 1. *Structures of carbinoxamine (CA), doxylamine (DO), and orphenadrine (OR).*

A number of capillary electrophoresis (CE) methods with chiral selectors [3–22] have been reported for the qualitative chiral separation of CA, DO, OR, and other chiral drugs. Among them, several chiral selectors were used for successful separation of CA, DO, or OR enantiomers under suitable CE conditions. Namely, CA enantiomers were separated with sulfated β-cyclodextrin (β-CD) [12]; DO

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enantiomers were separated with β-CD [3], carboxymethylated β-CD (CM-β-CD) [4,5,8], heparin sodium [6], hydroxypropyl-α-CD [20] or sulfated β-CD [21]; and OR enantiomers were separated with sulfated β-CD [12], hydroxypropyl-γ-CD [18], or hydroxypropyl-α-CD [20]. No reports are available for the simultaneous quantitation of chiral CA, DO, and OR. In this work, a capillary zone electrophoresis (CZE) method has been developed for the chiral quantitation of CA, DO, and OR with readily available sulfated β-CD as a chiral selector. The results indicate that the method is simple and efficient for the quantitation of CA, DO, and OR enantiomers.

EXPERIMENTAL

Apparatus and CE conditions. A Beckman P/ACE system 2200 (Fullerton, CA, USA) equipped with a filter ultraviolet (UV) detector and a liquid-cooling device was used. CD-mediated CZE was performed in an uncoated fused silica capillary (37 cm × 50 μm inner diameter, 30 cm effective length, Polymicro, Phoenix, AZ, USA). Samples were injected at the cathode end by pressure (0.5 psi) for 2 s and the applied voltage was 15 kV. Enantioseparation was performed at about 25°C in Tris buffer (100 mM; pH 4.60) with sulfated β-CD (15 mg/mL). Detection was carried out by the on-column measurement of UV absorption at 200 nm (anode at the detection side). A Beckman Gold software system was used for data processing.

Chemicals and related solutions. CA, DO, OR, diphenhydramine · HCl (DP), dexchlorpheniramine maleate (d-CP), and dexbrompheniramine maleate (d-BP) (Sigma, St. Louis, MO, USA), α-CD, β-CD, and γ-CD (TCI, Tokyo, Japan), CM-β-CD polymer (Cyclolab, Budapest, Hungary), sulfated β-CD with a degree of subsitution 7–11 (Aldrich, St. Louis, MO, USA) and tris(hydroxymethyl)aminomethane (Tris) (E. Merck, Darmstadt, Germany) were used without further treatment. Milli-Q (Millipore, MA, USA) treated water (water) was used for the preparation of buffer and related aqueous solutions. Solutions of various Tris buffer at pH 4.60 were prepared by treating appropriate Tris solution with H_3PO_4. Reference solutions of CA, DO, and OR at various concentrations and internal standard solution of DP at 200 μM were prepared in water.

RESULTS AND DISCUSSION

Enantioseparation of CA, DO, and OR racemates each at 200 μM by CZE was briefly tested with common chiral selectors (α-CD, β-CD, and γ-CD, each at 10 mM or CM-β-CD at 4 mg/mL) in Tris buffer (100 mM; pH 2.50) with other CE conditions as stated under "Apparatus and CE conditions" except that a normal polarity mode was used, i.e., detection at the cathode side. Simultaneous separation of CA, DO, and OR is unattainable using the common chiral selectors under the tested conditions (data not shown). Since sulfated β-CD was used in the qualitative analysis of various chiral drugs [9,12,21] with good separation results, simultaneous separation and quantitation of CA, DO, and OR in sulfated β-CD-mediated Tris buffer was studied by CE with reversed polarity mode (detection at the anode end). The main parameters for optimizing the enantioseparation of CA, DO, and OR were investigated, including buffer pH and concentrations of Tris buffer and sulfated β-CD.

pH of buffer. The effects of various acid pHs (3.50–5.00) on the separation of CA, DO, and OR in Tris buffer with sulfated β-CD are shown in Figure 2. The separation of CA, DO, and OR enantiomers is attainable at pH ⩾ 4.60. Incomplete separation found at a lower pH resulted mainly from the small electroosmotic flow (at low pH) and the high electrophoretic mobility of CD inclusion complexes/ion-pair species, unfavorably leading to fast migration and a narrow separation window.

Concentration of sulfated β-CD. Figure 3 shows the effects of various concentrations of sulfated β-CD in Tris buffer (100 mM; pH 4.60) on the separation of CA, DO, and OR racemates. Good resolution of CA, DO, and OR with suitable migration time is obtainable using sulfated β-CD at a concentration ⩾15.0 mg/mL. In this case, the migration time seems to be governed mainly by the

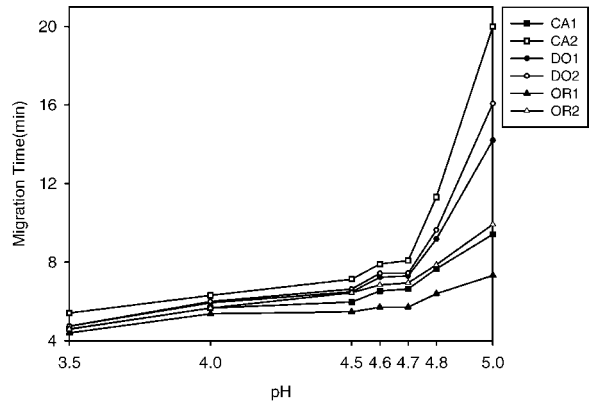

Figure 2. *Effects of buffer pH on the migration of the drugs in the presence of 15 mg/mL sulfated β-CD in Tris buffer (100 mM). Enantiomeric pairs of CA1/CA2, DO1/DO2, and OR1/OR2 for carbinoxamine maleate, doxylamine succinate and orphenadrine citrate, respectively, giving the first eluted enantiomer as 1 versus the second eluted enantiomer as 2 for each pair.*

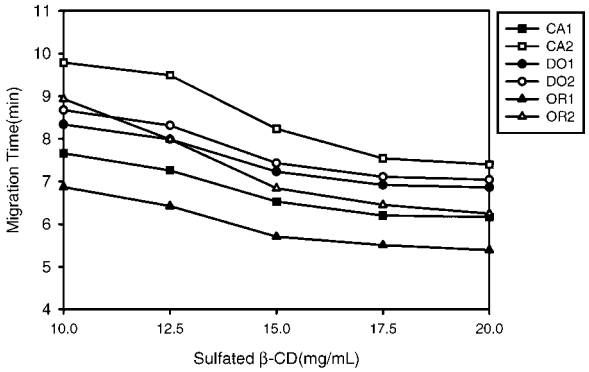

Figure 3. *Effects of sulfated β-CD concentrations on the migration of the drugs in the Tris buffer (100 mM; pH 4.60). See Figure 2 for the descriptors of the related drugs.*

electrophoretic mobility of the analyte-sulfated β-CD complex (a multiple negatively charged species).

Concentration of Tris buffer. The effects of various concentrations of Tris buffer (25−125 mM) at pH 4.60 with sulfated β-CD (15 mg/mL) on the separation of CA, DO, and OR enantiomers are shown in Figure 4. Baseline resolution of all enantiomer pairs is obtainable at a buffer concentration $\geqslant 75$ mM, and the buffer concentration at 100 mM is selected for the CE analysis. Based on the optimizing conditions, a typical electropherogram for the simultaneous separation of CA, DO, and OR is shown in Figure 5. Peak 0 in Figure 5 is equivalent to maleic acid (MA) (by a migration time test) from the free organic acid moiety of CA. MA is an unsaturated dicarboxylic acid (containing a conjugated chromophore) with significant absorbance at 200 nm used for present CE detection.

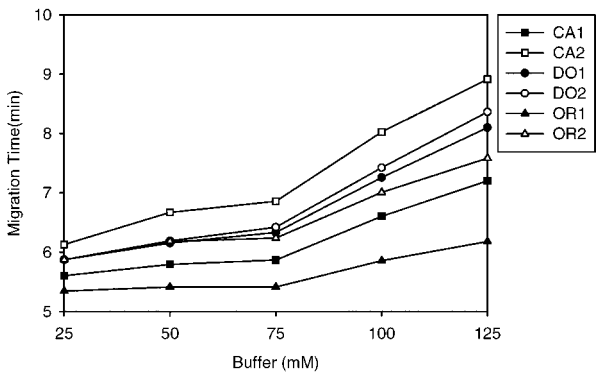

Figure 4. *Effects of buffer concentrations on the migration of the drugs in the presence of 15 mg/mL sulfated β-CD in the Tris buffer (100 mM; pH 4.60). See Figure 2 for the descriptors of the related drugs.*

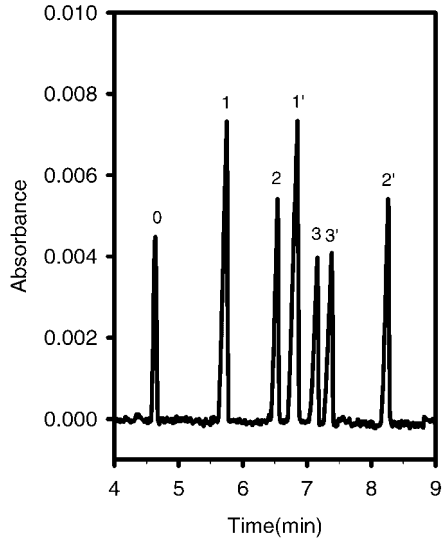

Figure 5. *Electropherogram of CD-mediated capillary zone electrophoresis of the drugs each at 200 μM in the presence of 15 mg/mL sulfated β-CD in the Tris buffer (100 mM; pH 4.60). Peaks: 0 for maleic acid; 1 and 1' for OR1 and OR2; 2 and 2' for CA1 and CA2; 3 and 3' for DO1 and DO2.*

Some separation considerations. The detailed mechanism for the separation and migration order of CA, DO, and OR enantiomers in Figure 5 is unknown. But some separation may be considered from their structural basis (Figure 1). The common feature of CA, DO, and OR is that they all have a 2-dimethylaminoethoxy moiety. That can be fully protonated under the present CE condition (at pH 4.60) because the related basic compound, ethanolamine [23] has a pK_a of 9.4. The main structural differences among CA, DO, and OR that affect separation may include the following. (i) A pyridine ring appears on CA or DO, but it is absent on OR that has an *o*-tolyl group instead. The pyridine nitrogen can be partly protonated at pH 4.6 (pyridine with a pK_a of 5.19), resulting in difference of inclusion formation or ion-pair interaction. (ii) A phenyl group is linked to DO or OR, but a *p*-chlorophenyl group instead is connected to CA. (iii) A small hydrogen atom is linked to the chiral center of CA or OR, but a methyl group is linked to that of DO. These variations in structures may make a difference in fitting CA, DO, or OR in the sulfated β-CD cavity.

No reference enantiomers are available for CA, DO, and OR, and the separated enantiomeric pairs of CA, DO, and OR cannot be assigned. By comparison with the structure-related chiral antihistamines of d-CP (an *S*-enantiomer) and d-BP (an *S*-enantiomer) (Figure 6), d-CP or d-BP behaves as the first

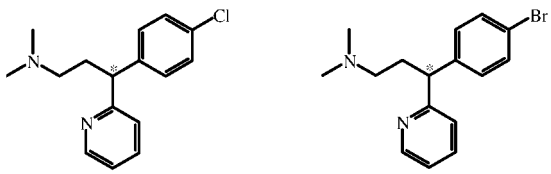

Figure 6. *Structures of chlorpheniramine (CP) and brompheniramine (BP).*

eluate of its enantiomeric pair (data not shown) under the present CE conditions. The *levo*-isomer of carbinoxamine is reported with an *S*-configuration and it is superimposed upon the *S*-configuration of d-CP [2]. The exact relationship between the first eluate of CA, DO, or OR and an *S*-configuration needs to be confirmed.

Analytical calibration. For studying the quantitative applicability of the method, five different concentrations of CA1:CA2, DO1:DO2, and OR1:OR2 pairs were each analyzed in the range of 25–125 μM, using DP as an internal standard (IS). The commercial CA, DO, and OR used for the preparation of the calibrated enantiomeric solutions were tested and proved acceptable as related racemates (Table I). CA1, DO1, and OR1, respectively, stand for the first eluate of each enantiomer pair versus their second migrating counterpart as CA2, DO2, and OR2, respectively. The linearity was evaluated between the normalized peak-area ratios (Y) of an analyte to the IS and the concentrations (X, μM) of the analyte. The linear regression equations ($n = 3$) obtained were $Y = (0.0060 \pm 0.0119) + (0.00404 \pm 0.00010)X$ ($r = 0.999$) for CA1, $Y = (-0.0074 \pm 0.0140) + (0.00418 \pm 0.00026)X$ ($r = 0.997$) for CA2, $Y = (-0.0005 \pm 0.0049) + (0.00296 \pm 0.00010)X$ ($r = 0.997$) for DO1, $Y = (0.0014 \pm 0.0049) + (0.00302 \pm 0.00013)X$ ($r = 0.998$) for DO2, $Y = (0.0188 \pm 0.0053) + (0.00685 \pm 0.00010)X$ ($r = 0.999$) for OR1, and $Y = (0.0210 \pm 0.0123) + (0.00681 \pm 0.00023)X$ ($r = 0.999$) for OR2.

Table I. *Analytical Results for the Enantiomeric Ratios of the Racemic Drugs (Expected: 1.00)*

Concentration (μM)	Normalized peak area ratio[a]
CA	
110	1.034 ± 0.052
80	1.027 ± 0.045
40	1.031 ± 0.040
Mean	1.030
SD	0.003
DO	
110	0.983 ± 0.018
80	0.977 ± 0.333
40	0.992 ± 0.069
Mean	0.984
SD	0.007
OR	
110	1.020 ± 0.026
80	1.041 ± 0.040
40	1.027 ± 0.043
Mean	1.029
SD	0.010

[a]Normalized peak area ratio = (peak area 1/time 1)/(peak area 2/time 2); 1 and 2 stand for the first eluted enantiomer versus the second eluted enantiomer of each enantiomeric pair, respectively ($n = 3$).

Table II. *Precision for the Determination of CA, DO and OR Enantiomers ($n = 3$)*

Enantiomer	Concentration known (μM)	Concentration found (μM)	RSD (%)
CA1	40	42.09 ± 0.31	3.12
	80	80.56 ± 1.08	1.34
	110	110.07 ± 1.47	1.33
CA2	40	42.14 ± 2.45	3.66
	80	79.33 ± 1.34	1.69
	110	109.47 ± 0.82	0.75
DO1	40	41.31 ± 0.96	2.33
	80	79.30 ± 2.40	3.02
	110	107.97 ± 3.35	3.11
DO2	40	41.31 ± 1.21	2.93
	80	79.12 ± 2.99	3.78
	110	112.35 ± 3.47	3.09
OR1	40	41.23 ± 1.27	3.08
	80	79.00 ± 0.84	1.06
	110	112.53 ± 1.44	1.28
OR2	40	39.75 ± 1.08	2.72
	80	78.50 ± 0.90	1.14
	110	109.58 ± 1.88	1.72

The results indicate that a good linearity between Y and X is attainable over the range studied. The relative standard deviations (RSDs) ($n = 3$) of the method evaluated for the concentration levels of 40, 80, and 110 μM of individual enantiomer are all below 3.8 % (Table II).

In conclusion, a simple and economic CD-mediated CZE method has been developed for the quantitative chiral analysis of CA, DO, and OR antihistamines. Application of the method to the analysis of enantiomeric CA, DO, or OR in a real sample (if available) can be expected.

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