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Ultra-fast chiral separation of basic drugs by capillary electrophoresis

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Abstract

Chiral separation-methods development is usually very time-consuming, due to the diversity in chemical structures of pharmaceutical drug substances as well as the suitable separation conditions and the problem to choose the appropriate chiral selector. This paper shows an ultra-fast, capillary electrophoresis based screening procedure which was developed for chiral separation of several basic pharmaceuticals using dimethyl-β-cyclodextrin as chiral selector. Complete enantiomeric separations of basic drugs (metaproterenol and isoproterenol) were achieved as fast as in 40–50 s, with an R.S.D. for the absolute migration time reproducibility of less than 0.75%. The peak efficiency of the separations was usually over one million theoretical plates per meter, which correspond to an efficiency generation rate above 30 000 plates/m s.

1. Introduction

A large number of pharmaceutical drugs contain one or more chiral centers and may exist in two or more enantiomeric forms [1]. In most instances, only one of the enantiomeric forms is active therapeutically [2] while the other enantiomer is either much less active, inactive, or sometimes even toxic. The drug regulatory agencies in many countries have expressed an interest in investigations of the stereoisomeric composition of the drugs and their associated therapeutic and toxicological consequences. In general, only highly biologically active enantiomers are used in the production of drugs [1,3]. Hence, there is a real necessity to develop rapid and sensitive chiral separation methods required in the investigation of drug enantiomers composition.

Chiral gas and liquid chromatography were the first tools employed in the analytical separation of enantiomers [4]. In the last several years, capillary electrophoresis (CE) has become a powerful technique as an alternative to chiral chromatography methods, because it is fast, inexpensive, and usually does not require the labor-intensive pre- or post-column derivatization steps [5]. This very efficient separation method can easily be adapted to an additional, fast chiral separation and enantiomeric purity validation method in the modern pharmaceutical industry [6]. The separation principle of CE is based on the different electrophoretic mobilities of solutes, which, in turn, depend on their charge densities [7]. Similar to high-performance liquid chromatography (HPLC), secondary equilibria also can be employed in CE separations by means of the use of special additives in the running buffer, such as inclusion com-

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plexation agents like cyclodextrins (CDs). CD inclusion complexes can be used for the optical resolution of enantiomers by capillary electrophoresis, based on the differences in electrophoretic mobility of the complexes arising from different complex-formation constants with the analyte [8]. It has been demonstrated that several factors impact peak resolution in chiral CE separations. Rawjee and Vigh [9] showed that basic chiral compounds can be involved in three different types of cyclodextrin-mediated capillary electrophoresis separations. The separation is desionoselective when only the non-dissociated enantiomer complexes selectively with the cyclodextrin. The separation is ionoselective when only the dissociated enantiomer complexes selectively, and the separation is duoselective when both the non-dissociated and dissociated enantiomer complex selectively with the chiral selector. Thus, separation methods development of basic compounds should be primarily based on the pH of the running buffer, besides the vital separation parameters of cyclodextrin type and cyclodextrin concentration. Once the initial separation conditions have been achieved (pH, CD type, CD concentration [10]), further enhancements can be made by adjusting the applied electric field strength, temperature, and capillary length in order to decrease analysis time.

There have been reports on the use of CDs to separate enantiomers of basic pharmaceutical drug compounds [11–24] by CE. In these chiral methods, low-pH buffers with various concentrations of modified β-CDs [11–13,16–18,25–28] were used, including dimethyl-β-cyclodextrin (DM-β-CD) which has been demonstrated to resolve optically the largest number of chiral compounds [13,18,25–28] in CE.

2. Experimental

In all these studies, the P/ACE system 5500 capillary electrophoresis apparatus (Beckman Instruments, Fullerton, CA, USA) was used in normal (anode on the injection side) polarity mode. The separations were monitored on-column at 200 nm. The temperature of the cartridge holding the capillary column was thermostatted at 15 ± 0.1°C by the liquid cooling system of the P/ACE instrument. The electropherograms were acquired and stored on an IBM 486/66 MHz computer and were evaluated with the System Gold software package (Beckman Instruments).

In all the capillary electrophoresis experiments the eCAP chiral methods development kit was used (Beckman Instruments), with a 7 and 20 cm effective length (27 cm total length) low electroosmotic flow (EOF $< 1.5 \cdot 10^{-5}$ cm^2/V s) neutrally coated capillary column (50 μm I.D. and 375 μm O.D.). The kit also contains all the necessary buffers and chiral selectors needed for separation-methods development, including DM-β-CD (substitution rate: 14) which was chosen during the experiments as chiral selector. Before each analysis, the capillary was rinsed at 138 kPa with 0.1 *M* HCl for 0.5 min, deionized water for 2 min, and the running buffer for 2 min. This procedure was also used when a new capillary was employed.

The racemates of metaproterenol and isoproterenol (Sigma, St. Louis, MO, USA) were dissolved in deionized water in 0.01 mg/ml concentration. The samples were injected electrokinetically (2 s, 10 kV) into the capillary and were stored at −20°C or freshly used. All the buffer solutions were filtered through a 0.20-μm pore size filter (Schleicher and Schuell, Keene, NH, USA) and carefully vacuum degassed before use.

3. Results and discussion

The water solubility of β-CD can be increased and its enantioselectivity can be altered by chemically modifying the CD with alkyl substituents [13,29–33], which also changes the size and flexibility of the CD cavity [31]. The orientation of bulky and/or charged substituents on the CD cavity can decrease the enantioselectivity by causing steric hindrance and/or coulombic repulsion, preventing guest–host inclusion complex formation [30,34]. DM-β-CD used in this work is an alkyl-modified β-CD and available in various forms with different molar proportion of methyl-

group substitution on the β-CD. The physical characteristics of DM-β-CD appear to make it an attractive chiral additive for CE in two respects: it is highly water soluble, in fact well in excess of the parent β-CD, and each grade is a complex cocktail of related substances varying in both the position and the number of methyl groups attached [35].

Usually, DM-β-CD is a complex mixture, where one of these CDs might form host–guest complexes with certain racemates that approaches the optimum for separation by CE whereas another might be more suitable in the CE separation of another racemate. This may explain the wider range of structural types resolved by CE with DM-β-CD compared to β-CD. We investigated the use of DM-β-CD with the average substitution rate of 14, in the CE separation of some chiral basic drugs.

Initial chiral separation conditions, such as pH and CD type were suggested to be pH 2.5 and DM-β-CD, respectively, based on the methods-development protocol and flow chart published in previous work [36]. Therefore, low-pH phosphate buffers (pH 2.5) were prepared with low (10 m*M*) and high (50 m*M*) DM-β-CD concentrations. Enantiomeric separations were attempted using normal polarity separation mode (anode at the injection side) with a low electroosmotic flow (EOF) neutrally coated capillary column. Since chiral separations were achieved for both compounds with both of the concentrations of the CD type chosen (DM-β-CD) in the low-pH running buffer, we concluded that separations obtained were iono- or duoselective types. Thus, further optimization steps were made to increase enantiomeric resolution by varying the concentration of the chiral selector between the preliminary low and high concentrations of 10 and 50 m*M*. It is important to note that, based on our previous results [10], the applied voltage and the separation temperature were maintained at the maximum level of 30 kV and minimum value of 15°C, respectively, during the CD concentration optimization. As Fig. 1 shows, using the above separation conditions of pH 2.5 buffer, 30 kV, and 15°C, the chiral selector concentration of 30 m*M* DM-β-CD was found to be optimal for the separation of the chiral pharmaceuticals metaproterenol and isoproterenol using a 20-cm effective length neutrally coated capillary column. Fig. 1 also exhibits the increase in migration time of the solute when the chiral selector concentration is increased. The use of the neutral coated, low-EOF capillary ensured the predicted migration direction of the basic solutes with the low-pH running buffer, i.e. the use of normal polarity (anode at the injection side). In other words, when EOF is present, at a given pH, the vectorial sum of the solute's electrophoretic mobility and the EOF defines the resultant mobility of the solute. This can cause confusion in the prediction of the migration direction if the EOF and the mobility of the solute are unknown. When no or minimal EOF is present, the migration direction of a basic solute can be easily defined if the p*K* of the solute and the running buffer pH are known. Fig. 2 shows the separation of metaproterenol under the above-found optimal conditions. The resolution achieved in this separation was $R_s = 6.38$ for the two enantiomers (migration times of 6.58 and 7.04 min, Fig. 2). The peak efficiency, i.e. the theoretical plates

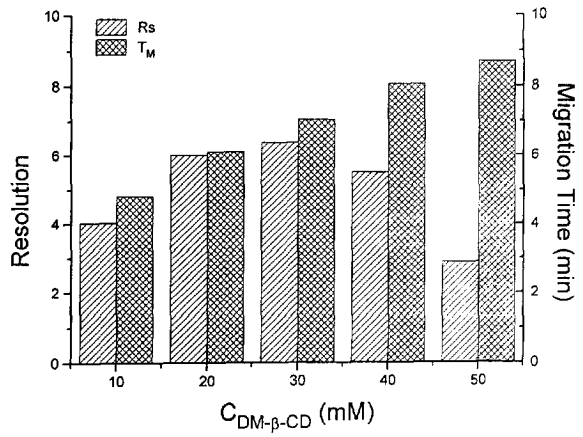

Fig. 1. Effect of the chiral selector concentration on the resolution (R_s) between the *R*- and *S*-enantiomers and the migration time (T_M) of the *S*-enantiomer of metaproterenol. Conditions: 27-cm neutrally coated capillary (20 cm effective length), low-pH buffer (25 m*M* phosphate, pH 2.5), detection: UV at 200 nm, $E = 1111$ V/cm (normal polarity), temperature: 15°C, injection: 2 s, pressure.

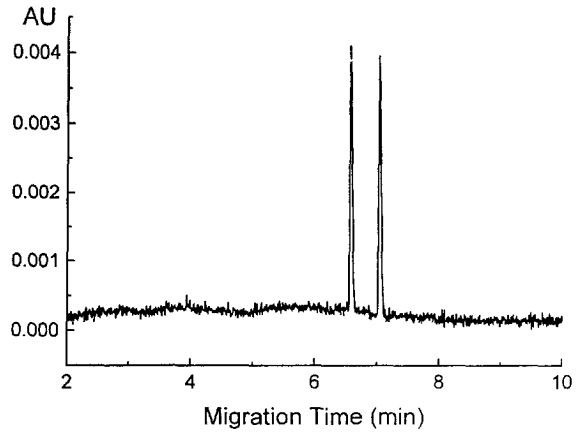

Fig. 2. Separation of the *R*- and *S*-enantiomers of metaproterenol. Conditions: 27-cm neutrally coated capillary (20 cm effective length), low-pH buffer (25 m*M* phosphate, pH 2.5) containing 30 m*M* DM-β-CD, detection: UV at 200 nm, $E = 1111$ V/cm (normal polarity), temperature: 15°C, injection: 2 s, pressure.

obtained for the two enantiomers, were 720 000/m and 696 500/m, respectively.

In order to decrease the analysis time, the separation capillary length was reduced from 20 to 7 cm. As Fig. 3 shows, baseline separation of the two enantiomers of metaproterenol was achieved in 45 s when using the shorter column, maintaining the same applied voltage of 30 kV and temperature of 15°C. The resolution attained was still greater than 2 ($R_s > 2.11$), which is enough for low enantiomeric contamination assessment [37]. The peak efficiencies of this separation were found to be $1.36 \cdot 10^6$ and $0.88 \cdot 10^6$ plates/m for the two enantiomers, respectively. These numbers correspond to an average efficiency generation rate of 30 000 plates/m s.

Fig. 4 shows the ultra-fast separation of another structurally alike basic drug, the isoproterenol enantiomers, using a 7-cm effective length capillary. Similar to the case of metaproterenol, the same concentration (30 m*M*) of chiral selector DM-β-CD was found to be optimal for the separation of the optical isomers of isoproterenol. Here, complete baseline separation of the two enantiomers was achieved in less than 40 s. The theoretical plate numbers achieved are similar to those in Fig. 3, and both are above 1 million plates per meter.

The migration-time stability of the enantiomers resolved was satisfactory, reflected by migration time %R.S.D. ranging from 0.3–0.8 for inter- and 0.6–0.7 for intra-day reproducibility (Table 1). The asymmetry factors of the

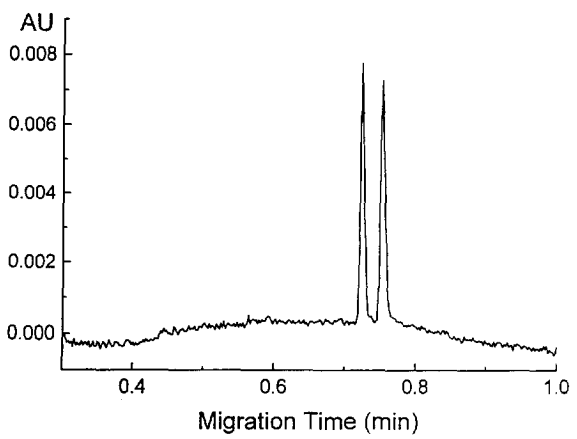

Fig. 3. Ultra-fast separation of the *R*- and *S*-enantiomers of metaproterenol. Conditions: 27-cm neutrally coated capillary (7 cm effective length), low-pH buffer (25 m*M* phosphate, pH 2.5) containing 30 m*M* DM-β-CD, detection: UV at 200 nm, $E = 1111$ V/cm (normal polarity), temperature: 15°C, injection: 2 s, 10 kV.

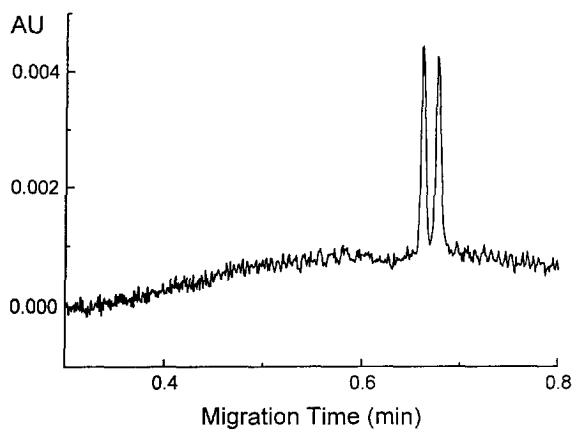

Fig. 4. Ultra-fast separation of the *R*- and *S*-enantiomers of isoproterenol. Conditions: 27-cm neutrally coated capillary (7 cm effective length), low-pH buffer (25 m*M* phosphate, pH 2.5) containing 30 m*M* DM-β-CD, detection: UV at 200 nm, $E = 1111$ V/cm (normal polarity), temperature: 15°C, injection: 2 s, 10 kV.

Table 1
Migration time reproducibility data

Compound		R	S	
Metaproterenol	Mean T_M	0.656	0.671	Day 1
	S.D.	0.0036	0.0037	
	%R.S.D.	0.56	0.49	
	n	10	10	
Isoproterenol	Mean T_M	0.725	0.754	
	S.D.	0.002	0.002	
	%R.S.D.	0.28	0.27	
	n	3	3	
Metaproterenol	Mean T_M	0.653	0.668	Day 2
	S.D.	0.0047	0.0049	
	%R.S.D.	0.73	0.75	
	n	19	19	
Metaproterenol	Mean T_M	0.655	0.667	Day-to-day
	%R.S.D.	0.65	0.62	
	n	29	29	

peaks in the enantiomeric separations were in the range 1.15–1.6, and can be improved by better mobility matching between the buffer coions and the solute ions, if necessary [38].

4. Conclusion

The rapid analysis time, characteristic of capillary electrophoresis, allowed the development of an easy to use and ultra-fast chiral separation method for basic drugs. Based on the size and the shape of the test compounds metaproterenol and isoproterenol, the chiral selector employed during this method development was chosen to be dimethyl-β-cyclodextrin. In the separation of basic solutes, the use of a low-pH phosphate buffer was the recommended choice with the use of a low-EOF, neutrally coated capillary. Once the appropriate separation pH was defined, and initial enantiomeric separation was achieved, further separation optimization steps were accomplished by varying the CD concentration, while the applied electric field strength and separation temperature were maintained the applicable maximum and minimum level, respectively. Under the optimized conditions of 30 m*M* DM-β-CD in the pH 2.5 phosphate buffer, 30 kV applied voltage, and 15°C temperature, with the use of a 7-cm neutrally coated capillary, ultra-fast (less than one minute) chiral separations were attained with extremely high efficiency (over 1 million plates/m) for the basic solutes of metaproterenol and isoproterenol. Employing this ultra-high-speed analysis technique, we are currently using this method for fast enantio-separation screening of another 35 basic chiral compounds of pharmaceutical interest, which will be reported later.

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