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Chiral separation of anticholinergic drug enantiomers in nonaqueous capillary electrophoresis

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Abstract

Nonaqueous capillary electrophoretic (NACE) method for the separation of nine structurally similar chiral anticholinergic drugs was developed. The eight drug enantiomers were separated on baseline within 18 min using 20 mM phosphoric acid and 10 mM NaOH, containing 10 mM heptakis(2,3-dimethyl-6-sulfato)-β-cyclodextrin (HDMS-β-CD) in methanol. The results were compared with those obtained in the high performance liquid chromatography system.

Keywords: Nonaqueous capillary electrophoresis; Methanol background electrolyte; Enantiomer separation; Heptakis(2,3-dimethyl-6-sulfato)-β-cyclodextrin; Anticholinergic drugs

1. Introduction

Capillary electrophoresis (CE) used for chiral separation offers a number of distinct advantages over HPLC, such as high efficiency, speed of analysis, flexibility of rapid incorporation of various chiral selectors, and feasibility of method development [1].

Ten years ago, Ye and Khaledi reported the first successful chiral separation of trimipramine with β-CD in nonaqueous capillary electrophoresis (NACE) [2]. Then, NACE separations of enantiomers have attracted many scientists' attention [3–9]. Cyclodextrins (CDs) and their derivatives have been successfully applied as chiral selectors in NACE. The applications of charged cyclodextrins have achieved better resolution in NACE chiral separation [3–7]. Especially, the application of oppositely charged CDs in NACE for separation of positively charged hydrophobic amines was better in the nonaqueous media [6]. As indicated by recent reviews [10–15] and monographs [3–7], over the years charged cyclodextrins have become the most widely used chiral resolving agents in CE.

This report has presented a continuation of the application of charged CDs in NACE chiral separation for nine structurally similar chiral anticholinergic drugs. The effects of CD types, CD concentrations, buffer ionic strength and acidity, and operating voltage on chiral separations were also studied. The results were compared with those of HPLC.

2. Materials and methods

2.1. Chemicals

The analytes I–IX were obtained as racemates from Beijing Institute of Pharmacology and Toxicology, and their chemical structures were listed in Fig. 1. Heptakis (2,3-dimethyl-6-sulfato)-β-cyclodextrin and HPLC-grade methanol were obtained from Aldrich (Milwaukee, WI, USA). Phosphoric acid and sodium hydroxide were of analytical reagent grade and were purchased from Fluka (Milwaukee, Wl, USA).

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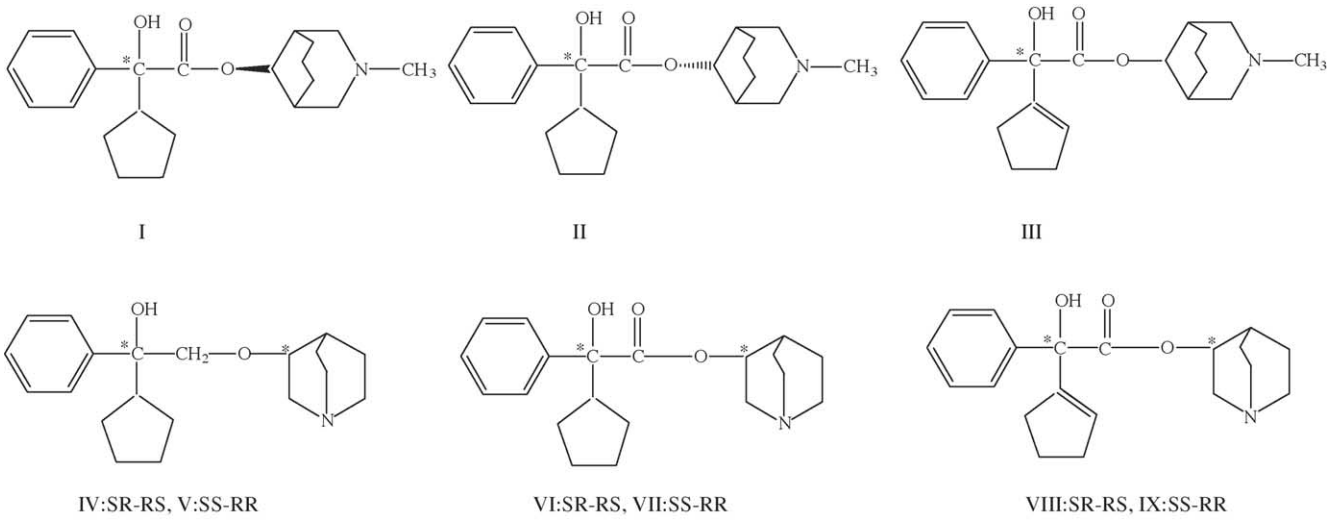

Fig. 1. Structures of the structurally similar chiral anticholinergic drugs.

2.2. Instrumentation

Chiral separations were carried out using an Agilent CE system (Model HP^{3D} CE; Agilent Technologies). The Instrument is equipped with a diode array detector which was used for UV detection only. A CE ChemStation (Agilent Technologies), using software Version Rev.A.06.01-403, was employed for instrument control, data acquisition and data handling. CE separations were performed using bare, fused-silica capillaries (50 μm I.D., 45 cm, or 55 cm effective length, and 53.5 cm, or 63.5 cm total length; Yongnian, Hebei Province, China).

2.3. Preparation of BGE and analysis solution

The acidic stock background electrolyte (BGE) solution was prepared by adding 0.02 mole phosphoric acid and 0.01 mole of sodium hydroxide to 1 L volumetric flask, then diluting to the mark with methanol. This acidic stock solution was used for the daily preparation of the 10 mM HDMS-β-CD BGE. Then the solution was filtered with a 0.45 μm filter before use.

A stock solution of each chiral anticholinergic drug was freshly prepared prior to use. The stock solution consisted of each drug dissolved in methanol at a concentration of 0.1 mg/mL.

2.4. CE procedures

All new capillary tubes were conditioned prior to use by rinsing with 1.0 M sodium hydroxide for 1 h and followed by 15 min with water. Samples were introduced into the capillary tube by positive pressure at 50 mbar for 1–4 s. The capillary tube was reconditioned prior to each sample introduction by rinsing with water for 2 min, 0.1 M sodium hydroxide for 3 min, water for 3 min, and with background electrolyte for 5 min. All separations were carried out at 20–30 kV and the capillary tube was maintained at 25 °C. The capillary zone electrophoresis (CZE) mode was carried out with UV detection at 200 nm wavelength for all analytes studied. When not use, all capillary tubes were rinsed with water for approximately 5 min and stored in air at 25 °C.

3. Results and discussion

In this paper, methanol was chosen as the solvent medium studying chiral separation. For these experiments, phosphoric acid and sodium hydroxide were added to the BGE to have the sufficient ion strength.

3.1. Influence of acidity of running buffer

It is well known that buffer acidity not only can control the number of negatively charged silianol on the inner wall of the capillary, but also affect the degree of dissociation of analytes. Because these structurally similar chiral anticholinergic drugs being investigated were weak basic, in order to study the effect of buffer acidity on resolution, 20 mM H_3PO_4 mixed with NaOH at four different concentrations (5, 10, 15 and 20 mM) were used to separate the nine chiral anticholinergic drugs. Table 1 showed the resolution of the compound IV in above different experimental conditions. So 20 mM H_3PO_4 and 10 mM NaOH in methanol were chosen as the optimum acidity and concentration of running buffer.

Table 1
Effects of acidity and BGE concentration on resolution

Items	1	2	3	4
H_3PO_4 concentration (mM)	20	20	20	20
NaOH concentration (mM)	5	10	15	20
Rs	0.86	1.59	1.02	–[a]

[a] Precipitation.

3.2. Influence of CDs types

The effect of CDs types on chiral separations is very important. In order to make the selection of a chiral selector for the enantiomeric separation, we carried out the enantioseparations of I, II, and IV using α-CD, CM-β-CD, HP-β-CD, HP-γ-CD, 6-sulfo-β-CD and HDMS-β-CD in the acidic methanol system. It was proved that HDMS-β-CD allowed the baseline separation of analytes I, II, and IV, while a partial separation was observed using HP-β-CD and no separation at all in α-CD, CM-β-CD, 6-sulfo-β-CD or HP-γ-CD NACE systems. In fact, benzene ring linking with some large group might be more difficult to be included into the cavity of α-CD than β-CD. In addition, the solubility of α-CD in this NACE media was also an important reason why no chiral separation was observed. These molecules were also unfit to the cavity of HP-γ-CD, and accordingly, inclusion complexes were not benefit to be formed. As to CM-β-CD and 6-sulfo-β-CD, they could not exhibit effects on the resolution of analytes I, II, and IV, which was mainly attributable to the insolubility of CM-β-CD and 6-sulfo-β-CD in methanol. So HDMS-β-CD was selected as the best selector.

The reason that the baseline enantioresolution of three chiral compounds could be obtained with HDMS-β-CD was probably related to the presence of negative charges on this CD derivative. Indeed, charged CDs were able to generate different separation selectivities compared to neutral CDs. Since the charge of HDMS-β-CD was opposite to that of the drug enantiomers (Fig. 1), a higher mobility difference between the free and the complexed enantiomers could be expected, providing an enhancement in separation selectivity. Moreover, the electrostatic interaction between the anionic HDMS-β-CD and the cationic analyte, even though it was not stereoselective, could contribute to the chiral recognition interaction. So HDMS-β-CD showed different interactions with the analytes and would therefore offer different separation possibilities.

3.3. Influence of the HDMS-β-CD concentration

The concentration of chiral selector plays a crucial role in the separation. The influence of HDMS-β-CD concentration on the resolution of nine enantiomers was studied in the range from 2 to 10 mM. As seen in Fig. 2, for compound VI, no separation was observed at 2 mM and 5 mM HDMS-β-CD (seen Fig. 2a and b). Enantiomer resolution was found to increase with increasing HDMS-β-CD concentration in the range from 8 to 10 mM HDMS-β-CD (seen Fig. 2c and d). Therefore, further studies were undertaken at HDMS-β-CD concentration of 10 mM.

3.4. Influence of the applied voltage and sample plug length

In capillary electrophoresis, voltage has a great influence on resolution and efficiency. Generally, higher voltage

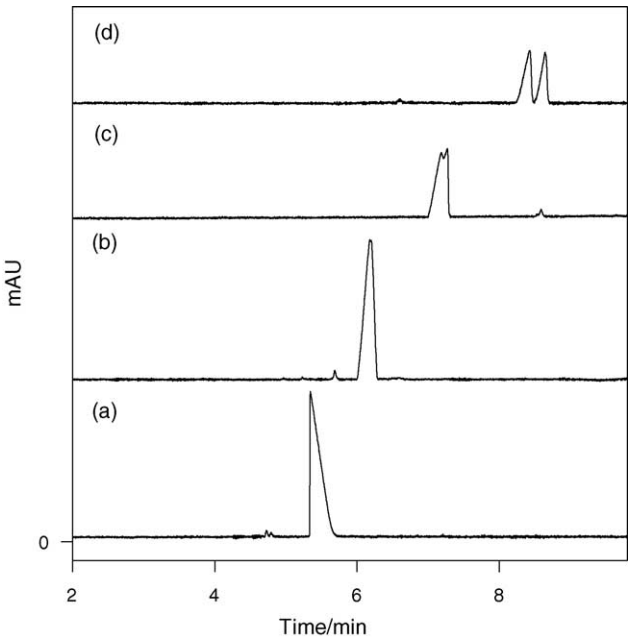

Fig. 2. The influence of HDMS-β-CD concentration on the separation of compound VI in 20 mM phosphoric acid and 10 mM sodium hydroxide methanol BGE. HDMS-β-CD concentration: (a) 2 mM, (b) 5 mM, (c) 8 mM, and (d) 10 mM. The length of the capillary was 45 cm. For details of experimental conditions seen Section 2.4.

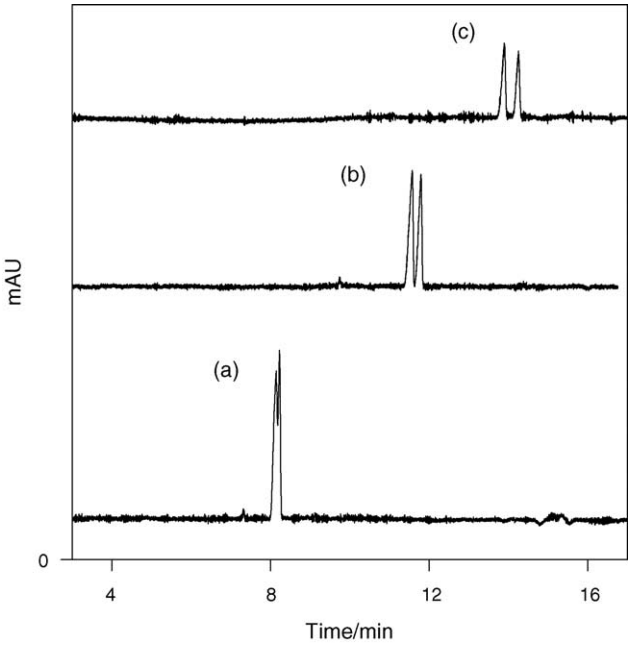

Fig. 3. The influence of applied voltage and sample plug length on chiral separation of compound II in 20 mM phosphoric acid and 10 mM sodium hydroxide methanol BGE containing 10 mM HDMS-β-CD. Condition: (a) 30 kV, 3 s; (b) 25 kV, 3 s; and (c) 22 kV, 2 s. The length of the capillary was 45 cm. For details of experimental conditions seen Section 2.4.

leads to better resolution and higher efficiency when Joule heating is small. But higher voltage always results in bigger current and when the current is too big to ignore the Joule heating effect, poorer resolution and lower efficiency will occur. The effect of applied voltage was studied in our experiments using 30, 25, and 22 kV (Fig. 3). Another parameter, the sample plug length, which influenced CE separations, was also investigated. During injection it was important that the sample plug length was minimized. If the length were much longer than dispersion zone caused by diffusion, efficiency and resolution would be sacrificed.

Fig. 3 showed effects of applied voltage and sample plug length on chiral separation of compound II. A poorer separation (Fig. 3a) was observed at 30 kV when sample was introduced into the capillary by a 50 mbar pressure injection for 3 s. With decrease of applied voltage, resolution could be improved. The baseline separation was obtained at 25 kV under the same sample plug length (Fig. 3b). The best separation (Fig. 3c) of compound II was achieved at 29 kV when

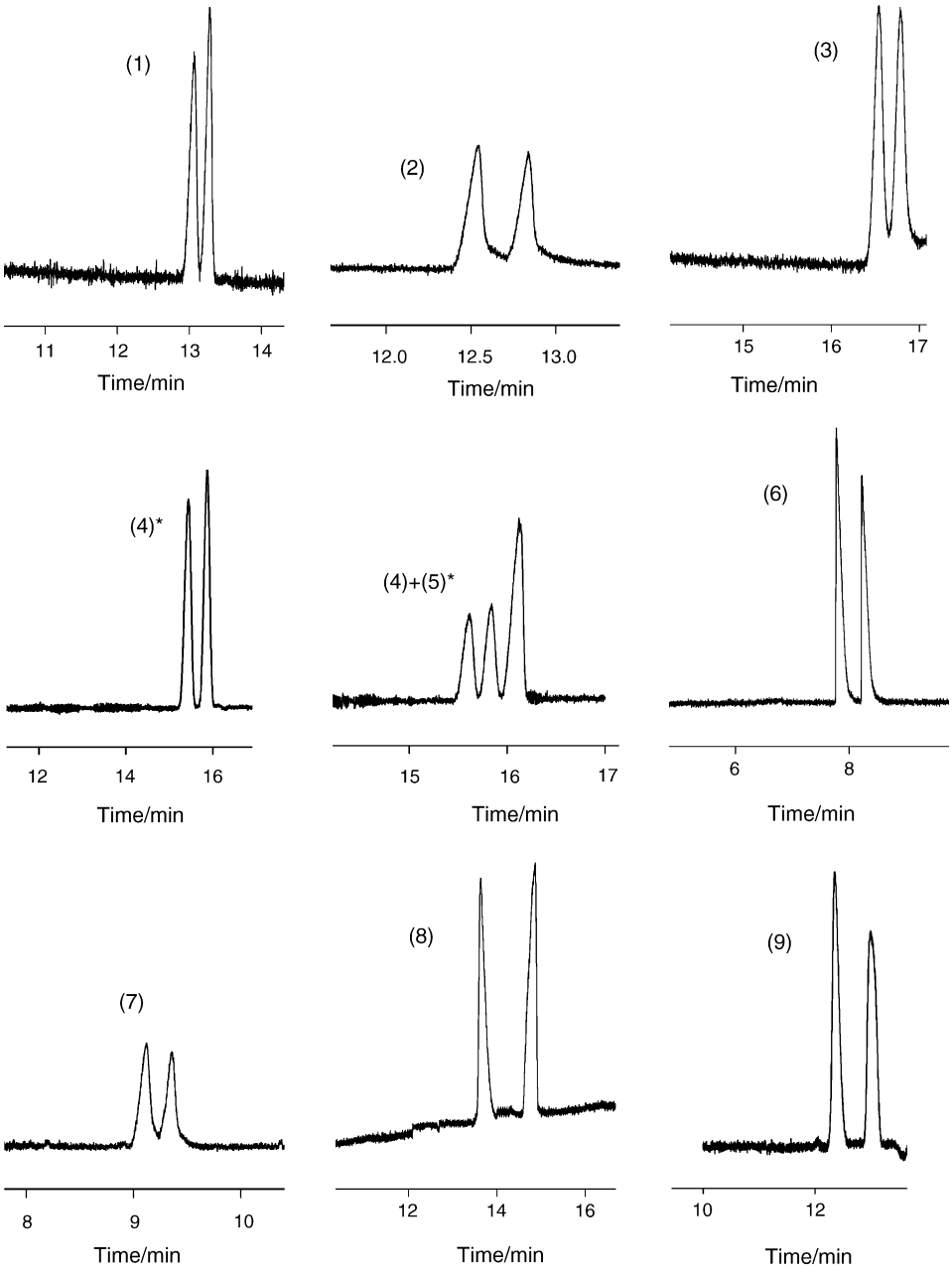

Fig. 4. Electrophoregrams of nine structurally similar chiral anticholinergic drugs using 20 mM phosphoric acid and 10 mM NaOH, containing 10 mM HDMS-β-CD in methanol. For details of experimental conditions seen Section 2.4. (1) Compound I; (2) compound II; (3) compound III; (4) compound IV; (5) compound V; (6) compound VI; (7) compound VII; (8) compound VIII; (9) compound IX. (*) 55 cm capillary tube in efficient length for compounds IV and V; 45 cm for others.

Table 2
Comparison between results of HPCE and HPLC

Methods	Compounds									
	I	II	III	IV	V	IV–V	VI	VII	VIII	IX
HPLC-M [16]	1.20	–[a]	–	1.06	1.08	1.35	–	–	–	–
HPLC-S [17]	2.80	1.79	NS[b]	1.44	0.75	NS	3.05	2.51	NS	NS
HPCE	1.57	1.89	1.27	1.80	NS	1.33	2.64	3.43	4.04	13.55

[a] No experiment.
[b] The enantiomers cannot be separated.

sample was introduced into the capillary by a 50 mbar pressure injection for 2 s.

3.5. Separation of the chiral anticholinergic drugs by NACE

In the above, we demonstrated the importance of acidity and CD concentration for the optimization of separation conditions in NACE. The optimum separation conditions for compound II or IV were used to separate other similar chiral anticholinergic drugs listed in Fig. 1. It was showed that an excellent separation of eight chiral drug enantiomers by NACE with 20 mM H_3PO_4, 10 mM NaOH, and 10 mM HDMS-β-CD was achieved as seen in Fig. 4. Chiral separation was not observed only for the studied compound V.

In order to give the method validation, we have also carried out the reproducibility experiments. The results showed that the relative standard deviation (RSD) of migration times was 1.8% ($n = 5$) for compounds IV and V in the repeatability study, the relative standard deviation (RSD) of migration times was 3.7% ($n = 6$) for compound II in the intermediate precision study.

As seen in above Fig. 4, all studied chiral anticholinergic drugs with ester bond to the chiral center could be completely separated using the capillary tube of 45 cm efficient length. Compound IV with ether bond to the chiral center could not be completely separated under the same condition, and baseline separation was obtained using the capillary tube of 55 cm efficient length. Compound V, isomeric compound of IV, was not resolved no matter how experimental conditions were changed. It could be concluded that the compounds with ester bond to chiral center was greatly favorable for chiral recognition of CDs or their enantioseparation. Our previous paper had reported there was the larger ratio of inclusion constants (K_R/K_S) for compound I than that of compounds IV and V by RP-HPLC with β-cyclodextrin as a mobile phase [16], which was consistent with the order of the result observed in this paper. It was rational reason that ester bond to chiral center was much preferable to forming hydrogen bond with the hydroxyl group beside the CDs and led the stronger chiral recognition of CDs. In addition, compound I and II were position isomers each other, but there was different resolution for them.

Here, we have compared the chiral resolution results of nine chiral anticholinergic drugs using HPCE and HPLC methods (seen Table 2). Although compounds IV and V had been completely baseline separated by RP-HPLC with β-cyclodextrin as a mobile phase as previous reported [16], only compound IV was completely separated, and compound V was not at all resolved for HPCE method established. This phenomenon was perhaps correlation with the higher polarity (3.25 DB) and melting point (m.p. 212–214 °C) of compound IV compared with the lower polarity (2.23 DB) and melting point (m.p. 178–180 °C) of compound V. Compounds III, VIII and IX had been completely baseline separated by NACE method, while no separation was observed by HPLC with β-cyclodextrin as chiral column. Moreover, it could give us a suggestion that there were different advantage and use for other methods although HPCE could provide us a powerful separation tool.

4. Concluding

The anionic CD derivative HDMS-β-CD has been proved to be a powerful tool to achieve the enantioseparation of eight chiral anticholinergic drugs in NACE. The type of CDs as well as its concentrations was clearly identified as factors influencing enantiomeric selectivity. The baseline enantioseparations for the eight anticholinergic drugs were obtained using 10 mM HDMS-β-CD, 20 mM phosphoric acid and 10 mM NaOH in methanol as BGE.

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