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Enantioseparation of rabeprazole and omeprazole by nonaqueous capillary electrophoresis with an ephedrine-based ionic liquid as the chiral selector

Zheng Ma, Lijuan Zhang, Lina Lin, Ping Ji and Xingjie Guo*

ABSTRACT: An ephedrine-based chiral ionic liquid, (+)-*N*,*N*-dimethylephedrinium-bis(trifluoromethanesulfon)imidate ($[DMP]^+[Tf_2N]^-$), served as both chiral selector and background electrolyte in nonaqueous capillary electrophoresis. The enantioseparation of rabeprazole and omeprazole was achieved in acetonitrile–methanol (60:40 v/v) containing 60 mM $[DMP]^+[Tf_2N]^-$. The influences of separation conditions, including the concentration of $[DMP]^+[Tf_2N]^-$, the electrophoretic media and the buffer, on enantioseparation were evaluated. The mechanism of enantioseparation was investigated and discussed. Ion-pair interaction and hydrogen bonding may be responsible for the main separation mechanism. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: chiral ionic liquid; enantioseparation; nonaqueous capillary electrophoresis

Introduction

One of the most successful applications of capillary electrophoresis (CE) is chiral separation. A recent surge in the number of reports clearly indicates its feasibility and great application prospect. Nonaqueous capillary electrophoresis (NACE), using organic solvents as major electrophoretic separation media, is a promising technique for enantioseparation. In NACE, non-aqueous medium provides stronger intermolecular interactions (e.g. diopole–diopole, hydrogen bonding) and a higher degree of ion-pair formation, which favor enantioseparation (Tjornelund and Hansen, 1999).

Ionic liquids (ILs) refer to a group of organic salts that are liquid at room temperature. Owing to their unique characteristics, including being environmental benign, nonvolatility, conductivity and good solubility for a wide range of both inorganic and organic substances, they have been considered as an alternative to conventional molecular solvents. In the analytical chemistry field, great progress has been made in the application of ILs in electrochemistry (Kavan *et al*., 2004), chromatography (Armstrong *et al*., 1999; Berthod *et al*., 2001), especially in CE. Generally, ILs have been reported to be utilized as background electrolytes (BGEs) or chiral selectors in CE. Yanes *et al*. (2001) developed a CE method using imidazole-based ILs as aqueous BGEs for separating phenolic compounds extracted from grape seeds. The authors proposed that, in the presence of ILs in running electrolyte, the cations (i.e. imidazolium ions) were coated on the capillary wall, and thus rendered anodic electroosmotic flow (EOF). Also, ILs have shown great potential in separation of compounds including carboxylates (Laamanen *et al*., 2005), benzoic acid, chlorophenoxy acid herbicides (Yu *et al*., 2005) and anthraquinones (Tian *et al*., 2007). The application of ILs in non-aqueous medium was first reported by Vaher *et al*. (2001). In their non-aqueous system employing acetonitrile as separation medium, the IL served as the electrolyte and led to the charging of the analytes. The separation of dyes in non-aqueous media was thus accessible.

Chiral ILs have been employed in enantiomeric separation of chiral compounds. Two chiral ILs [ethyl- and phenylcholine of bis(trifluoromethysulfonyl)imide] were reported as additives to cyclodextrins in CE for enantiomeric separation (Francois *et al*., 2007b). The authors proposed the ability of ILs to assist in separation indirectly, which was a decrease in EOF resulting from an increase in salt concentration and possible wall adsorption. However, no enantioselectivity was observed in the aforementioned chiral ILs. Tran and Mejac (2008) performed enantioseparation of pharmaceutical product by CE in aqueous separation media; however, the chiral IL alone was inefficient for chiral separation. Chiral separation can only be achieved by the addition of another chiral anion (i.e. cholate) or chiral neutral compound (i.e. 1-s-octyl-β-D-thioglucopyranoside). Rizvi and Shamsi (2006) reported an efficient method using amino acid-derived chiral ILs and their polymers as chiral selectors in micellar electrokinetic capillary chromatography (MEKC) to separate two acidic chiral analytes. This was the first successful example of chiral separation employing chiral ILs as chiral selectors.

* Correspondence to: Xingjie Guo, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, China. E-mail: gxjhyz@yahoo.com.cn

Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, China

Abbreviations used: BGE, background electrolyte; EOF, electroosmotic flow; IL, ionic liquid; NACE, nonaqueous capillary electrophoresis.

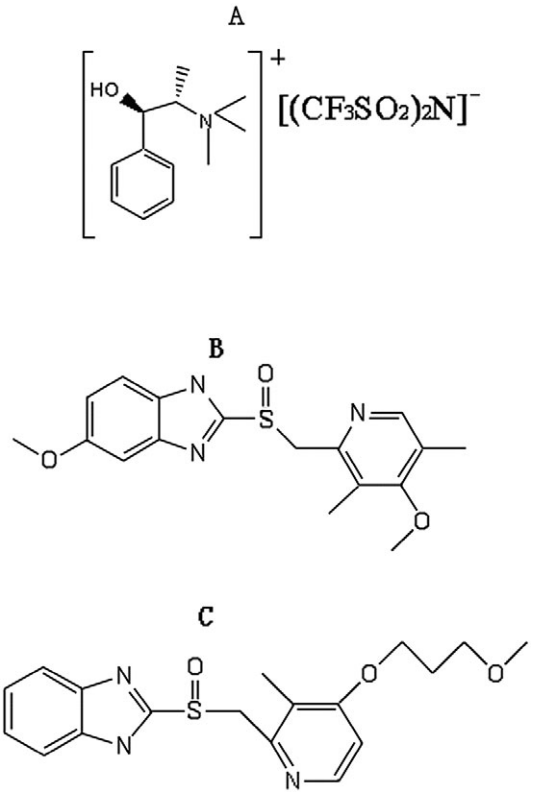

Figure 1. Structures of $[DMP]^+[Tf_2N]^-$ and analytes. (A) $[DMP]^+[Tf_2N]^-$; (B) omeprazole; (C) rabeprazole.

The ephedrine-based chiral IL, (+)-*N*,*N*-dimethylephedrinium-bis(trifluoromethanesulfon)imidate ($[DMP]^+[Tf_2N]^-$, Fig. 1), was utilized as the stationary phase in gas chromatography for enantioseparation of alcohols, diols, sulfoxides, epoxides and acetylated amines by Ding *et al*. (2004). In this paper, the $[DMP]^+[Tf_2N]^-$ was introduced in NACE as both chiral selector and BGE. The effects of the $[DMP]^+[Tf_2N]^-$ concentration, the buffers and the composition of organic solvents were studied. The discussion of separation mechanism was further undertaken.

Experimental

Chemicals and Reagent

Lithium bis(trifluoromethanesulfonyl) (>99%) was purchased from Sigma. Omeprazole and rabeprazole were provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile, methanol and ethanol (HPLC-grade) were obtained from Corncord Tech (Tianjin, China). The synthesis of $[DMP]^+[Tf_2N]^-$ was accomplished via a simple anion exchange reaction (Wasserscheid *et al*., 2002) and its characterization was performed employing ^{1}H NMR and MS.

Capillary Electrophoresis Instrumentation

The experiment was performed with a CL1030 capillary electrophoresis system equipped with HW-2000 Chemstation (Cailu Separation Technology Co. Ltd, Beijing, China). Bare-fused silica capillaries with 49 cm × 50 μm i.d. (effective length 40 cm) were purchased from Yongnian Capillary Factory (Hebei, China). The new capillary was flushed with 1 M sodium hydroxide for 10 min and distilled water for 15 min. At the beginning of each working day, the capillary was flushed successively with 10 mM sodium hydroxide, water, non-aqueous solvent (without $[DMP]^+[Tf_2N]^-$) and the BGEs. Between each injection, the capillary was conditioned by flushing with the non-aqueous electrolytes for 5 min and then the BGEs for 10 min. Injections were made in hydrodynamic mode for a period of 5 s. The electrophoresis was performed at an applied voltage of 10 kV with UV detection set at 295 nm on cathodic side. The reversed polarity mode (–10 kV) was used to measure the anodic EOF.

Sample Preparation

The sample solutions were prepared by dissolving rabeprazole or omeprazole in the mixture of acetonitrile and methanol (6:4, v/v) at the concentration of 500 μg/mL. The BGEs were prepared by dissolving appropriate amount of $[DMP]^+[Tf_2N]^-$ in acetonitrile–methanol at various ratios. The BGEs and the samples to be analyzed were filtered through 0.45 μm membrane filters and degassed before used.

Calculation

The mobility of the analyte was calculated from the observed migration times with the equation:

$$\mu_{ep} = \frac{lL}{V}\left(\frac{1}{t_m} - \frac{1}{t_{eo}}\right)$$

where μ_{ep} is the electrophoretic mobility of the analyte tested, t_m and t_{eo} are the measured migration time of the analyte and EOF marker (acetone) measured directly from the electropherogram, L is the total length of capillary, l is the effective length and V is the applied voltage. The resolution (R_s) is presented as Kaiser factors (Kaiser, 1960) calculated as f/g. A straight line is drawn between the two peak maxima, g is defined as the distance from this line to the extended baseline through the valley between the two peaks and f is the distance from the same line to the valley.

Results and Discussion

Influence of $[DMP]^+[Tf_2N]^-$ Concentration

It was observed that the addition of the $[DMP]^+[Tf_2N]^-$ led to reversed EOF (anodic flow), probably due to the adsorption of the cations on the capillary wall as already mentioned by Yanes *et al*. (2001). To study the effect of $[DMP]^+[Tf_2N]^-$ concentration on EOF, experiments were carried out in the reversed polarity mode using acetone as the EOF marker. The influence of $[DMP]^+[Tf_2N]^-$ concentration was investigated in the range of 30–70 mM in acetonitrile–methanol (6:4 v/v). The EOF and the electrophoretic mobility of rabeprazole as a function of IL concentration are given in Fig. 2. With the concentration of $[DMP]^+[Tf_2N]^-$ increased, the absolute value of EOF decreased, which may in part be attributable to the enhanced ionic strength and possible capillary wall adsorption.

In the presence of $[DMP]^+[Tf_2N]^-$, the analytes could only be detected at the cathode side; the mobility was in the opposite direction to the EOF. This observation implied that the addition of $[DMP]^+[Tf_2N]^-$ led to a charging of the analytes. This phenomenon can be explained by the interactions between IL and analytes as advocated by Vaher *et al*. (2001). Figure 2 illustrates that the electrophoretic mobility ($\mu_{ep} = \mu_{ap} - \mu_{eo}$) of rabeprazole increased as the concentration of $[DMP]^+[Tf_2N]^-$ increased up to 60 mM, probably owing to the decreasing in the absolute value of μ_{eo} and increased interaction degree. In most cases shorter migrate time had a negative effect on enantioseparation, since it provided less opportunity for interactions between chiral selector and analyte, thereby achieved lower resolution. However, in the case of $[DMP]^+[Tf_2N]^-$, this detrimental effect on enantioseparation may

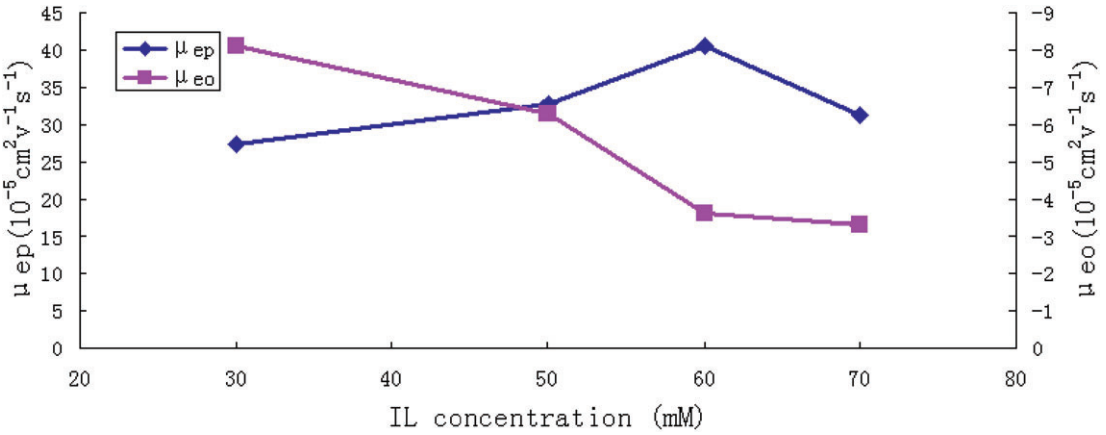

Figure 2. The effect of $[DMP]^+[Tf_2N]^-$ concentration on the effective mobility of rabeprazole and on the EOF.

be compensated for by the increased extent of ion-pairing formation occurringd at higher concentration of $[DMP]^+[Tf_2N]^-$. A tendency towards enantioseparation was observed for rabeprazole by addition of 50 mM $[DMP]^+[Tf_2N]^-$. As shown in Fig. 3, improved chiral resolution of rabeprazole (R_s from 0 to 0.87) was obtained with increasing concentration (from 30 to 60 mM) of $[DMP]^+[Tf_2N]^-$. However, the baseline noise increased greatly with the IL concentration up to 70 mM (Fig. 3D). The optimal concentration was found to be 60 mM. It was also observed that migration time of analytes was increased at the $[DMP]^+[Tf_2N]^-$ concentration of 70 mM; this phenomenon might be related to the higher viscosity of the electrophoretic system.

Influence of Electrophoretic Media

In NACE, the types of electrophoretic media have a significant influence on enantioselectivity. Various nonaqueous media can offer different selectivities. The choice of acetonitrile as main separation medium was based on its possessing a sufficiently high dielectric constant ($\varepsilon = 37.5$), which allowed a dissociation of IL. Furthermore, the $[DMP]^+[Tf_2N]^-$ is miscible with acetonitrile, which makes the concentration adjustment simple. The possibility of enantiosparation was investigated in pure acetonitrile; however, no enantioseparation was observed. Enantioseparations of rabeprazole and omeprazole were observed in acetonitrile with methanol up to 20%. Table 1 summarizes the effect of volume ratio of acetonitrile to methanol on the enantioseparation of rabeprazole and omeprazole. As indicated, as the proportion of methanol increased, the mobility of analytes increased. This behavior could be explained by the fact that the addition of methanol gave rise to lower EOF resulting from the lower ε (dielectric constant)/η(viscosity) ratio of methanol. Interestingly, despite the relative shorter migration time, better resolutions were found with 40% methanol. Figures 3(C) and 4 illustrate that satisfactory enantioseparation was achieved for rabeprazole and omeprazole using an acetonitrile and methanol (60:40) mixture containing 60 mM $[DMP]^+[Tf_2N]^-$. It is well known that methanol possesses both hydrogen donating and accepting ability in contrast to acetonitrile, which can only act as a hydrogen acceptor. Our results suggested that the mixture bearing hydrogen bonding properties benefitted enantioseparation. However, further increase in the ratio of methanol led to no enantioseparation, which indicated a more complex mechanism. Other electrophoretic media including acetonitrile–ethanol and acetonitrile–isopropyl alcohol were applied for the enantioseparation of rabeprazole and omeprazole; unfortunately, no resolutions were obtaind. Thus, acetonitrile–methanol was chosen as the electrophoretic media in this study.

Influence of Buffers

Buffers are usually added to BGEs in CE in order to ionize chiral selector/analytes and to improve peak shapes. In some cases, the addition of buffers can also provide better enantioselectivity. Ammonium acetate is usually used as a buffer as it has fair solubility in most organic solvents. However, in the present study, with the existence of 5 mM ammonium acetate in the BGEs, no enantioseparations were observed for either rabeprazole or omeprazole. One explanation for this behavior could be ascribed to competitive non-steroselectivity of ammonium acetate.

Acidic or basic conditions were applied in an attempt to improve the enantioselectivity. In the presence of 5 mM triethylamine, the direction of EOF was changed (cathodic flow). The migration time of analytes (about 3 min) was almost the same as the EOF marker. In the case of 5 mM acetic acid, no enantioseparation was observed. It is assumed that in acidic media the protonation of rabeprazole and omeprazole may take place, which would have a negative effect on analyte–chiral IL interactions. Finally, the optimal separation condition was an acetonitrile and methanol (60:40) mixture containing 60 mM $[DMP]^+[Tf_2N]^-$.

Separation Mechanism

IL is a salt. When added to the separation medium, it becomes a charged ion. In this experiment the cations of $[DMP]^+[Tf_2N]^-$ were responsible for the key role. Both the cations coated on the capillary walls and the free cations in the BGEs interacted with the analytes (Francois *et al*., 2007a). The two analytes (Fig. 1) are ampholytic with p*K* values of about 3–4 for the protonation of the N-pyridme and 8–9 for deprotonation of the benzimidazole–NH. The experiment showed that the addition of $[DMP]^+[Tf_2N]^-$ led to a slightly acid environment of the BGEs (data not shown). The analytes may uncharged or partly negatively charged in the BGEs. Moreover, a method was designed to justify the state of the two analytes. Firstly, the apparent pH of BGE was measured in acetonitrile–methanol (6:4) containing 60 mM $[DMP]^+[Tf_2N]^-$. Then CE was performed in the same apparent pH system (adjust with acetic acid) which consisted of plain solvent. The result

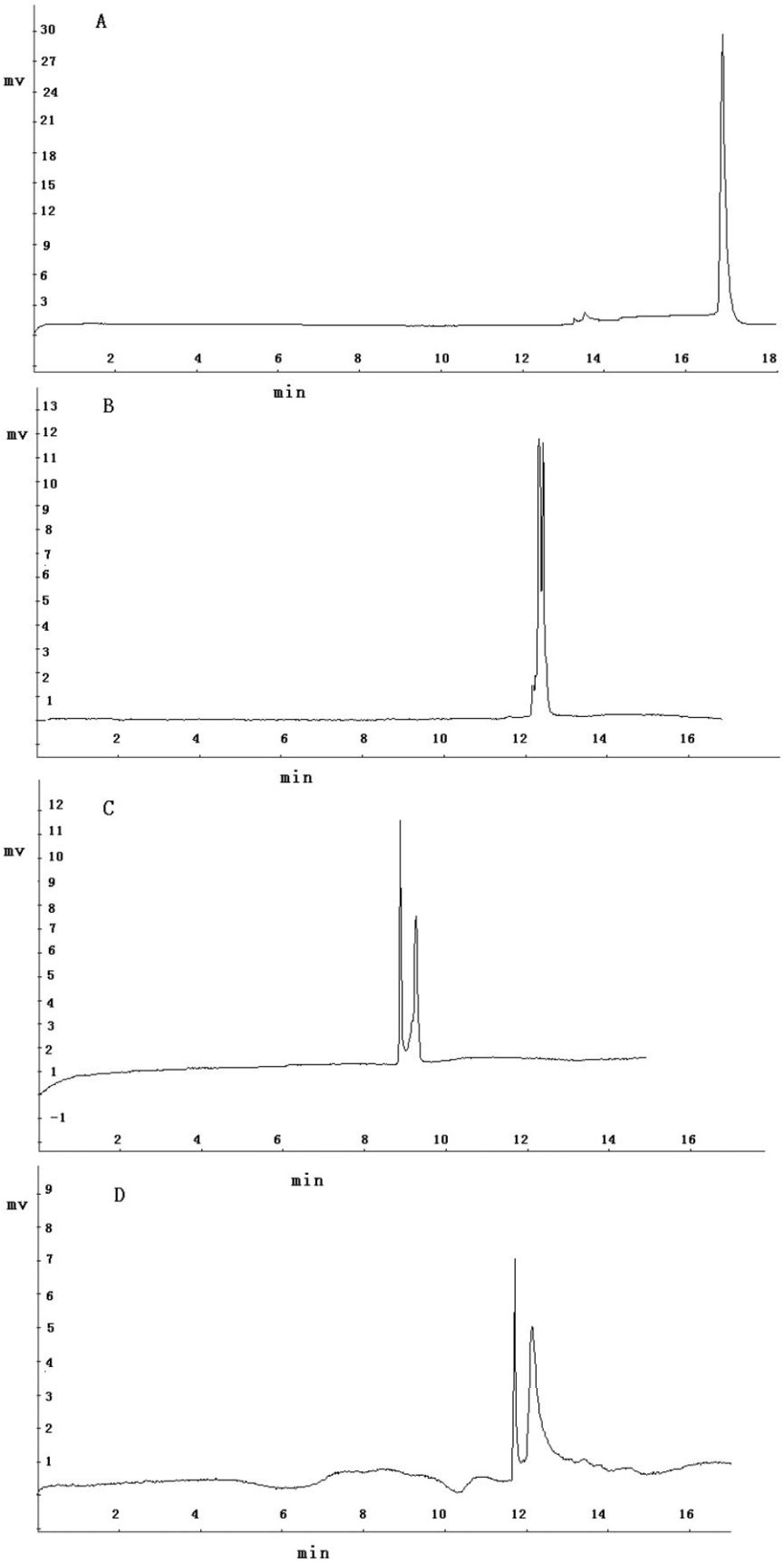

Figure 3. Enantioseparation of rabeprazole using (A) 30 mM, (B) 50 mM, (C) 60 mM and (D) 70 mM $[DMP]^+[Tf_2N]^-$ in acetonitrile–methanol (60:40 v/v).

Table 1. The effect of volume ratio of acetonitrile to methanol on the enantioseparation of omeprazole and rabeprazole

Parameter	Acetonitrile : methanol							
	8:2		7:3		6:4		5:5	
	Omeprazole	Rabeprazole	Omeprazole	Rabeprazole	Omeprazole	Rabeprazole	Omeprazole	Rabeprazole
t_{m1} (min)	16.45	13.72	10.76	9.54	10.46	8.87	9.94	8.53
t_{m2} (min)	16.48	13.77	10.92	9.70	10.70	9.23	—	—
μ_{ap1} (10^{-4} cm^2 V^{-1} s^{-1})	1.99	2.38	3.04	3.42	3.12	3.68	3.55	3.83
μ_{ap2} (10^{-4} cm^2 V^{-1} s^{-1})	1.98	2.37	2.99	3.37	3.05	3.53	—	—
α	1.01	1.00	1.01	1.02	1.02	1.04	1.00	1.00
R_s	0.16	0.06	0.87	0.81	0.95	0.83	0	0

Experimental conditions: 60 mM chiral IL in various ratios of methanol; +10 kV.
$\alpha = \mu_{ap1}/\mu_{ap2}$.

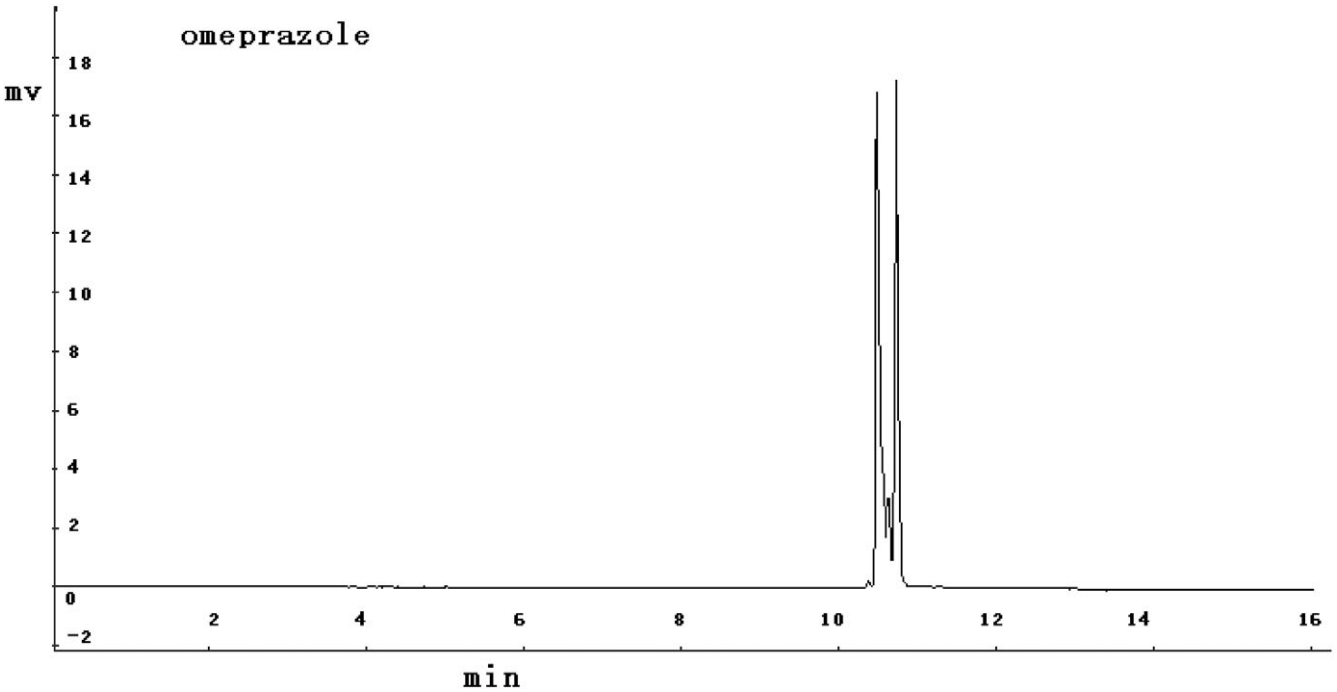

Figure 4. Enantioseparation of omeprazole. BGE: 60 mM $[DMP]^+[Tf_2N]^-$ in acetonitrile–methanol (60:40 v/v).

showed that the electropherograms of the two analytes were at the front of the EOF, which indicated anionic analytes without chiral IL–analyte interactions being taken into account. Therefore, ion-pairs may be formed between the ephedrine cation and the negatively analytes. The separation was achieved based on (a) the different ion-pair formation equilibrium constants between the IL cation and negatively charged enantiomers and (b) the different mobility of the free forms of the analytes and the ion pairs. The presence of ammonium acetate led to a poor stereoselectivity, which probably related to its competitive non-stereoselective ion-pairs formation. These findings are in accordance with the observations utilizing ion-pair selectors in literature (Carlsson *et al.*, 2001).

In addition, hydrogen bonding was supposed to afford a supplementary intermolecular interaction for stereoselectivity. The $[DMP]^+[Tf_2N]^-$ bears two chiral centers in its structure. One is directly connected to the hydroxy group and aromatic ring; the other is connected to the alkyl and amino groups. The hydroxy group connected to the chiral carbon exhibited stereoselectivity, as proved by Pégot *et al.* (2004), who developed a method using similar ephedrine-based IL as reaction media in their asymmetric synthesis. When the hydroxy group was replaced by an acetyl group, much lower enantiomeric excesses were obtained. In the case of rabeprazole and omeprazole, we supposed that the hydroxy group of $[DMP]^+[Tf_2N]^-$ participated in hydrogen bonding with the sulfoxide group of the analytes. Although other interactions such as π–π interaction and diopole–diopole interaction may also take place., the main mechanism for enantioseparation should be ion-pairing and hydrogen bonding.

Conclusion

A simple, efficient NACE method utilizing $[DMP]^+[Tf_2N]^-$ as both chiral selector and BGE was developed for the enantioseparation of rabeprazole and omeprazole. The occurrence of ion-pair inter-

action as well as the supplementary hydrogen bonding accounted for the main separation mechanism.

Acknowledgements

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