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Chiral separation in non-aqueous media by capillary electrophoresis using the ion-pair principle

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Abstract

Chiral separation by capillary electrophoresis using the principle of diastereomeric ion-pair formation for chiral separation in non-aqueous media is presented. As the test counter-ions, the enantiomeric pure (+)- or (−)-camphorsulphonates are used. Fifteen basic chiral drug substances were used as test solutes. Cisapride and substances with a β-amino alcohol configuration could be separated into their enantiomers. The principle, the field of application as well as the theory are discussed.

Keywords: Enantiomer separation; Ion-pairing reagents; Camphorsulfonate; Cisapride; Basic drugs; Amino alcohols

1. Introduction

The principle of diastereomeric ion-pair formation is well established and is a useful method for chiral separation in HPLC [1,2]. In principle, it should be a general method for the separation of charged, chiral substances, as the technique takes advantage of non-selective ionic interactions. So, in theory, it should be possible to separate all ionizable racemates by adding an oppositely charged chiral selector, such as camphorsulphonate, tartrate, maleate or lactate. However, experiences from HPLC separations have shown that ion-pair formation alone does not lead to chiral separation and that at least a two-point interaction between the substances and the counter-ions is necessary [2]. This paper indicates that the same pattern can be applied to CE.

Recently, non-aqueous capillary electrophoresis has gained more and more interest [3–10]. Non-aqueous media can be useful for the separation of compounds with low water solubility and they have been shown to be useful for obtaining fast and very effective separations. Furthermore, it is often possible to obtain different selectivities in non-aqueous media than those found in water [7,10]. As nonpolar solvents with low dielectric constants provide a better environment for achieving ion-pair interactions than water, the ion-pair formation as a principle for chiral separation in non-aqueous media should be optimal.

In the present paper, the ion-pair formation principle for the separation of chiral substances in non-aqueous media is presented.

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Camphorsulphonate was chosen as the ion-pair reagent as it has previously been used as such for chiral separation in HPLC [2]. Furthermore, many of its salts are soluble in the organic solvents used, it has a low UV absorption and it is fairly inexpensive.

1.1. Theoretical model

The theory behind the ion-pair separation principle is analogous to the theory for chiral separation with cyclodextrins, described by Wren and Rowe [11]. If the enantiomeric analytes interact with the chiral counter-ion, two diastereomeric ion-pairs, which differ both chemically and physically, will be formed. If the enantiomers have different formation equilibrium constants (K_1 and K_2) with the chiral counter-ion, the enantiomers will be retained to a different extent and chiral separation can be achieved. As the diastereomeric pairs are neutral, they have no migration of their own but migrate with the same velocity as the electroosmotic flow (EOF). Thus, separation can only be achieved if the diastereomeric ion-pairs have different equilibrium constants.

The following equilibria exist for a pair of cationic enantiomers and an anionic chiral additive:

$$A_R^+ + C^- \rightleftharpoons A_RC \quad (1)$$

$$A_S^+ + C^- \rightleftharpoons A_SC \quad (2)$$

A_R^+ is the *R*-enantiomer, A_S^+ is the *S*-enantiomer and C^- is the chiral counter-ion. The equilibrium constants can be expressed as followed:

$$K_1 = \frac{[A_RC]}{[A_R^+][C^-]} \quad (3)$$

and

$$K_2 = \frac{[A_SC]}{[A_S^+][C^-]} \quad (4)$$

The apparent mobility ($\mu_{A_R^+}$, $\mu_{A_S^+}$) of the enantiomers in the presence of the chiral counter-ion, C^-, is a function of the proportion of time they spend as a part of the diastereomeric ion-pair and as the free analytes. The apparent mobility, e.g. of the *R*-enantiomer ($\mu_{A_R^+}$) in the presence of the chiral counter-ion can therefore be expressed by Eq. 5.

$$\mu_{A_R^+} = \frac{[A_R^+]}{[A_RC] + [A_R^+]}\mu^0_{A_R^+} = \frac{\frac{[A_RC]}{K_1[C^-]}}{[A_RC] + \frac{[A_RC]}{K_1[C^-]}}\mu^0_{A_R^+} = \frac{1}{K_1[C^-]+1}\mu^0_{A_R^+} \quad (5)$$

$\mu^0_{A_R^+}$ is the apparent mobility of the *R*-enantiomer when no counter-ion is present in the electrophoresis medium. A similar equation can be set up for the *S*-enantiomer.

Separation can only be achieved if the two enantiomers have different mobilities ($\mu_{A_R^+} \neq \mu_{A_S^+}$) and the optimum separation is achieved when the difference in mobility ($\Delta\mu$) is as large as possible.

$$\Delta\mu = \mu_{A_R^+} - \mu_{A_S^+} = \frac{\mu^0_{A_R^+}}{K_1[C^-]+1} - \frac{\mu^0_{A_S^+}}{K_2[C^-]+1} \quad (6)$$

As the mobility of the enantiomers is the same in the absence of the chiral counter-ion ($\mu^0_{A_R^+} = \mu^0_{A_S^+}$), Eq. 6 can be expressed as Eq. 7.

$$\Delta\mu = \frac{[C^-]\mu^0_A(K_2 - K_1)}{[C^-]^2K_1K_2 + [C^-](K_1 + K_2) + 1} \quad (7)$$

As can be seen from Eq. 7, the separation depends on K_1, K_2 and $[C^-]$. If $K_1 = K_2$, no separation will be achieved as $\Delta\mu = 0$. As pointed out by Wren and Rowe [11], obtaining maximal $\Delta\mu$ is not only dependent on the difference in equilibrium constants but also on the absolute value of these. Furthermore, it can be seen that at extreme concentrations of counter-ion, i.e. when no counter-ion is added or when the concentration of counter-ion is very high, no separation will be obtained. Between these two extremes there is an optimum concentration of counter-ion where the maximum $\Delta\mu$ ($\Delta\mu_{max}$) is obtained and thus the maximum separation of the two enantiomers can occur.

The *percentage difference* in equilibrium constants is important for the magnitude of $\Delta\mu_{max}$. Thus, a high *percentage difference* in equilibrium constants leads to high values of $\Delta\mu_{max}$. Whereas the *absolute values* of equilibrium constants are important for determining which concentration of counter-ion will

lead to $\Delta\mu_{max}$. Thus $\Delta\mu_{max}$ is obtained at lower concentrations of counter-ion when the equilibrium constants have higher *absolute values*.

2. Experimental

2.1. Capillary electrophoresis system

A HP^{3D} capillary electrophoresis system (Hewlett-Packard, Waldbronn, Germany) equipped with a diode array detection (DAD) system was used. A detection wavelength of 214 nm was used for all samples. The separation was performed in fused-silica capillaries (64.5 cm in total×50 μm I.D.) (Polymicro Technologies, Phoenix, AZ, USA). The separation was also performed in a methyl cyanopropyl phenyl deactivated capillary (64.5 cm in total×50 μm I.D.) from Polymicro Technologies and in a polyvinylalcohol-coated capillary (64.5 cm in total×50 μm I.D.) from Hewlett-Packard. The capillary surrounding was thermostated to 25°C by air. Samples were kept at ambient temperature in the autosampler by water cooling of the tray and they were injected by applying a pressure of 5 kPa (50 mbar) for 3 s. A voltage of 30 kV was applied during analysis. A Coolnics Circulator CTE 32 from Yamato-Komatsu (Tokyo, Japan) was used for water cooling the sample tray.

Prior to use, the capillaries were rinsed with 1 *M* sodium hydroxide for 60 min, 0.1 *M* sodium hydroxide for 20 min, distilled water for 20 min, 1 *M* acetic acid in methanol–acetonitrile for 10 min and electrophoresis medium for 10 min. Between analysis, the capillaries were flushed with electrophoresis medium for 2 min. However, when the media contained Tween 20 or more than 20 m*M* camphorsulphonate, the capillaries were flushed with 0.1 *M* sodium hydroxide for 2–10 min and then with electrophoresis media between runs.

2.2. Chemicals and reagents

Atenolol, ephedrine hydrochloride, epinephrine hydrochloride, metoprolol and trimipramine maleate were obtained from Sigma (St. Louis, MO, USA). Atropine sulphate, pindolol, salbutamole sulphate, acetic acid, (+)-camphorsulphonic acid sodium salt [(+)-*S*-camphorsulphonate], (−)-*R*-camphorsulphonic acid and Tween 20 were obtained from Wako (Osaka, Japan). (+)-*S*-camphorsulphonic acid was obtained from Ferak Berlin (Berlin, Germany). The (−)-*R*- and (+)-*S*-camphorsulphonic acids were converted to the sodium salt, the potassium salt and the lithium salt, respectively, by mixing equal amount of the acids and metal hydroxide in absolute ethanol. The salts were precipitated, washed with diethyl ether and dried.

Bisoprolol fumarate, promethazine hydrochloride and propranolol hydrochloride were obtained from Tanabe Seiyaku (Osaka, Japan). Bupivacaine was obtained from Eisai (Tokyo, Japan). Bunitrolol and mexiletin hydrochloride were obtained from NBI (Hyogo, Japan). Flecainide acetate was obtained from 3M Health Care (Loughborough, UK). Cisapride and four synthetic impurities were obtained from Janssen Pharmaceutica (Beerse, Belgium). Methanol and acetonitrile (MeCN) were obtained from Nacalai Tesque (Kyoto, Japan).

2.3. Sample preparation

All test samples were dissolved in methanol at concentrations of 0.2–0.3 mg/ml.

Cisapride and impurities were dissolved in methanol at concentrations of 0.04 mg/ml each.

3. Results and discussion

3.1. Choice of solvent

Not all organic solvents are equally usable as media for non-aqueous CE separations [12]. They must not be too flammable, too viscous or too easily oxidized and they must be able to dissolve the electrolytes.

MeCN, dioxane and tetrahydrofuran (THF) should be good solvents for obtaining ion-pairs, as they are aprotic solvents with dielectric constants that are lower than water. MeCN has the lowest UV cut off value of these three solvents and also has the lowest viscosity. Therefore, it would be preferable to use media with a fairly high content of MeCN as these will provide the lowest detection limits and the highest EOF, as the latter depends on the viscosity of

the medium. Dioxane and THF are less polar than MeCN and should theoretically be more favourable for achieving ion-pair formation. However, MeCN was chosen for the experiments discussed in this paper because is was the only medium where camphorsulphonate could be dissolved in sufficient concentrations. Acetic acid was added to adjust the acidity of the medium and to increase the solubility of camphorsulphonate.

3.2. Chiral separation by diastereomeric ion-pair formation

Camphorsulphonate (Fig. 1) was chosen as the counter-ion as it has previously been used as such for chiral separation in HPLC [2]. Furthermore, many of its salts are reasonably soluble in organic solvents, it has a low UV absorption and it is very inexpensive. Other counter-ions such as tartrate, maleate and lactate were also tried but could not be used due to solubility problems. The sodium salt (as well as the potassium salt) of camphorsulphonate can be used, although the potassium salt was preferred due to its higher solubility in MeCN. The lithium salt of camphorsulphonate was practically insoluble in MeCN. Although the triethyl ammonium salt of camphorsulphonate was very soluble in MeCN, no chiral separation could be obtained with this salt.

Fifteen basic chiral drug substances (Fig. 1) were chosen as test compounds to investigate whether the ion-pair formation principle could be used for the separation of enantiomers in a non-aqueous medium. No separations were obtained when an **aqueous** electrophoresis medium containing 30 m*M* (+)-*S*-camphorsulphonate was used.

Separation of the test substances was investigated in a non-aqueous medium with increasing concentrations of camphorsulphonate (Fig. 2). At 5 m*M* camphorsulphonate, a small separation of salbutamole could be seen and separation was improved with up to 30 m*M* camphorsulphonate. No further separation was achieved at higher concentrations of camphorsulphonate (Fig. 2). The tendency shown in Fig. 2 was the same for all substances that could be separated.

Only substances with a β-amino alcohol configuration (Fig. 1 substances 1–9) could be separated. This fact corresponds very well with results obtained

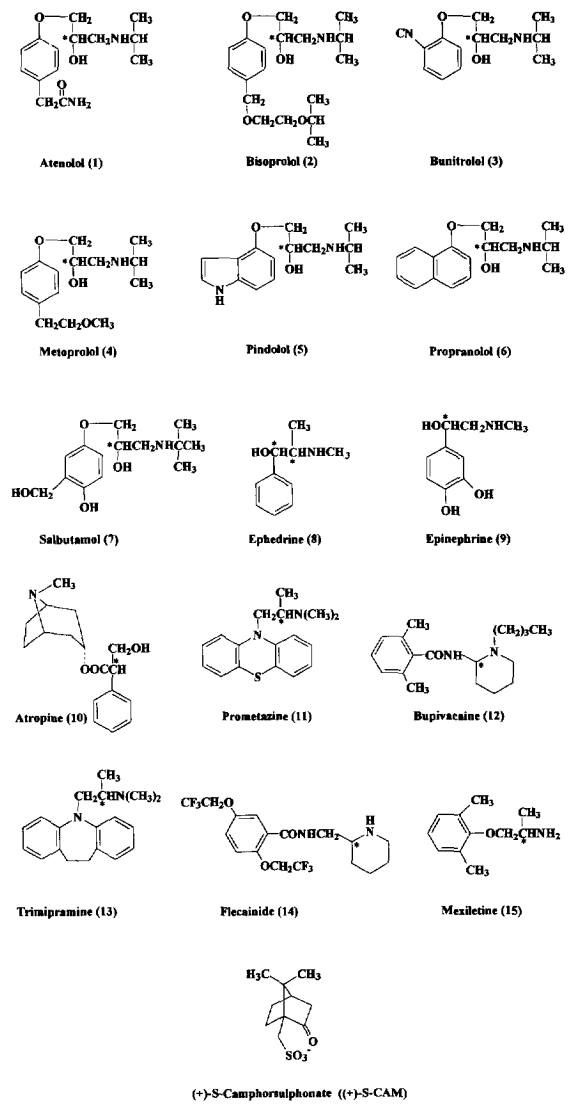

Fig. 1. Structure of the test substances.

using HPLC. Pettersson and Schill [2] found that stereoselective retention of diastereomeric ion-pairs in HPLC could only be achieved if the counter-ion and the analytes were capable of making a two-point binding interaction. The two-point binding of anionic camphorsulphonate can be achieved by the charged sulphonate group and by the hydrogen-accepting oxo-group that can give a strong interaction with the hydrogen-donating alcohol in the cationic β-amino

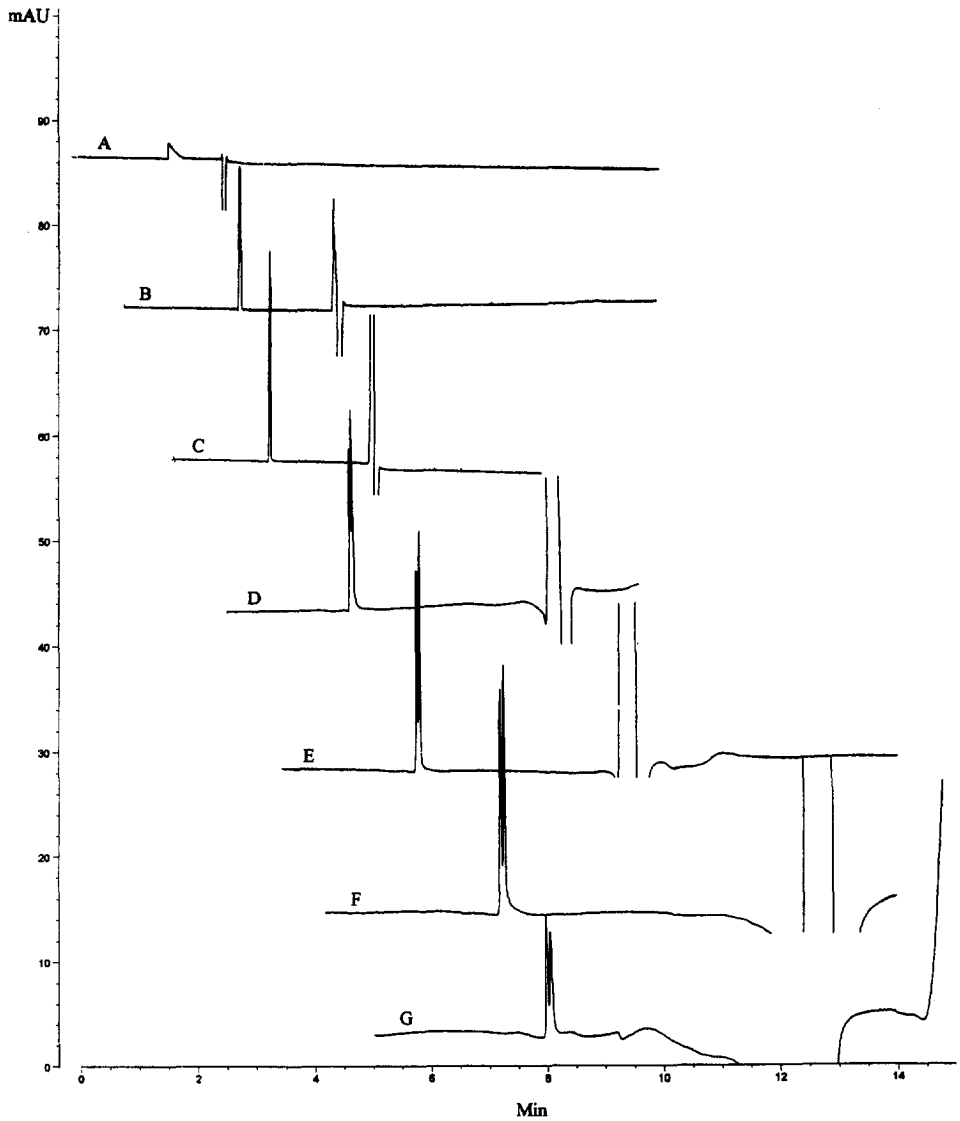

Fig. 2. Electropherograms of 0.3 mg/ml salbutamol with increasing concentrations of (+)-*S*-camphorsulphonate in the electrophoresis medium. Apparatus settings are as described in Section 2. Electrophoresis medium: 1 *M* acetic acid in MeCN. (A) no (+)-*S*-camphorsulphonate added; (B) 5 m*M* (+)-*S*-camphorsulphonate; (C) 10 m*M* (+)-*S*-camphorsulphonate; (D) 20 m*M* (+)-*S*-camphorsulphonate; (E) 30 m*M* (+)-*S*-camphorsulphonate; (F) 40 m*M* (+)-*S*-camphorsulphonate and (G) 60 m*M* (+)-*S*-camphorsulphonate.

alcohol. The substitution pattern in the aromatic ring seems to have no significant effect on the stereoselectivity. However, the size of the substituents at the nitrogen seems to influence the separation of the enantiomers. Thus the chiral selectivities towards the methylamines, ephedrine (8) and epinephrine (9), were poorer than toward the isopropylamines (1–6) and *tert.*-butylamine (7).

At camphorsulphonate concentrations above 20 m*M*, the EOF was no longer reproducible and the capillary had to be flushed with sodium hydroxide in between runs.

3.3. The addition of Tween 20 and investigation of coated capillaries.

In order to slow down the EOF, Tween 20 was added in small concentrations (0.1–0.2 m*M*) (Fig. 3). The EOF decreased from around 10 min to 20 min after the addition of 0.1 m*M* Tween 20 and after the addition of 0.2 m*M* Tween 20 no EOF could be seen for 120 min. The separation also improved (Fig. 3), but reproducible results were hard to obtain. The problems could partly be solved by flushing the capillary for 10 min with 0.1 *M* sodium hydroxide between runs. These and similar problems that arose when the camphorsulphonate concentration was increased are probably due to wall-coating phenomena. Therefore, several coated capillaries were investigated in order to avoid the addition of Tween 20. Two coated capillaries from different suppliers were investigated. One of the capillaries showed a noisy baseline and a quite high EOF, even when none was expected. The second capillary provided broad peaks, unstable baselines and after a few runs (ca. ten), the outside coating of the ends of the capillary were degradated by the medium.

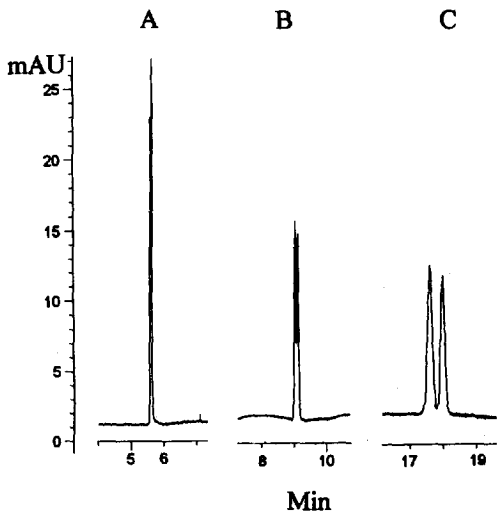

Fig. 3. Electropherograms of 0.2 mg/ml atenolol with increasing concentrations of Tween 20 added to the electrophoresis medium. Apparatus settings are as described in Section 2. Electrophoresis medium: 1 *M* acetic acid in MeCN. (A) 30 m*M* (+)-*S*-camphorsulphonate; (B) 30 m*M* (+)-*S*-camphorsulphonate, 0.1 m*M* Tween 20 and (C) 30 m*M* (+)-*S*-camphorsulphonate, 0.2 m*M* Tween 20.

3.4. Chiral selectivity

To emphasize that it is a chiral separation, a racemic mixture of *RS*-metoprolol was mixed with *S*-metoprolol and only the area of one of the peaks increased confirming the chiral separation (Fig. 4).

A non-racemic mixture of *R*- and *S*-metoprolol was then analyzed in an electrophoresis medium containing (+)-*S*-camphorsulphonate and (−)-*R*-camphorsulphonate, respectively. As can be seen in Fig. 5, the migration order of *S*- and *R*-metoprolol was reversed. This result proved that the separation of the enantiomers is due to formation of diastereomeric ion-pairs.

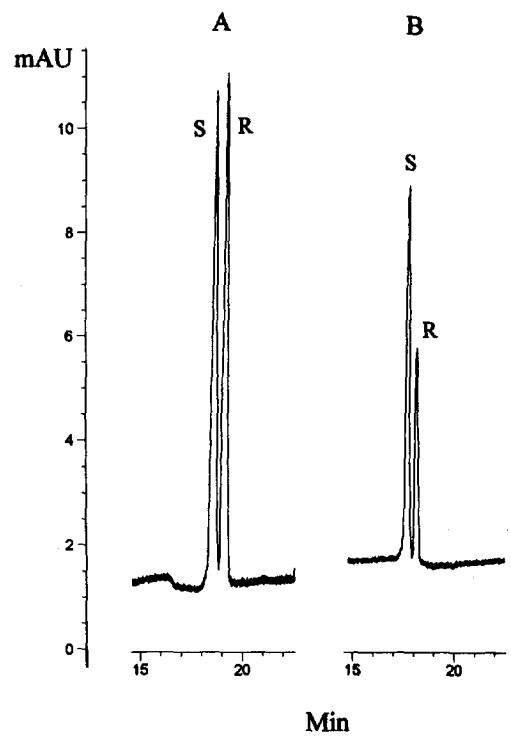

Fig. 4. Electropherograms of racemic and non-racemic metoprolol. Apparatus settings are as described in Section 2. Electrophoresis medium: 30 m*M* (+)-*S*-camphorsulphonate–0.2 m*M* Tween 20–1 *M* acetic acid in MeCN. (A) *RS*-metoprolol and (B) *RS*-metoprolol spiked with *S*-metoprolol.

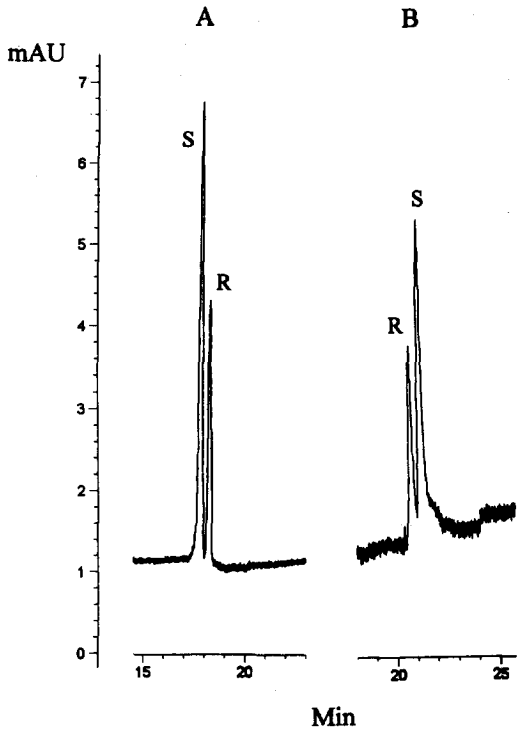

Fig. 5. Electropherograms of non-racemic metoprolol. Apparatus settings are as described in Section 2. Electrophoresis medium: 0.2 m*M* Tween 20–1 *M* acetic acid in MeCN. (A) 30 m*M* (+)-*S*-camphorsulphonate and (B) 30 m*M* (−)-*R*-camphorsulphonate.

When acetate was added to the camphorsulphonate-containing media, the test substances could no longer be separated. This is probably due to competitive interactions by the non-chiral acetate counter-ion.

3.5. Application

Chiral separation by capillary electrophoresis using the principle of diastereomeric ion-pair formation for chiral separation in non-aqueous media can be used both for the separation of highly water-soluble racemates and for the separation of substances with low water solubility. The method is superior for analysis of very lipophilic ionic racemates that are difficult to analyze in aqueous media, due to precipitation.

Cisapride racemate and four synthetic impurities (Fig. 6) show a low water solubility. In particular,

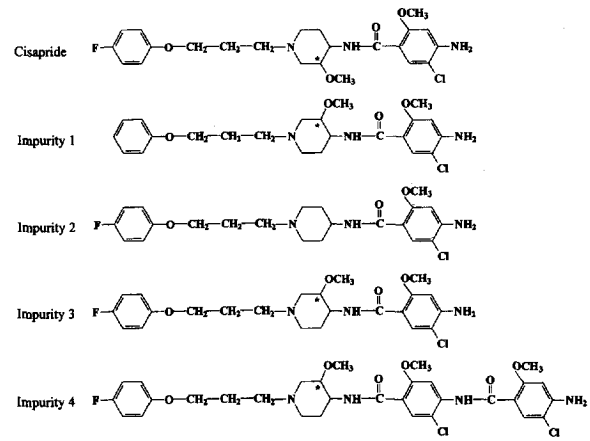

Fig. 6. Structure of cisapride and four synthetic impurities.

impurity 4 has a low water solubility. We tried to separate all substances in a system containing 30 m*M* (+)-*S*-camphorsulphonate, 0.2 m*M* Tween 20, 1 *M* acetic acid in MeCN (Fig. 7). As can be seen from Fig. 7, some separation were achieved of both cisapride and the three racemic impurities. Furthermore, it is interesting to note that none of these substances are β-amino alcohols.

4. Conclusion

The principle of diastereomeric ion-pair formation can be used for chiral separation by capillary electrophoresis using MeCN as the solvent for the electrophoresis medium. This principle can be useful for the separation of ionizable drug substances, especially for those with a low water solubility.

Camphorsulphonate can be used as an anionic counter-ion for the separation of cationic β-amino alcohols, but separation of other substances such as cisapride is also possible.

The best separations were achieved using 30 m*M* camphorsulphonate, 0.2 m*M* Tween 20, 1 *M* acetic acid in MeCN as the electrophoresis medium. Further improvements of the method would involve solving the wall coating problems that occur when high concentrations of chiral additive are used. Furthermore, several chiral counter-ions should be

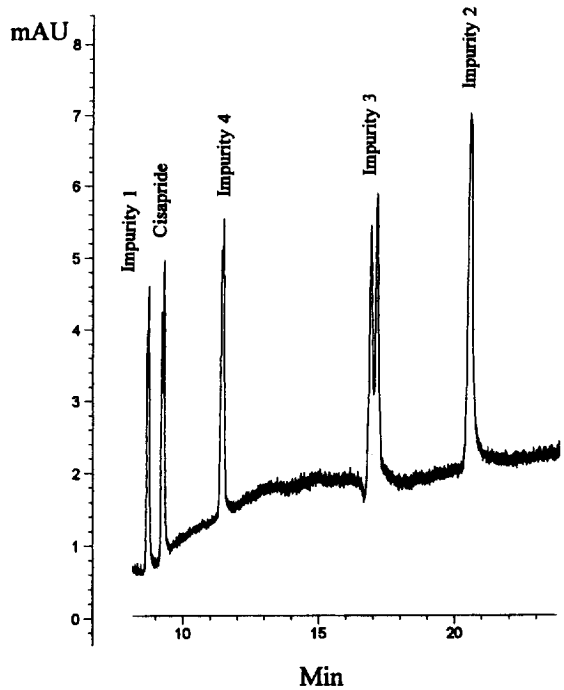

Fig. 7. Electropherograms of cisapride and four synthetic impurities. Apparatus settings are as described in Section 2. Electrophoresis medium: 30 m*M* (+)-camphorsulphonate, 0.2 m*M* Tween 20 and 1 *M* acetic acid in MeCN.

tested in order to show whether the principle is generally applicable.

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