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Short communication

Azithromycin as a new chiral selector in capillary electrophoresis

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ABSTRACT

In capillary electrophoresis (CE), separation of enantiomers of a chiral compound can be achieved through the chiral interactions and/or complex formation between the chiral selector and the enantiomeric analytes on leaving their diastereomeric forms with different stability constants and hence different mobilities. A great number of chiral selectors have been employed in CE and among them macrocyclic antibiotics exhibited excellent enantioselective properties towards a wide number of racemic compounds. The use of azithromycin (AZM) as a chiral selector has not been reported previously. This work reports the use of AZM as a chiral selector for the enantiomeric separations of five chiral drugs and one amino acid (tryptophan) in CE. The enantioseparation is carried out using polar organic mixtures of acetonitrile (ACN), methanol (MeOH), acetic acid and triethylamine as run buffer. The influences of the chiral selector concentration, ACN/MeOH ratio, applied voltage and capillary temperature on enantioseparation are investigated. The results show that AZM is a viable chiral selector in CE for the enantioseparation of the type of chiral drugs investigated.

1. Introduction

The chirality is a major consideration in drug discovery because more than one-half of commercialized drugs are chiral in nature. A number of synthetic chiral drugs are still distributed as racemic mixture in spite of the fact that one enantiomer possesses very different and significant pharmacological, toxicological activities from the antipode and may even disturb other biological processes and cause catastrophic side effects [1]. Thus, the enantiomeric separation and analysis of chiral drugs have become essential in the pharmaceutical field [2]. According to the guidelines of the U.S. Food and Drug Administration (FDA), the pharmaceutical companies have to develop therapeutically active enantiomers of chiral drugs. However, the enantiomers of the drugs should be separated and studied their pharmacological and metabolic pathways [3]. Hence, synthesis and enantioseparation of chiral drugs carry equal significance in pharmaceutical investigations such as pharmacological and toxicological studies.

The chiral separation has become one of the most significant identity in the field of analytical science. Among the separation techniques capillary electrophoresis (CE) has been proven to be an efficient and powerful technique to resolve different classes of enantiomeric compounds [4,5]. CE is a direct separation method that comes with such advantages as high efficiency and separation speed, low cost, availability of a broad variety of chiral selectors, enormous flexibility of enantioseparation and method development, minimal amount of the analyte and organic waste [6,7]. The chiral selector is simply added to background electrolyte (BGE) [8] which gives a solution-based chiral discrimination and hence offers a greater flexibility in the optimization of the separation than the stationary phase-based separation, often with higher resolution and chiral selectivity [9].

The chiral selectors of different types, including cyclodextrins and their derivatives, macrocyclic antibiotics, crown ethers, polysaccharides, proteins, chiral metal complexes, chiral surfactants, chiral ion-pairing reagents [4,10,11], have been utilized for the chiral separation with high resolution capability and separation efficiency. Among various chiral selectors, macrocyclic antibiotics have been widely employed in CE after their introduction by Armstrong and co-workers [12–14] and shown to resolve a wide variety of enantiomeric compounds. The macrocyclic antibiotics including glycopeptides, ansamycins, aminoglycosides and polypeptides have been proved to be a new class of powerful chiral selectors. They possess many stereogenic centers and different functional groups, which allow for multiple interactions with the analyte enantiomeric molecules, resolving a wide range of enantiomers including those of pharmaceutical interest often with very high selectivities.

The chiral separation using azithromycin (AZM) antibiotic as the chiral selector has not been reported previously. AZM is a semi-synthetic macrolide antibiotic derived from erythromycin; however it differs from erythromycin in that a methyl-substituted nitrogen atom is incorporated into the lactone ring (Fig. 1). AZM

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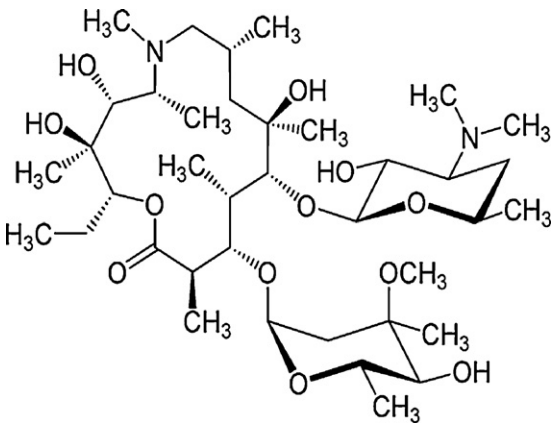

Fig. 1. Structure of azithromycin.

has been approved to be an effective antibiotic for the treatment of sexually transmitted diseases, respiratory tract infections, otitis media and skin structure infections [15–17]. AZM is composed of a characteristic fifteen-membered ring structure having two sugar moieties, several hydroxyl groups, two tertiary amino groups and one oxycarbonyl group. It is only slightly soluble in water but highly soluble in lower alcohols and yet shows low viscosity in these solvents, and absorbs UV very weakly due to lack of aromatic rings in the structure. Due to the presence of multiple chiral centers and different functional groups that can undergo multiple interactions with the analyte enantiomeric molecules, AZM is thought to be a potential chiral selector for chiral separation by CE. We reports in this work the preliminary results of the chiral separation of a set of five chiral drugs and one amino acid by CE in polar organic mixtures composed of acetonitrile (ACN), methanol (MeOH), acetic acid (AA) and triethylamine (TEA) containing AZM as the chiral selector. The effects of AZM concentration, ACN/MeOH ratio, applied voltage and capillary temperature on chiral separation are investigated.

2. Experimental

AZM was purchased from Sigma–Aldrich (St. Louis, USA). The chiral compounds investigated in this work, carvedilol (CAR), cetirizine (CET), citalopram hydrobromide; (CIT), darifenacin (DAR), sertraline hydrochloride (SER) and tryptophan (TRY), were purchased from Aldrich (Milwaukee, USA) or TCI (Tokyo, Japan). Fused silica capillaries (50 μm I.D., 365 μm O.D.) were obtained from Polymicro Technologies (Phoenix, USA). HPLC-grade acetonitrile and methanol were obtained from J.T. Baker (Phillipsburg, USA). Sodium hydroxide, hydrochloric acid, acetic acid and triethylamine were purchased from Sigma-Aldrich (St. Louis, USA). All reagents were reagent grade or better and used as received without further purification. Water was purified with an Elgastat UHQ water purification system (Bucks, UK).

An Agilent HP ^{3D}CE System (Palo Alto, USA), equipped with a diode-array UV detector, a ±30 kV high voltage power supply and an external nitrogen pressure (up to 10 bars) was used to perform electrophoretic experiments. The enantioseparations were carried out at 20, 25 and 30 °C and constant voltages in a range of 10–20 kV, and monitored at 200, 214, 254, 265 and 280 nm. Sample injections were performed by an applied voltage of 15 kV for 3 s and the migration times of two consecutive injections were in agreement within 3%.

Unmodified 50-μm I.D. fused-silica capillaries with a total length of 35 cm (effective length, 25 cm) were initially pretreated at the beginning of each series of experiments as follows. Capillaries were washed with 1 M HCl, 1 M NaOH and water successively for 20 min each, and then finally with BGE for 30 min. The subsequent injections were preceded by a purge with 0.1 NaOH and water for 5 min, and then with run buffer for 15 min. The capillary was washed successively with 1 M HCl, 1 M NaOH and water successively for 10 min each at the end of each day.

The background electrolyte used was a mixture of ACN, MeOH, AA and TEA in the volume ratio of 80:20:0.1:0.1 unless stated otherwise and was degassed by sonication under vacuum before use. The BGE containing AZM was freshly prepared by dissolving AZM in the BGE. The sample solutions of 0.5 mg/mL concentration were prepared by dissolving the chiral drugs in the BGE and TRY in water. The migration time of acetone as the neutral marker was used to obtain electroosmotic mobilities.

3. Results and discussion

Apart from HPLC, CE is conceived as a complementary analytical technique for enantioseparation [11], which is mainly due to high efficiency, separation speed, small amounts of buffers and analytes required. The present work describes enantioseparations of a set of six chiral compounds, CAR, CET, CIT, DAR, SER and TRY, in polar organic BGEs composed of MeOH/ACN/TEA/AA containing AZM as the chiral selector. This specific polar organic BGE was first utilized in CE with cyclodextrin as the chiral selector by Armstrong group [18] and also used with the macrocyclic antibiotics as the chiral selectors [19–21]. TEA and/or AA are added to regulate retention and selectivity [22].

Macrocyclic antibiotics possess several stereogenic centers and functional groups which can undergo multiple interactions with analytes including, ionic or electrostatic, π–π, dipole–dipole, hydrophobic and steric repulsions, host–guest inclusion complexation and hydrogen bonding, which are responsible for chiral recognition [10,23,24]. Fig. 2 shows the electropherograms for enantioseparation of the chiral analytes in the BGEs containing AZM. AZM has multiple stereogenic centers and a number of functional groups including several hydroxyl groups, two amino groups and one ester group. These functional groups on AZM can undergo multiple interactions including ionic, dipole–dipole and steric repulsions with functional groups (at the stereogenic centers) of the chiral analyte, which are needed for chiral recognition in a similar manner to other macrocyclic antibiotic chiral selectors [12,13]. AZM also has two amino groups whose pK_a values are 8.7 and 9.5 [25], both of which are present in the protonated form in the polar organic run buffer (apparent pH 8.1), and possible inclusion complexation of the analyte in the cavity of AZM led by strong electrostatic interactions can also affect chiral recognition.

The BGEs containing 2, 4, 6 and 8% (w/v) AZM were used to see the effect of the AZM concentration on enantioseparation (Table 1). At 2% AZM, chiral separations of all analytes except CIT and SER are achieved. At 4% AZM, all enantiomers are baseline separated, and a further increase in AZM concentration to 6% gives even better resolution and selectivity, especially for CET, CIT and SER. The highest enantioresolution is observed with SER (R_s = 3.74), which is followed by CIT (R_s = 3.08). With increasing AZM concentration migration times increase for all analytes. Similar behavior in migration times upon increasing the chiral selector concentration was also observed with other antibiotic chiral selectors [26,27]. The reason for the increasing migration time with AZM concentration is three-fold: (i) increased adsorption of AZM on the capillary wall; (ii) increased buffer viscosity, and (iii) increased complexation between AZM and analytes, all of which will cause migration times to increase. Electroosmotic flow (EOF) in CE is inversely related to the viscosity of the BGE. Increased viscosity of the BGE with increasing AZM concentration leads to decreased EOF and hence increased migration times of the analytes. Resolution of the drug enantiomers

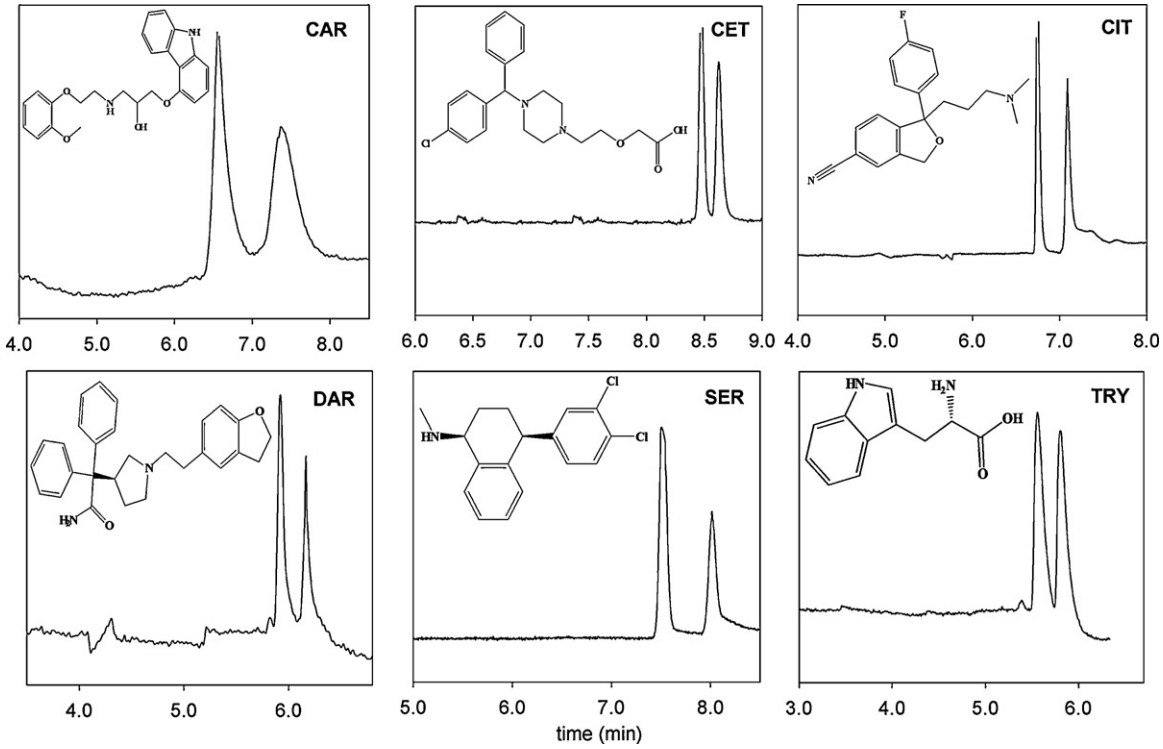

Fig. 2. Electropherograms for the chiral separation of CAR, CET, CIT, DAR, SER and TRY. Conditions: capillary, 35 cm (25 cm effective length) × 50 μm I.D.; BGE, ACN/MeOH/AA/TEA (80:20:0.1:0.1%, v/v/v/v) containing 4% of AZM for CAR, DAR, TRY and 6% for CET, CIT, SER; applied voltage, 20 kV; injection, 15 kV, 3 s; capillary temperature, 20 °C; detection, 254 nm.

increases with increasing concentration of AZM from 2% to 6%, and then it decreases at 8%. The lower resolution at 8% AZM is likely due to peak broadening caused by saturated interaction between the chiral solute and AZM [28]. Upon change of the AZM concentration, enantioselectivity changes only slightly. Little variation in enantioselectivity with AZM concentration is likely an indication of weak complexation between AZM and the analyte. However, chiral recognition mechanism is not clear at present and subject to further study.

The composition of the polar organic BGE strongly affects the enantioresolution due to its influence on EOF, electric current and peak efficiency [28,29]. BGEs having ACN/MeOH ratios of 90:10, 80:20, 70:30 and 60:40 were investigated to see the effect of this ratio on the enantioseparation by keeping the amounts of TEA and AA constant. Table 1 shows the enantioseparation results of two representative solutes, CET and TRY in the BGEs containing 6% AZM. MeOH has a lower dielectric constant/viscosity ratio (ε/η) than ACN, and thus increasing MeOH content will generate decreasing EOF, which is proportional to the ε/η ratio. This will result in increasing migration times of the analytes with increasing MeOH composition. MeOH is more viscous than ACN and thus the increase in MeOH content causes the viscosity of the BGE to increase, which also make the migration time of the analytes to increase. As the MeOH content increases from 10% to 20% resolution increases while enantioselectivity decreases. Further increase in the MeOH content results in decreasing resolution for both solutes. MeOH is more dipolar than ACN, and MeOH is capable of undergoing hydrogen-bonding interactions with the analyte and AZM while ACN is not [30]. Increased dipolar and hydrogen bonding interactions by MeOH with AZM may cause chiral interactions between AZM and the analyte to decrease, thereby leading to weakened chiral recognition. In addition, with increasing MeOH concentration peak broadening was observed, which will lead to decreased separation efficiencies. The best chiral resolutions can thus be obtained in the BGE with ACN/MeOH ratio of 80:20.

Effect of applied voltage on enantioseparation was studied for two representative analytes, CAR and TRY, by varying the voltage over a range of 10–20 kV in the BGE having 4% AZM. Table 1 lists migration times, resolutions, plate numbers and enantioselectivities for the analytes observed at different applied voltage. The increased applied voltage would cause EOF to increase, giving reduced migration times. Increasing voltage will give increased peak sharpness due to less time allowed for longitudinal diffusion of the analyte molecules [31], thereby showing reduced peak broadening. The plate number and resolution increase while enantioselectivity shows no appreciable change with increasing voltage.

Temperature is one of the operational parameters in CE to adjust migration time and enantioseparation. As enantioseparation is based on the different binding ability of two enantiomers to the chiral selector, temperature can affect the binding stability and hence the extent of enantioseparation as well as the migration time. In Table 1 are listed enantioseparation results of DAR and TRY in ACN/MeOH/AA/TEA (80:20:0.1:0.1) having 4% AZM at 20, 25 and 30 °C. Better enantioresolution is usually obtained at lower temperatures [21,32]. Resolution factor and the plate number improve upon increase in temperature from 20 to 25 °C while enantioselectivity remains unchanged. The increased temperature results in decreased viscosity of the BGE, which provides a faster equilibrium between AZM and the run buffer, thereby resulting in increased efficiency. Within the temperature range investigated, resolution and plate numbers change appreciably with temperature, showing maxima at 25 °C. Further increase in temperature results in an even lower viscosity which will cause increased longitudinal diffusion of the analyte molecules, leading to worsened separation efficiency.

In this work, we tested the viability of AZM as a novel antibiotic chiral selector in CE for a set of six chiral compounds including five chiral drugs and one native amino acid. The enantioseparation results of the analytes indicate that AZM is a viable chiral selector in polar organic BGE comprised of ACN/MeOH/AA/TEA. The effect of the AZM content in the BGE on the chiral separation

Table 1
Enantioseparation data in ACN/MeOH/AA/TEA.

Solute	AZM concentration (w/v%)[a]											
	2			4			6			8		
	t_1/t_2[b]	R_S[c]	α[d]	t_1/t_2[b]	R_S[c]	α[d]	t_1/t_2[b]	R_S[c]	α[d]	t_1/t_2[b]	R_S[c]	α[d]
CAR	5.64/5.88	1.41	1.04	6.56/7.36	2.24	1.07	7.75/8.84	2.05	1.07	8.65/9.32	1.21	1.07
CET	5.02/5.21	0.94	1.01	6.58/6.97	1.48	1.08	8.47/8.63	1.55	1.11	12.95/13.16	0.88	1.07
CIT	5.56/5.79	2.69	1.12	5.74/6.37	2.87	1.15	6.75/7.08	3.08	1.16	12.77/15.08	2.18	1.14
DAR	5.54/5.78	1.14	1.08	5.92/6.18	2.02	1.12	7.83/8.56	1.76	1.10	8.73/9.38	1.10	1.07
SER	5.61/5.87	2.58	1.11	6.27/7.40	3.44	1.16	7.52/8.02	3.74	1.18	13.38/16.34	2.23	1.17
TRY	5.42/5.61	1.00	1.04	5.54/5.79	1.72	1.05	6.71/6.96	1.66	1.06	8.85/9.31	1.47	1.05
Solute	ACN composition[f]											
	90% ACN			80% ACN			70% ACN			60% ACN		
	t_1/t_2[b]	R_S[c]	α[d]	t_1/t_2[b]	R_S[c]	α[d]	t_1/t_2[b]	R_S[c]	α[d]	t_1/t_2[b]	R_S[c]	α[d]
CET	5.62/5.85	1.34	1.11	8.47/8.63	1.55	1.09	10.69/11.05	1.42	1.08	12.93/13.25	1.02	1.05
TRY	5.29/5.53	0.96	1.07	6.71/6.96	1.66	1.06	8.73/9.33	1.22	1.05	11.13/11.56	0.88	1.05
Solute	Applied voltage[g]											
	10 kV				15 kV				20 kV			
	t_1/t_2[b]	R_S[c]	α[d]	N_1[e]	t_1/t_2[b]	R_S[c]	α[d]	N_1[e]	t_1/t_2[b]	R_S[c]	α[d]	N_1[e]
CAR	14.76/15.71	2.23	1.06	34450	9.63/10.27	2.30	1.07	35840	6.56/7.36	2.24	1.07	38880
TRY	11.89/12.42	1.62	1.05	25540	8.28/8.72	1.65	1.05	27200	5.54/5.79	1.72	1.05	29250
Solute	Column temperature[g]											
	20 °C				25 °C				30 °C			
	t_1/t_2[b]	R_S[c]	α[d]	N_1[e]	t_1/t_2[b]	R_S[c]	α[d]	N_1[e]	t_1/t_2[b]	R_S[c]	α[d]	N_1[e]
DAR	5.92/6.18	2.02	1.12	38,200	5.62/5.86	2.10	1.12	44,300	5.36/5.49	1.95	1.12	40,800
TRY	5.54/5.79	1.72	1.05	29,250	5.48/5.67	1.62	1.05	28,100	5.38/5.62	1.50	1.05	28,410

[a] Conditions: capillary, 35 cm (25 cm effective length) × 50 μm I.D.; BGE, ACN/MeOH/AA/TEA (80:20:0.1:0.1%, v/v/v/v) containing different amounts of AZM; applied voltage, 20 kV; injection, 15 kV, 3 s; capillary temperature, 20 °C; detection, 254 nm.
[b] Migration times of the first and second eluted enantiomer.
[c] Resolution factor.
[d] Apparent enantioselectivity factor given by t_2/t_1.
[e] Number of the theoretical plates.
[f] AZM concentration, 6% (w/v).
[g] AZM concentration, 4% (w/v).

was investigated. It was found that 4–6% (w/v) of AZM provided good enantioseparation of the analytes tested. The effects of the ACN/MeOH ratio in the BGE, applied voltage and column temperature on the enantioseparation were also examined. The best chiral resolutions were obtained in ACN/MeOH/AA/TEA (80:20:0.1:0.1%, v/v/v/v) at 25 °C with applied voltage of 15 kV. AZM is more stable in polar organic BGE than other macrocyclic antibiotic chiral selectors. The present study indicates that AZM can be added as a newcomer to the group of macrocyclic antibiotic chiral selectors and deserves further evaluation and continued development of chiral analyses for more variegated chiral analytes in different analytical techniques.

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