Review

Chiral analysis of pharmaceuticals by capillary electrophoresis using antibiotics as chiral selectors

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ABSTRACT

The review summarizes the use of the chiral capillary electrophoresis (CE) with different class of antibiotics as chiral selectors in the pharmaceutical field. Basic factors influencing the enantioseparation are shortly discussed. Non-aqueous capillary electrophoresis is also included as well as the coupling of CE to MS. The selection of a chiral selector according the ionic state and structure of the analyte is described. Summary of pharmaceutical applications of chiral CE is given.

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1. Introduction

Macrocyclic antibiotics were the first group of antibiotics introduced as a chiral selector (CS) by Armstrong et al. in 1994 [1]. Many papers were published devoted to their use as chiral selectors in HPLC [2–6], CE [7–12], and TLC [13]. Although cyclodextrins and their derivatives are still the most popular selectors, in the past few years the interest in antibiotics has been continuously increasing [14–22]. Thanks to the diversity in chemical groups, antibiotics exhibit a variety of interactions (inclusion, electrostatic, hydrogen bond) which enable them to achieve high chiral resolution with a

wider range of analytes (acidic, basic, or neutral). To point a few, they are carboxylic and phosphonic acids, amino acids, amino alcohols, etc. Almost all of them are biologically active compounds. Enantioseparations of such pharmaceuticals as NSAIDs (profens), β-blockers, vasodilators, anti-cancer drugs and many others have been reported [23–25].

Different reviews covering advances in chiral separations, including pharmaceutical applications, using different chiral selectors were published [24,26–35]. At present, six groups of antibiotics are reported to be used as a chiral selector in CE: glycopeptides, polypetides, ansamycins, aminoglycosides, macrolides, and lincosamides. Several reviews dealing with chiral CE with macrocyclic antibiotics (MAs) were published [33,36–38], the latest being available starting with 2001. Authors essentially summarize the advantages of glycopeptides for the enantioseparations of different aromatic carboxylic acids and N-blocked amino acids (AQC-, CBZ-, dasnsyl-, etc.).

The aim of this work is to review the use of all the above antibiotics for enantioseparations of pharmaceuticals, such as NSAIDs (ibuprofen, ketoprofen, flurbiprofen, etc.), anticoagulant (warfarin, coumachlor), β-blockers (pindolol, propranolol, atenolol, etc.), vasodilators (bamethan), hormones (epinephrine, DOPA, etc.), and other, while amino acid derivatives are not included. Some suggestions on selection of the appropriate chiral selector according to the ionic state of analyte are going to be given. In the last part of this review, the practical applications (impurity testing, quantification in pharmaceutical preparations, or in biological samples) are to be discussed.

1.1. Relevant properties of antibiotics

The size of the antibiotic and the sterical arrangement of functional groups define its enantioselective properties. The primary interactions in antibiotics-based enantioseparations are thought to involve electrostatic interactions. Electronegative atoms in molecules like oxygen, nitrogen, and halogens can form hydrogen bonds and provide dipole–dipole interactions. Moreover, π–π interaction is possible if one or more aromatic rings are present in the analyte structure. By nature, antibiotics can be acids, bases, amphoteric or neutral compounds.

The glycopeptides (GAs) (vancomycin, ristocetin A, eremomycin, etc.) contain a peptide core of amino acids and linked phenolic moieties, one or more sugar moieties (so-called aglycone), and one or more amino saccharides. The aglycone is semirigid and can be in a "basket"-like form. There is a long hydrophobic chain in the teicoplanin molecule; therefore, it has surfactant-like properties (CMC 0.21 mM 50 mM phosphate buffer, pH 7.4) [39]. In the enantiorecognition process, enantiomers are settled in aglycone basket of the GA in different ways. It appears to be beneficial when acidic or anionic moiety (carboxylate, phosphate groups, etc.) is in α- or β-position to the stereogenic center. Enantiomeric separation seems to be enhanced when a chiral compound contains a carbonyl group, an aromatic ring, or an amide nitrogen close to the stereogenic center. The secondary amine in the aglycone "basket" plays a key role in the enantiorecognition process [40].

GAs, except for A82846B and hepta-tyr, are easily soluble in water, some of them are soluble in aprotic polar solvents. In aqueous solution, vancomycin, eremomycin, balhimycin tend to dimerize, depending on the type of solution and its concentration [41,42]. They contain several ionizable groups: isoelectric point pI of many antibiotics is in the pH range 7–8.5. Due to the presence of amino groups antibiotics can be protonated at pH < pI. That is why the adsorption of the chiral selector phenomenon on the capillary wall is observed. The adsorption level is greater when the numbers of amino groups in the antibiotic and overall positive charge are greater. Therefore, compared to the usual rinsing

longer and more intensive post-run washing is needed to achieve reproducible results. In addition, in aqueous solutions glycopeptide antibiotics are known to decompose with time; they are particularly unstable at elevated temperatures and in basic solutions. The antibiotic degradation leads to the elongated migration times and noisy baseline, the enantiomer resolution decreases as well.

Glycopeptides significantly absorb in the UV region (max around 285 nm) that decreases the detection sensitivity. To overcome the problem some approaches described below have been developed (see Section 3).

The ansamycins, rifamycin B and rifamycin SV, have a characteristic ansa structure consisting of a ring structure or chromophore spanned by an aliphatic bridge, which can be substituted. They are well soluble in water and low-molecular weight alcohols. Rifamycin B is a dibasic acid with pK_a 2.8 and 6.7. The members of this class absorb in the UV–vis region, their solutions are of orange color [7]. They are used at concentrations of 20–25 mM; separations are usually monitored via indirect detection. When 350 nm wavelength was used, direct detection was possible and improved sensitivity was observed [43].

Erithromycin, one of the macrolides, consists of one macrocyclic lactone ring to which two sugar moieties are attached. The macrocycle has a "basket"-like form [44]. Erithromycin is a weak base; it is poorly soluble in water. With the lack of the aromatic ring in the structure, it exhibits very weak UV absorption that turns out to be an advantage.

Though the aminoglycoside streptomycin and lincosamide clindamycin phosphate are not macrocyclic antibiotics they are often discussed together with MAs. Streptomycin contains three glycosidic rings. It has apparent pK_a 8.7 and is soluble in water. This antiobiotics belong to the cationic chiral selectors [45]. Clindamycin phosphate is the newest member of the antibiotic family used as a chiral selector [18]. It consists of an amido portion, an amino portion (tertiary amine), a phosphate ester portion, and an aglycon moiety in each molecule. It possesses high solubility and low viscosity in both water and alcohol. The common advantage of these antibiotics is the lack of the absorbance in UV region.

2. Basic factors, influencing the enantioseparation

2.1. Influence of the composition and pH of the run buffer

As all the antibiotics possess many different functional groups capable of dissociation (hydroxyl, amino, carboxylic groups), hence pH of run buffer governs the charge and mobility of both the chiral selector and analytes [36,37]. The electromigration of the chiral selector opposite to the direction of the analyte is known to be beneficial for the enantioseparation since the difference between migrations of chiral selector–enantiomer complex and free enantiomer increases [46]. Therefore, to enantioseparate anionic compounds (for example, profens, warfarin) it is reasonable to choose the pH ranges of the protonated selector (pH < pI). Taking into account the macrocyclic antibiotic stability considerations, run buffer pH range 4.0–8.0 should be used. Ansamycins were used in buffers with pH 5.0–9.0 [7]; and lincosamide clindamycin phosphate was used in buffers with pH 6.6–8.8 [18]. In general, enantioresolution vs. pH has a maximum around 6.0–7.0 [22,47]. To separate enantiomers of cationic compounds (atenolol, octopamine, oxprenolol, ephedrine, etc.), run buffer with pH 7.0–7.6 was used [7,18]. By tradition, in capillary electrophoresis these pH ranges are covered with phosphate buffer. The selection of the buffer is usually determined by the pH value needed. However, some authors used Good buffers or TRIS-H$_3$PO$_4$ buffers [11,48–50]. Vespalec and co-authors [10] compared Na-phosphate, TRIS-MOPS, and MOPS-TRIS buffers and showed that the worst

results (baseline noise and peak broadening) were obtained using Na-phosphate buffer. When Na cation was substituted with TRIS, higher (15–20 kV) voltage could be applied due to the lower run buffer electroconductivity [22]. Some authors [51–53] prefer the Britton-Robinson buffer because wide pH range is then available. Acetic acid–ammonium acetate buffer (pH 4.8) containing vancomycin meets several important requirements for chiral CE–MS coupling, i.e. (a) it is volatile, (b) vancomycin is positively charged and migrates away from the detector [54].

To reduce the adsorption of such antibiotic as vancomycin, eremomycin, streptomycin on the capillary wall concentrated (50–100 mM) run buffer are recommended [37]. However, the high electroconductivity of these buffers and as a consequence the high current can negatively influence the CS stability during the analysis [8,39]. Moreover, the ionic strength increase leads to the EOF decelerating [55].

2.2. Effect of the chiral selector concentration

The concentration of the chiral selector as well as the run buffer pH is an important variable controlling the chiral recognition in CE [8,14,15,18,21,39,46,50,51,56–60]. One of the advantages of the GAs as a chiral selector is the low concentration of antibiotics in the run buffer. It is usually sufficient to use only 1–5 mM solution. To achieve the same enantioresolution with ansamycins and erythromycin 20–30 mM solutions should be used. Macrolides and lincosamines should be added to the buffer at 50–100 mM, fortunately they do not absorb in UV region [15,18,44].

In general, higher concentrations of the chiral selector produce higher enantioresolutions and longer separation times. The reason for the increase in migration time is that higher concentrations of CS tend to slow down the electroosmotic flow. Hence, the improvement in the enantioseparations at higher concentrations seems to be the result of a greater time of association between the chiral selector and enantiomer due to mass action and the decrease in the EOF [8,39,46]. Certain antibiotics (eremomycin, A82846B) so strongly adsorb on the uncoated capillary wall that the reversal of the EOF can be observed. In that case carrying out the analysis demanded applying a positive pressure [21,61]. Introduction of 0.9 mM eremomycin solution in the capillary was shown to reverse the EOF [17]. In this case the selector behaves as a pseudostationary phase [46,48], while analyte migration order and enantioselectivities change.

2.3. Effect of the organic modifier additive

To change or to enhance the separation selectivity, the organic modifier of the run buffer can be added. The addition of some organic modifier can change or enhance the separation selectivity. In CE methanol, 2-propanol, and acetonitrile are most frequently used. They usually constitute no more than 10–20% of the run buffer volume. Greater percentage leads to the migration slowing down and sometimes to the separation deterioration. When 30% of 2-propanol was added to the ristocetin A solution, and already good enantioseparation of ketoprofen (Rs = 2.6) doubled while the migration time increased by three times [8]. It is possible that the increase in resolution can be attributed to a decrease of EOF and adsorption of the CS on the capillary wall. When adding alcohols (methanol, 2-propanol), the migration times usually become longer than when acetonitrile is added. This effect can probably be explained by the fact that methanol/water mixtures (up to 40%) exhibit a higher viscosity than acetonitrile/water solutions. However, to achieve the optimal enantioseparation of anti-hepatitis drug, diphenyldimethylester, and its derivatives using erythromycin, it is useful to increase the methanol content in phosphate buffer up to 50% [59].

When using vancomycin, eremomycin, it is often preferable not to use the organic solvent. This is not the case of antibiotics poor soluble in water (for example, LY307599) and detergent-like antibiotics (avoparcin and reifamycin B). In the former case the role of the organic modifier is to improve the solubility, in the latter solvent prevents the aggregation of the antibiotic. Specifically, actaplanin A is dissolved in aqueous buffer containing 2-methoxyethanol (15–30%) [62]. Hepta-tyr is also poor soluble in water. Fanali and co-authors studied the effect of acetone, acetonitrile, n-propanol, 2-propanol, and trifluoroethanol on the solubility of the CS and separation of selected profens when using it [54]. It was found that the best enantioresolution at shortest possible time was achieved using the 20% additive of acetonitrile. The optimal separation does not always take a little time. Even in borate buffer, pH 9.2, containing 4 mM LY307599, 15% methanol the analysis of flurbiprofen lasted 40 min [56], the resolution factor Rs being 1.5.

It was demonstrated that the aggregation of surface-active antibiotics could inhibit the enantiorecognition [47,58,63]. The enantioseparation of carprofen significantly improved when 20% was added [39]. To prevent the self-association of teicoplanin, avoparcin, and rifamycin B the buffer with acetonitrile (in the first case) and 2-propanol should be used.

To our knowledge, only one paper devoted to the use of antibiotics in non-aqueous CE (NACE) has been published [14]. The macrolide antibiotic erythromycin was used to enantioseparate basic compounds (duloxetine, propranolol) in methanol media. Addition of some ionic substances can ensure interactions like ion pairing and stronger electrostatic interactions. The migration times of duloxetine were approx. 25 min at 15–20 kV in TRIS-H$_3$BO$_3$ buffer. It is probably due to the both high buffer and chiral selector (100 mM) concentration. Boric acid seems to determine the interaction between analyte and erythromycin. Hydrogen bonding between both the amino and hydroxyl groups both in the CS and drugs molecules, and as well as electrostatic interactions is supposed to be responsible for the recognition process.

The enantiorecognition in non-aqueous media seems to be more effective than in aqueous. Thus, in spite of lack of aromatic rings in erythromycin lactobionate molecule the separation selectivity $\alpha = t_2/t_1$ was 1.06 and 1.12 for duloxetine and propranolol enantiomers, respectively. In phosphate buffer (pH 7.0) no changes in electrophoretic mobilities of propranolol were observed [64], while in methanol-based system the resolution factor 1.78 was achieved [14]. This demonstrates the complimentary potential of NACE to aqueous CE for chiral separations.

2.4. Effect of the micelles formation

The effective approach to diminish the negative adsorption is to use run buffer containing surfactants capable to form micelles. When achiral ionogenic surfactant (for example, sodium dodecyl sulphate, SDS) is added to the run buffer containing vancomycin, the latter is solubilized into micelles. The surfactants that can be used include sodium thiosulphate (STS), sodium octyl sulphate (SOS), and SDS. The SDS is preferable, because it provides better baseline stability [15]. Moreover, propranolol, chlorpheniramine, trypthophane, and nefopam are better enantioseparated in clindamycin-based buffer with SDS additive. It was demonstrated [9] that the beneficial effect on the efficiency (1 order of magnitude), analysis time, and resolution of enantiomers is achieved with the addition of sodium SDS micelles to vancomycin solutions. For most compounds studied, the enantioresolution goes through maximum (49 mM SDS). This effect can be due to the competition of SDS monomer with the analyte for the chiral selector. The mixture of three profens was separated with 30 mM SDS in half as less time than in free solution CE. Further increasing SDS concentration slightly rises the migration times. The changes in enantiomer

migration order of some analytes were observed. The same situation occurs probably with teicoplanin-based separations in micellar electrokinetic chromatography (MEKC). When it comes to the addition of SDS to a ristocetin A-based buffer, enantioselectivity can either increase or decrease. The above effects are highly variable and depend on the antibiotic type and SDS concentration.

2.5. Strategies to enhance the separation and to increase the detection sensitivity

To achieve the best enantioseparation, many parameters have to be considered. Chen et al. [18] compared the influence of all the parameters on the resolution factor of basic drugs by means of the multivariate analysis of variance as a calculation method. They found that buffer pH and CS concentration are the most significant factors to be controlled. An interesting example of experimental design methodology to optimize the resolution and migration times of a NSAID candidate (DF-1770y) is disclosed in [53]. The response surface methodology and the desirability function approach simplified the determination of optimal conditions and allowed to baseline (Rs = 2.5) enantioseparate 2-[(4′-benzoyloxy-2′-hydroxy)phenyl]propionic acid (DF-1770y) in 8 min.

As mentioned above, the strong adsorption of GA and aminoglycoside streptomycin on the capillary wall complicates the analysis. To overcome the problem, modified capillaries were suggested. Two modification ways are possible. The first one is to bond chemically the modifier on the wall (for example, poly(acrylamide) [45,51–51,57,65], poly(dimethylacrilamide) [22]). The other way is to use the dynamic coating (hexadimethrine bromide, HDB [11,42,49,50], coupled chitosan [17]).

One of the first poly(acrylamide) was introduced as a capillary modifier [66]. With over 3 weeks of stability, it is very often used [51–54,57,65,67,68]. The antibiotic adsorption is then suppressed. Kang et al. suggested HDB as a dynamic coating of the wall. In this capillary, the EOF is reversed: it is directed towards anode, as well as anions [11]. The injection form the cathode end is used. Compared with the analysis in the conventional fused-silica capillary, the migration times are reduced and its reproducibility is better. Some disadvantage of the modified capillaries sometimes could be lower enantioselectivity, which could be explained by less chiral selector–enantiomer interaction time. Fortunately, it is compensated with rapidity and high efficiency of the separation.

The coated capillaries are usually employed in the combination with partial filling technique (PFT) first reported by Valtcheva et al. [69]. The capillary is filled with CS run buffer up to the detector cell. CS and analytes are supposed to be oppositely charged and migrate in opposite directions, selector migrating away from the detector and removing background absorbance. Each enantiomer reaches the cell after passing through the selector zone. The combination of PFT with countercurrent process is often used [57,68]. The wall adsorption effects are virtually eliminated improving efficiency (a); the amount of the CS required is minimal (b); the detection sensitivity is improved (c).

In literature, there are many examples of enantiomer separation in coated capillaries showing the above advantages. For example, when short-end injection of the sample in the HDB coated capillary was used, the enantioseparation took only 2.5 min. The resolution factor was 1.86 and the number of theoretical plates was not less than 1×10^5 [49]. Ketoprofen, flurbiprofen, pirprofen, and thiaprofenic acid were separated within 5–7 min [50].

An alternative to the UV-detection is the capacitively coupled contactless conductivity detection (C^{4}D) which allows the direct determination of any charged compounds. The main advantages of this detection technique, which implies monitoring of the conductivity changes of the run buffer, are high sensitivity towards non-UV-absorbing species, simple electronic circuitry, good suitability for conventional and microchip capillary electrophoresis and also relatively low cost [70,71]. Pormsila et al. reported the enantioseparations of five aliphatic α-hydroxy acids of pharmaceutical interest (including aspartic and glutamic acids) in vancomycin-based solutions (pH 7.35) [20]. Their determination was performed using C^{4}D and no PFT was required. The analysis of the mixture (DL-α-hydroxybutyric, DL-α-hydroxycaproic, DL-α-hydroxyoctanoic, and DL-α-hydroxyoctanoic acids) took approx. 22 min.

3. Approaches to selection of the appropriate chiral selector

Having very diverse structures (macrolides, polypeptides, glycopeptides, etc.), antibiotics present a wide variety for the enantioseparations of different pharmaceutical compounds. Although the precise mechanism has not yet been unequivocally established, there is evidence that it involves inclusion into hydrophobic cavities, dipole–dipole interactions, hydrogen bonding and electrostatic or π–π interactions. This variety of potential interactions allows these selectors to enantioresolve analytes with widely different structures [72]. Some groups of separated compounds using antibiotics are shown in Fig. 1. Unfortunately, it cannot be yet predicted a priori which antibiotic would give the best resolution of the analyte. Thus, although GAs are of the same structural family, they have similar but not identical enantioselectivities. Armstrong and Nair [33] supposed that MAs are complementary to one another. Relying on the principle of the complementary separations, the studies of synergistic effect of MA on the separation were carried out [67,73]. It was found that the use of the equimolar mixture (2 mM) of vancomycin/ristocetin A allows to broaden the range of analytes (carprofen, mandelic acid, and 2-(3′-chlorophonoxy)propionic acid) and to separate multiple chiral compounds in a single analysis. It is worth noting that the compounds (e.g., carprofen), which exhibited any enantioresolution with vancomycin, always produced superior enantioseparations when using a mixture of vancomycin and ristocetin A in the run buffer vs. either chiral selector alone. For the compounds that exhibited greater enantioresolution with ristocetin A , the 4 mM ristocetin A buffer usually produced better separations than the mixed chiral selector buffer (e.g. mandelic acid) [67].

Table 1 summarizes the examples of enantioseparation of different compounds of pharmaceutical interest. For convenience, they are divided into three parts according to their ionic state in solutions. As it can be seen, to enantioseparate anionic compounds, the selectors of choice are GAs and possibly streptomycin. More chiral analytes have been resolved using this class of antibiotic (GAs) than other types of antibiotics. GAs appear to be mostly selective towards molecules with carboxylic group. It seems that ristocetin A has the broadest enantioselectivity, while teicoplanin has the fewest [58]. Ristocetin A and vancomycin are equally selective towards ketoprofen. The antineoplastic agent, methotrexate, which possesses not only two carboxylic groups but also several amino groups, was baseline resolved with ristocetin A, vancomycin, and teicoplanin. The enantioselective properties of eremomycin are largely similar to those of vancomycin. However, it was mostly selective towards flurbiprofen and indoprofen (α = 1.35). The optimal concentration of eremomycin was equal to that of ristocetin and balhimycin (2–2.5 mM). The teicoplanin analogue, hepta-tyr, was more selective towards profens than warfarin or loxiglumide.

Balhimycin and its analogues bromobalhimycin and dechlorobalhymicin were used to separate 16 anionic compounds, including ketoprofen, pirprofen, and flurbiprofen [50]. Ketoprofen, flurbiprofen, and thiaprofenic acid are resolved within 5–7 min in the run buffer containing balhimycin or bromobalhimycin.

Table 1
Enantiomeric separation of racemic compounds by CE using antibiotics as chiral selector.

Compounds	Chiral selector	Run buffer	Comments	Ref.
Acidic compounds				
Flurbiprofen	LY307599 (4 mM)	100 mM borate buffer (pH 9.2), 15% methanol		[56]
Flurbiprofen	A82846B (5 mM)	50 mM phosphate buffer (pH 7.0)		[61]
Flurbiprofen fenoprofen, ibuprofen, indoprofen, ketoprofen, suprofen	Actaplanin A (0.5 mM)	40 mM phosphate buffer (pH 5.0 or 6.0), 15–30% 2-methoxyethanol		[62]
Flurbiprofen fenoprofen, indoprofen, ketoprofen, suprofen, carprofen, cycloprofen, naproxen, warfarin, acenocoumarol, loxiglumide	Hepta-tyr (0.55–1.10 mM)	50 mM Britton-Robinson buffer (pH 5.0), 20% acetonitrile	Polyacrylamide coated capillary	[52]
Flurbiprofen, ibuprofen, indoprofen, ketoprofen, suprofen, carprofen, cycloprofen, naproxen	Vancomycin (2.5 or 5 mM)	75 mM Britton-Robinson buffer (pH 5.0)	Polyacrylamide coated capillary	[51]
Flurbiprofen, fenoprofen, ibuprofen, indoprofen, ketoprofen, suprofen, carprofen	Vancomycin (1 or 2 mM)	100 mM phosphate buffer (pH 6.0)	Polyacrylamide coated capillary	[57]
2-[(4′-Benzoyloxy-2′-hydroxy)phenyl]propionic acid	Vancomycin (7 mM)	50 mM Britton-Robinson buffer (pH 6.4)	Polyacrylamide coated capillary	[53]
Flurbiprofen, indoprofen, ketoprofen, suprofen	Vancomycin (2 mM)	50 mM phosphate buffer (pH 7.0), 21–103 mM SDS		[9]
Flurbiprofen, ibuprofen and its metabolites, ketoprofen, carprofen, naproxen, etodolac and its metabolites	Vancomycin (5 mM)	50 mM acetic acid-ammonium acetate (pH 4.8)	Polyacrylamide coated capillary	[54]
Ketoprofen	Vancomycin (2 mM)	50 mM TRIS-phosphate buffer (pH 6.0), 0.002% HDB	HDB coated capillary	[11]
Fenoprofen, ketoprofen	Vancomycin (2 mM)	50 mM TRIS-phosphate buffer (pH 6.2), 0.001% HDB	HDB coated capillary	[49]
Fenoprofen, ketoprofen	Vancomycin (2 mM)	100 mM phosphate buffer (pH 6.0)		[58]
Flurbiprofen fenoprofen, indoprofen, ketoprofen, carprofen, naproxen, iopanoic acid, iophenoxic acid, methotrexate, folinic acid (leucovorin), progulomide	Vancomycin (5 mM)	100 mM phosphate buffer (pH 7.0)		[46]
S-(−)-ofloxacin, DU-6859	Vancomycin (5 mM)	100 mM acetate buffer (pH 4.0)		[74]
α-hydroxybutyric, α-hydroxycaproic, α-hydroxyoctanoic, α-hydroxyoctanoic, aspartic, glutamic acids	Vancomycin (5 mM)	10 mM TRIS, 4.4 mM maleic acid, 0.03 mM CTAB (pH 7.35)	Contactless conductivity detection	[20]
Aspartic, glutamic acids	Vancomycin (10 mM)	10 mM sorbic acid/histidine (pH 5.0)	Polyacrylamide coated capillary	[75]
S-carboxymethylcysteine, N-acetamido-S-carboxymethylcysteine	Vancomycin (10 or 1 mM)	10 mM sorbic acid/histidine (pH 5.0)	Polyacrylamide coated capillary	[65]
Warfarine, coumachlor	Vancomycin (2 mM)	100 mM phosphate buffer (pH 7.0), 25–50 mM SDS, 0–95% methanol or 10% acetonitrile		[76]
Dimethyl diphenyl bixarboxylate and its derivatives	Vancomycin (6 mM)	40 mM TRIS-phosphate buffer (pH 6.0), 0.001% HDB	HDB coated capillary	[77]
Clofibric acid, 2-(6-chlorobenzothiazol-2-ylsulfanyl)-propionic acid, 2-(6-methoxybenzothiazol-2-ylsulfanyl)-propionic acid, 2-(quinoline-2-yloxy)-propionic acid, 2-(2-chloroquinoline-2-yloxy)-propionic acid	Vancomycin (5 mM)	25 mM acetic acid/ ammonium acetate (pH 5.0)	Polyacrylamide coated capillary	[60]
2-[(5′-Benzoyl-2′-hydroxy)-phenyl]-propionic acid, 2-[(4′-benzoiloxy-2′-hydroxy) phenyl]-propionic acid	Vancomycin (5 mM)	50 mM Britton-Robinson buffer (pH 5.0)	Polyacrylamide coated capillary	[78]
Fenoprofen, indoprofen, ketoprofen, suprofen, carprofen	Teicoplanin (2 mM)	100 mM phosphate buffer (pH 6.0), 0–10% acetonitrile		[39]
Aspartic, glutamic acids	Teicoplanin (10 mM)	10 mM sorbic acid/histidine (pH 5.0)	Polyacrylamide coated capillary	[75]
Fenoprofen, ketoprofen	Teicoplanin (2 mM)	100 mM phosphate buffer (pH 6.0)		[58]
Flurbiprofen, fenoprofen, indoprofen, ketoprofen, suprofen, carprofen, naproxen, proglumide, amephoterine, folinic acid (leucovorin)	Ristocetin A (2 mM)	100 mM phosphate buffer (pH 6.0)		[8]

Table 1 (*Continued*).

| Compounds	Chiral selector	Run buffer	Comments	Ref.
Fenoprofen, ketoprofen, methotrexate	Ristocetin A (2 mM)	100 mM phosphate buffer (pH 6.0)		[58]
Indoprofen, ketoprofen	Ristocetin A (2 mM)	100 mM phosphate buffer (pH 6.0), 25 mM SDS		[58]
Fenoprofen, ketoprofen, indoprofen, carprofen	Ristocetin A (2 mM)	100 mM phosphate buffer (pH 6.0)	Polyacrylamide coated capillary	[68]
Ketoprofen, piroprofen, flurbiprofen, tiaprofenic acid, 2-(2,4,5-trichlorophenoxy)propionic acid	Balhimycin (2 mM)	50 mM TRIS-phosphate buffer (pH 6.0), 0.002% HDB	HDB coated capillary	[50]
Ketoprofen, piroprofen, flurbiprofen, tiaprofenic acid, suprofen	Balhimycin (2 mM)	50 mM TRIS-phosphate buffer (pH 6.0), 0.001% HDB	HDB coated capillary	[79]
Ketoprofen, piroprofen, flurbiprofen, tiaprofenic acid, suprofen	Dechloromobalhimycin (2 mM)	50 mM TRIS-phosphate buffer (pH 6.0), 0.001% HDB	HDB coated capillary	[79]
Ketoprofen, piroprofen, flurbiprofen, tiaprofenic acid, suprofen	Bromobalhimycin (2 mM)	50 mM TRIS-phosphate buffer (pH 6.0), 0.002% HDB	HDB coated capillary	[79]
Ketoprofen, pirprofen, flurbiprofen, tiaprofenic acid, 2-(2,4,5-trichlorophenoxy)propionic acid	Bromobalhimycin (2 mM)	50 mM TRIS-phosphate buffer (pH 6.0), 0.002% HDB	HDB coated capillary	[50]
Fenoprofen, ketoprofen, indoprofen, surprofen, naproxen	Avoparcin (0.4 mM)	50 mM phosphate buffer (pH 6.0)		[47]
Ibuprofen, indoprofen, ketoprofen, fenoprofen, flurbiprofen	Eremomycin (2.5 mM)	50 mM phosphate buffer (pH 6.1)		[21]
Ibuprofen, indoprofen, ketoprofen, fenoprofen, flurbiprofen	Eremomycin (0.75–1.6 mM)	20 mM acetate buffer (pH 4.5–6.2), 0–0.005% chitosan	Coupled chitosan coated capillary	[17]
Carprofen, fenoprofen	Vancomycin (0–4 mM)/Ristocetin A (4–0 mM)	100 mM phosphate buffer (pH 6.0)	Polyacrylamide coated capillary	[67]
Clentiazem intermediate	Streptomycin (3%)	20 mM phosphate buffer (pH 8.1), 30% methanol	eCAP™ neutral capillary	[45]
Dimethyl diphenyl ester	Erithromycin (30 mM)	50 mM phosphate buffer (pH 6.0), 40% 2-propanol		[44]
Dimethyl diphenyl ester	Erithromycin (20 mM)	50 mM phosphate buffer (pH 6.0), 50% methanol		[59]
Basic compounds				
Alprenolol, amphetamine, epinephrine, oxprenolol, metaprolol, norepinephrine, normetanephrine, octopamine, pindolol, propanolol	Rifamycin B (25 mM)	100 mM phosphate buffer (pH 7.0), 70% 2-propanol		[43]
Atenolol, alprenolol, bemethan, ±-ephedrine, ±-ψ-ephedrine, epinephrine, isoproterenol, metanephrine, metaproterenol, metaprolol, normetanephrine, norepinephrine, norphenylephrine, octopamine, oxprenolol, salbutamol, synephrine, terbutaline	Rifamycin B (25 mM)	100 mM phosphate buffer (pH 7.0), 40% 2-propanol		[7]
Duloxetine, propranolol	Erithromycin (100 mM)	50 mM TRIS, 100 or 150 mM boric acid in methanol		[14]
Nefopam, citalopram, tryptophan methyl ester, tryptophan, metoprolol, chlorphenylamine, propranolol, atenolol	Clindamycin (60 or 80 mM)	40 mM borax buffer (pH 7.0–7.6), 20% methanol		[18]
Propranolol, chlorphenylamine, citalopram, tryptophan methyl ester, nefopam, cetirizine, tryptophan, metoprolol	Clindamycin (60 or 80 mM)	40 mM phosphate buffer (pH 7.5), 25% 2-propanol, 40 mM SDS		[15]
Neutral compounds				
Dimethyl diphenyl ester	Erithromycin (20 mM)	50 mM phosphate buffer (pH 6.0), 50% methanol		[59]
Bendroflumethiazide, 5-(4-hydroxyphenyl)-5-phenylhydantoin	Vancomycin (2 mM)	100 mM phosphate buffer (pH 7.0), 25-50 mM SDS, 0-95% methanol or 10% acetonitrile		[76]
Glutethimide, hexobarbital	Rifamycin SV (25 mM)	100 mM phosphate buffer (pH 7.0), 70% 2-propanol		[43]
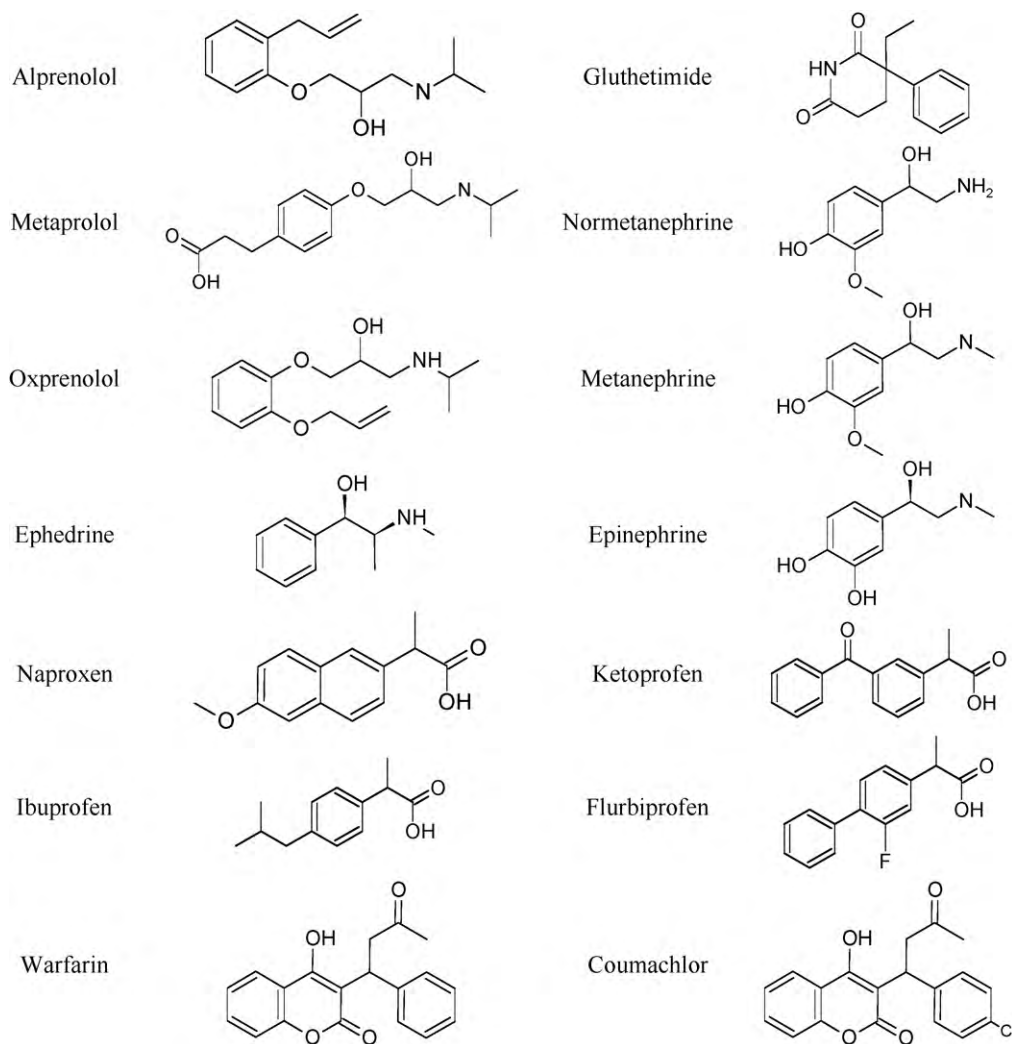
Fig. 1. Structures of selected pharmaceuticals.

For NSAIDs balhymycin showed a higher enantioresolution than dechlorobalhymycin [79]. Compared with vancomycin-mediated separations, better enantioresolutions were achieved for almost all 16 racemic compounds. However, tiaprofenic acid was better-resolved using vancomycin solution (see Fig. 2). Kang et al. supposed that the balhimycin susceptibility to dimerize plays an important role in the enantiomer recognition process [42]. The chlorine substituents in balhimycin, which are closely inserted into the cavity of the other molecule of the dimer, are believed to promote dimerization and then enantioresolution for tested analytes. It appears to be the true statement when the constants of dimerization of antibiotics are compared [41,80].

When antibacterial agents, ofloxacin and its analogues, were separated using vancomycin, it was shown that the molecular size of quinolones fit the hydrophobic pocket. Hydrogen bonding and amido linkages may occur with piperidino, keto and carboxylic groups. No enantioseparations were achieved, when carboxylic group was substituted with hydrogen. Aromatic interactions may occur with naphthyl ring of the quinolones [74]. One of the calcium channel blockers, clenthiazem intermediate, is known to be successfully separated using only streptomycin, while kanamycin sulfate and fradiomycin failed [45].

Ansamycins are applicable for enantioseparations of amine-containing compounds [7], it makes them complimentary to GA. Vasoconstrictors (epinephrine, sunephrine), bronchodilators (salbutamol), and β-blockers (pindolol, atenolol) are successfully resolved in rifamycin B and rifamycin SV media. Under usual conditions rifamycin B is anion (pK_a 2.8 and 6.7) while rifamycin SV is neutral. The former is more selective towards cationic compounds and the latter resolves better anionic and neutral analytes (e.g., glutethimide). It was noted [7], that hydroxy group in α-position to the aromatic ring enhanced the enantiorecognition. In the presence of rifamycin B as a chiral selector, the secondary amines were separated better than the primary ones (metanephrine better than normetanephrine). It was also shown that one aromatic ring is preferable (propranolol vs. pindolol). Due to the presence of the phosphate group, which allows clindamycin phosphate to be negatively charged at pH 6.6–8.0, it shows strong ionic interactions with the basic compounds, such as nefopam, metoprolol, atenolol, and others. It is obvious that the hydrogen bonding exists between the amino and hydroxyl groups in the basic drugs and the amino and hydroxyl groups in the CS. Profens and hypoglycemic agents mitiglinide and nateglinide, possessing carboxyl group, were not enantioresolved [18].

Enantioseparations of neutral analytes are possible using erythromycin lactobionate or GAs in MEKC mode. Four candidates for anti-hepatitis drugs, derivatives of biphenylmethylester, were very well resolved in phosphate buffer (pH 6.0)/ methanol mixture (50/50) [59]. At the same time Ha and co-authors completed an extensive screening (21 compounds) of the enantioselective
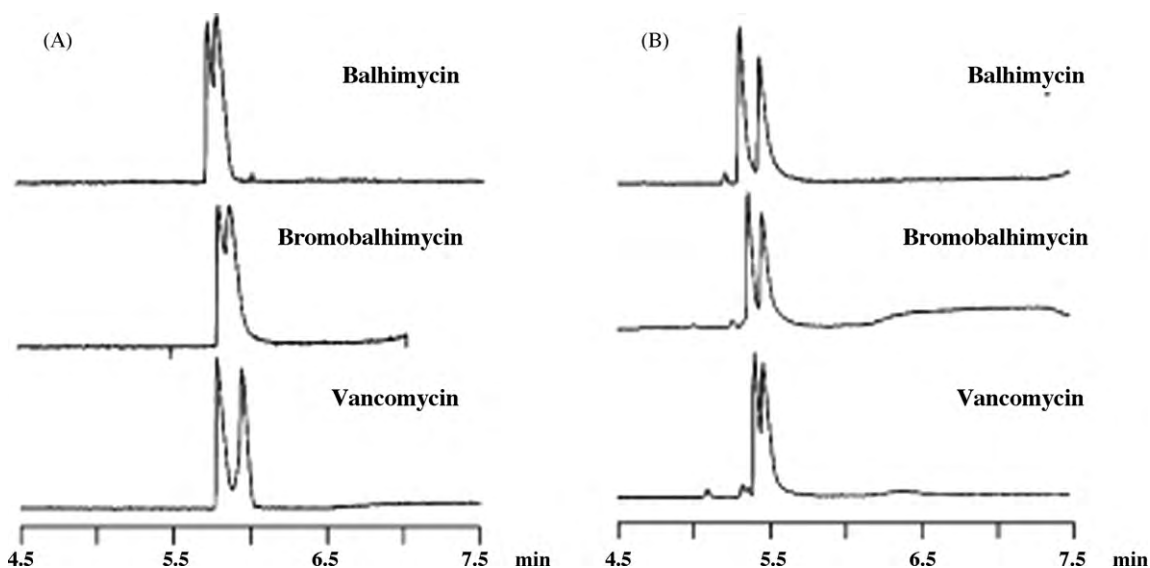
Fig. 2. Electropherograms of enantioseparation of several racemates with three different glycopeptides as chiral selectors. Conditions: 50 mM TRIS-phosphate buffer solution (pH 6.0) containing 0.001% (w/v) HDB; 2 mM balhimycin (bromobalhimycin or vancomycin): (A) tiaprofenic acid; (B) pirprofen. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Adapted with permission from Ref. [50].

properties of this CS and its five derivatives [64]. Some changes in electrophoretic mobilities with increasing erythromycin concentration were observed only for some anionic analytes (DOPA, carbidopa, ketoprofen, indoprofen, and leucovorine). Despite other optimization condition (run buffer pH, different erythromycins) no enantioseparations were achieved. Erythromycin appears to be a powerful chiral selector for enantioseparation of compounds possessing at least two benzene rings and a carboxylic group [44,59].

Therefore, the selection of a chiral selector is in first determined by the structure of the compound to be enantioseparated.

4. Pharmaceutical applications

In the review of Ha et al. of 2006 the achievements of the chiral CE for the pharmaceutical analysis are disclosed [24]. The analysis of profens, including enantiomers, in different matrices was reviewed in [81]. In the present work, the practical applications of enantioseparations of various compounds of pharmaceutical interest will be discussed. The examples of the antibiotics use for the enantiomer determination in real samples are not numerous.

Ofloxacine and DU-6859 are two fluorinated quinolones exhibiting marked bactericidal activity. S-(−)-ofloxacine (known as levofloxacine) is much more active than its racemate or enantiomer. The enantioseparation of ofloxacines and its 10 analogues were conducted in acetate buffer (pH 4.0) using vancomycin [74]. The limit of DU-6859 enantiomers quantification was 0.5%. The repeatability (RSD) was 7.1% for $c = 1.0\%$ spiked samples ($n = 6$).

The interesting example of the amino acid analysis was published by Fanali et al. [65]. The mucolitic substances S-carboxymethylcysteine (DF-1794Y) and its precursor N-acetamido-S-carboxymethylcysteine (DF-1796A) were determined in vancomycin media (10 and 1 mM, respectively) via indirect detection at 254 nm. The PFT use enabled a very fast enantioseparation; the analysis took no more than 4 min.

The authors [54] determined etodolac and its phase I metabolites (etodolacglucoronide, 7-hydroxyetodolac, etc.) as well as ibuprofen and its phase I metabolites in urine. The run buffer consisted of acetic acid/ ammonium acetate (pH 4.8) and 12.5 mM vancomycin. To achieve the adequate sensitivity and to protect the MS detector against the CS presence, the PFT was used. The analysis time was less than 15 min (15 kV). Etodolacglucoronides,

one of the glucuronides of the isomeric 7-hydroxyetodolac, and endogenous hippuric acid were observed. The described method is advantageous for peak identification, peak purity testing and for selective monitoring of overlapping peaks.

Flurbiprofen, suprofen, and naproxen can be determined in 75 mM Britton-Robinson buffer (pH 5) with 5 mM vancomycin [51] using 1-naphthalenesulfonic acid sodium salt as the internal standard. The optimized method enabled to carry out the analysis within a short period of time (6 min) and with high efficiency ($5.5–27.0 \times 10^8$). The precision of migration time and corrected peak areas of naproxen were very good (RSD, % was 0.9 and 1.80, respectively).The detection limit of flurbiprofen was 1×10^{-6} M, naproxen and suprofen – 5×10^{-6} M. The detector linearity for the analysis was studied in the concentration range $1–10 \times 10^{-5}$ M ($n = 10$). Compared to heptakis-2,3,6-tri-O-methyl-β-cyclodextrin, heptamethylamino-β-cyclodextrin vancomycin provided the same precision of migration time and corrected peak areas, and better detection sensitivity.

The method to determine loxiglumide enantiomers in the liquid pharmaceutical formulation was suggested by Fanali and co-author [82]. Vancomycin (3 mM) was used as a chiral selector and S-(−)-naproxen was the internal standard. The chiral selector filled only part of the polyacrylamide coated capillary and allowed a chiral resolution in less than 12 min using a 50 mM phosphate buffer at pH 6. The PFT allowed obtaining a detection limit of 0.5 μg/ml for each enantiomer. Good reproducibility was obtained both for the migration times and normalized peak areas (RSD for the second migrating enantiomer 1.1% and 1.87%, respectively, $n = 10$). The linearity ranges were 0.5–17.5 and 0.5–4 μg/ml for D- and L-loxiglumide, respectively.

It is sometimes useful to know the enantiomer migration order. Enantiomeric purity determination is one of the important objectives of the pharmaceutical analysis. In CE the enantiomer migration order depends on not only the affinity to the CS, but also on the run buffer pH and polarity [83]. S-(+)-ketoprofen and S-(+)-ibuprofen migrate faster than their R-(−)-enantiomer, when eremomycin containing run buffer is used in normal polarity [21]. Flurbiprofen, suprofen, and naproxen demonstrated the same migration order in vancomycin-based enantioseparation with reversal polarity [51]. It was shown that L-loxiglumide migrates slower than D-loxiglumide [82]. The study revealed that

0.2% (w/w) of L-loxiglumide can be easily detected and thus the optimized method can be successfully used for chiral purity control of D,L-loxiglumide in pharmaceutical preparations. The authors [7] demonstrated that although 20 mM rifamycin B solution significantly absorb, enantiomeric impurity determination of D-isoprotenerol (1%) in the presence of 99% L-isoprotenerol via indirect detection was possible.

5. Conclusions

The data presented are evidence of great possibilities of antibiotics as chiral selectors. To bridge over the above difficulties, the special techniques were developed and new detection systems were introduced, which allow both enantiomer quantifications and optical purity control. The number of enantioseparated compounds with different structures and properties is great. However, the necessity to enlarge the chiral selector family is still remaining because new biologically active compounds are continuously synthesized and installed. The above can be confirmed by the numerous scientific research publications devoted to the use of new antibiotics as chiral selectors.

Acknowledgments

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