ISSN 1061-9348, Journal of Analytical Chemistry, 2018, Vol. 73, No. 11, pp. 1064–1075. © Pleiades Publishing, Ltd., 2018.
Original Russian Text © E.N. Shapovalova, I.A. Fedorova, I.A. Anan'eva, O.A. Shpigun, 2018, published in Zhurnal Analiticheskoi Khimii, 2018, Vol. 73, No. 11, pp. 843–856.

REVIEWS

Macrocyclic Antibiotics as Chiral Selectors in High-Performance Liquid Chromatography and Capillary Electrophoresis

E. N. Shapovalova[a], I. A. Fedorova[a], I. A. Anan'eva[a, *], and O. A. Shpigun[a]

[a] *Department of Chemistry, Moscow State University, Moscow, 119991 Russia*

**e-mail: irishan@mail.ru*

Received March 1, 2018; in final form, May 28, 2018

Abstract—Data on the use of macrocyclic antibiotics (vancomycin, teicoplanin, teicoplanin aglycone, and eremomycin) for the enantioseparation of amino acids, various amino acid derivatives, α-phenylcarboxylic acids, β-blockers, and some pharmaceutical preparations in reversed-phase and polar-organic HPLC modes are summarized. It is shown that mixed chiral selectors (eremomycin–vancomycin, eremomycin–bovine serum albumin) combine the properties of two selectors. Eremomycin and macrolides (azithromycin, erythromycin, and clarithromycin) are successfully used as chiral selectors in capillary electrophoresis. Aqueous and aqueous–organic supporting electrolytes (**SEs**) with the addition of eremomycin or nonaqueous supporting electrolytes with the addition of a macrolide are used for enantioseparation. The use of nonaqueous supporting electrolytes decreases the adsorption of the selectors on the quartz capillary surface and enables the separation of enantiomers at a low concentration of a chiral selector. Additions of boric acid into the supporting electrolyte improve the selectivity of separation.

Keywords: chiral selectors, macrocyclic antibiotics, chiral chromatography, nonaqueous capillary electrophoresis, mixed selectors

DOI: 10.1134/S1061934818110114

Macrocyclic antibiotics are now among the most widely used chiral selectors (**CSs**), which offer the separation of enantiomers of different classes of substances by HPLC and capillary electrophoresis. A large number of original and review articles were devoted to the possibilities of such chiral selectors [1–6]. The structure of macrocyclic antibiotics typically contains several chiral centers, various functional groups (amino acids, *N*-substituted amino acids, small peptides, α-hydroxycarboxylic acids) and three or four cavities (cyclic amides or neutral cyclic amines). Such a structure ensures multiple interactions of an antibiotic with a chiral analyte via hydrogen bonds, π–π interactions, a variety of dipole, electrostatic, and hydrophobic interactions (hydrophobic inclusion complexes or associates with a hydrophobic "pocket"), as well as steric hindrance, leading to high enantioselectivity [7, 8].

Use of macrocyclic antibiotics for the separation of enantiomers in HPLC. The first works on the use of glycopeptide antibiotics for chiral separation were performed by Armstrong et al. and published in 1994 [9, 10]. The use of three chiral selectors (vancomycin, rifamycin B, and thiostrepton) covalently bound to the stationary silica phase in HPLC was described. These antibiotics showed high enantioselectivity to various compounds in both reversed-phase (**RP**) and normal-phase (**NP**) chromatography modes. The authors separated enantiomers of 70 compounds, including the enantiomers of amino acids, their dansyl, carbobenzoxy (**CBZ**), and benzyl derivatives, β-blockers, and lactones. Later, macrocyclic antibiotics of teicoplanin and teicoplanin aglycone were used as chiral selectors [11]. We also investigated the enantioseparation using macrocyclic antibiotics teicoplanin (a Chirobiotic T column), teicoplanin aglycone (a Chirobiotic Tag column), and vancomycin (a Chirobiotic V column) [12, 13]. Macrocyclic antibiotics exhibit high chiral activity in RP, polar-organic (**PO**), and NP modes of liquid chromatography. The selection of an operating mode is determined by the nature of the substances to be separated [12].

One of the essential problems in many areas of chemistry, biology, medicine, and pharmacology is the separation of isomers of optically active amino acids. The regularities of the retention of amino acid derivatives on the chiral stationary phases of Chirobiotic T, Chirobiotic Tag, and Chirobiotic V in reversed-phase chromatography (Table 1) were studied [13]. It is known that for most enantiomeric separations in the reversed-phase mode, retention and selectivity are mainly determined by the concentration and nature of the organic solvent and the pH value of the mobile phase. The nature and concentration of the buffer solution used have a smaller effect. It is found that, for all amino acid derivatives, the L-isomer is eluted first.

It was possible to separate 39 amino acid derivatives using a Chirobiotic T column, 32 of them with a resolution of $R_S > 1.5$. Using a Chirobiotic Tag column, 18 enantiomers of amino acid derivatives were separated, and 16 of them were separated with $R_S > 1.5$. Enantiomers of 20 derivatives were separated using a Chirobiotic V column; the separation was incomplete. Thus, teicoplanin is the most successful chiral selector with respect to amino acid derivatives. Among the compounds studied, the highest selectivity of separation with the Chirobiotic T column was achieved for CBZ, 2,4-dinitrophenyl (DNP), *N*-9-fluorenylmethoxycarbonyl (FMOC), and benzoyl derivatives of amino acids; it was from 1.95 for CBZ-DL-tryptophan to 4.53 for CBZ-DL-alanine, from 2.11 for DNP-DL-norleucine to 3.46 for DNP-DL-methionine, from 2.25 for FMOC-DL-valine to 3.36 for FMOC-DL-norleucine, and from 1.41 for benzoyl-DL-arginine to 3.20 for benzoyl-DL-valine [12, 13].

One of undeniable advantages of macrocyclic antibiotics is a possibility of the separation of amino acids without preliminary derivatization [14]. The retention and separation of the optical isomers of amino acids on chiral stationary phases with teicoplanin (Chirobiotic T, Chirobiotic Tag) and vancomycin (Chirobiotic V) was studied using mixtures of acetonitrile, methanol, ethanol, isopropanol, and a buffer solution of triethylamine acetate or water as mobile phases. It was found that, for all amino acids, the L-isomer is eluted first. It turned out that the column with vancomycin is not selective for D,L-isomers of amino acids; the other two columns yielded the complete separation of enantiomers of amino acids. It is found using Chirobiotic T as an example that a decrease in the acetonitrile concentration in the mobile phase from 20 to 2 vol % leads to a slight increase in the retention times of amino acids and does not affect the selectivity of the separation of their enantiomers or the efficiency of the column. The best results were obtained with an acetonitrile concentration of 5 vol %; a further decrease in its concentration led to the deterioration of the efficiency of the column and its resolving power. The replacement of acetonitrile with methanol or ethanol ensures an increase in the selectivity of separation of the enantiomers of most of the amino acids studied. The complete separation of a larger number of amino acid enantiomers is achieved when eluting with a mixture of ethanol and water in a volume ratio of 1 : 1.

The difference in the structures of macrocyclic antibiotics of teicoplanin and its aglycone significantly affects the chiral properties of the column. Using the Chirobiotic Tag column, it was possible to separate 20 amino acids with complete resolution, while the Chirobiotic T column enabled the separation of only 16 amino acids, and only 10 of them were resolved entirely (Table 2). This may be because the sugar residues create steric hindrances and block chiral recognition sites, which limits the access of other molecules to them.

The Russian antibiotic eremomycin has been studied as a chiral selector to a lesser extent. The structures of eremomycin and vancomycin molecules differ in the number of chlorine atoms (one in the eremomycin molecule and two in the vancomycin molecule). There is an additional carbohydrate residue with a primary amino group in the eremomycin molecule, eremosamine (4-epivancosamine), which differs from the analogous vancomycin residue in the disaccharide branch by the reverse configuration of the C4 carbon atom [15]. A chiral adsorbent for HPLC with eremomycin as a chiral selector was synthesized for the first time in 2006 [16]. It is a silica bearing epoxy groups with eremomycin bonded to them. The synthesized adsorbent exhibited a high ability for enantiomeric recognition of amino acids (especially acids containing an aromatic moiety or imino group) (Table 2) using aqueous methanol eluents containing various buffer solutions or addition of acetic acid [17, 18].

Using chiral stationary phases with eremomycin, it is possible to separate the enantiomers of not only nonderivatized amino acids but also dansyl, CBZ, benzoyl, and *tert*-butoxy (**BOC**) derivatives of amino acids; for all amino acid derivatives, the L-isomer is eluted first. The dansyl derivatives are most long retained on silica modified with eremomycin; therefore, their enantiomers were separated in the polar-organic mode upon elution with methanol with additions of acetic acid and triethylamine [19]. The enantiomers of the remaining derivatives were separated under RP HPLC conditions upon elution with mixtures of methanol or isopropanol and a phosphate buffer solution with pH 7.0 and 8.0 [20]. The concentration of methanol and isopropanol was varied from 1 to 30 vol %. It was found that for the same amino acid when the structure of the derivative (in the series of BOC > benzoyl > CBZ) becomes more complicated, its retention increases, and its resolution and selectivity of the peaks improve. Among all the amino acid derivatives studied, the CBZ derivatives are best retained under identical conditions, which is due to the presence of a benzene ring and a carbonyl group in their structure that ensure the strongest interaction of the adsorbate with eremomycin. The aromatic part of the molecules of the derivatives is responsible for the formation of π–π complexes between the adsorbate and the stationary phase containing π electrons and double-bond systems, and the presence of the group >C=O creates a possibility of additional π–π and dipole interactions and hydrogen bonding.

α-Phenylcarboxylic acids (α-hydroxyphenylacetic (mandelic), α-methoxyphenylacetic, 2-phenylpropionic, and 2-phenylbutanoic acids) are the class of substances, the enantiomers of which can be separated using a chiral adsorbent with eremomycin. A mixture of acetonitrile and a phosphate buffer solution with

Table 1. Separation of the enantiomers of amino acid derivatives in columns with macrocyclic antibiotics [12–14]

Substance*	Chirobiotic T	Chirobiotic Tag	Chirobiotic V
Dansyl-DL-valine	+	–	+
Dansyl-DL-norvaline	+	–	+
Dansyl-DL-leucine	+	+	+
Dansyl-DL-norleucine	+	–	+
Dansyl-DL-serine	+	–	+
Dansyl-DL-α-aminobutyric acid	+	–	–
Dansyl-DL-phenylalanine	+	+	+
Dansyl-DL-tryptophan	+	–	–
CBZ-DL-leucine	+	+	–
CBZ-DL-norleucine	+	+	+
CBZ-DL-alanine	+	+	+
CBZ-DL-valine	+	+	–
CBZ-DL-methionine	+	+	–
CBZ-DL-lysine	+	+	–
CBZ-DL-tyrosine	+	+	+
CBZ-DL-phenylalanine	+	+	+
CBZ-DL-tryptophan	+	+	+
Benzoyl-DL-valine	+	+	–
Benzoyl-DL-alanine	+	+	+
Benzoyl-DL-phenylalanine	+	+	+
Benzoyl-DL-arginine	+	–	+
Benzoyl-DL-methionine	+	–	+
FMOC-DL-valine	+	–	–
FMOC-DL-leucine	+	–	–
FMOC-DL-norleucine	+	–	–
FMOC-DL-methionine	+	–	–
FMOC-DL-tryptophan	+	–	+
Phthalyl-DL-valine	+	–	–
Phthalyl-DL-methionine	+	–	–
DNP-DL-norvaline	+	+	–
DNP-DL-norleucine	+	+	–
DNP-DL-methionine	+	+	–
DNP-DL-ethionine	+	+	–
OPA-DL-leucine	+	–	–
OPA-DL-phenylalanine	+	–	–
OPA-DL-tyrosine	+	–	+
OPA-methyl-DL-tryptophan	+	–	–
OPA-DL-glutamine	+	–	–
OPA-DL-arginine	+	–	–
OPA-DL-asparagine	+	–	–
OPA-DL-histidine	+	–	–

* Dansyl, 5-dimethylaminonaphthalene-1-sulfonyl; FMOC, *N*-9-fluorenylmethoxycarbonyl; DNP, 2,4-dinitrophenyl; OPA, *o*-phthalic aldehyde.

Table 2. Separation of the enantiomers of amino acids in columns with macrocyclic antibiotics [12, 16, 17]

Substance	Chirobiotic T	Chirobiotic Tag	Chirobiotic V	Nautilus-E
Phenylalanine	+	+	–	+
Valine	+	+	–	+
2,4-Dihydroxy-phenylalanine	+	+	–	+
Tryptophan	+	–	–	+
Norvaline	+	+	–	+
Arginine	+	+	–	–
Isoleucine	+	+	–	+
Isoserine	+	+	–	+
Leucine	+	+	–	+
Methionine	+	+	–	+
Alanine	+	+	–	+
Tyrosine	+	+	–	+
Asparagine	+	+	–	–
Aspartic acid	+	+	–	–
Lysine	+	+	–	+
Glutamic acid	+	+	–	–
Threonine	+	+	–	+
Norleucine	–	+	–	+
Histidine	–	+	–	–
Glutamine	–	+	–	–
Serine	+	+	–	+

pH 4.7 (30 : 70 vol) was used as an eluent. The retention of these acids on the adsorbent increases in the series 2-phenylpropionic acid < α-methoxyphenylacetic acid < mandelic acid as the CH_3O– and OH– polar groups appear in the α-methoxyphenylacetic and mandelic acids, respectively, which can interact with the active sites of the antibiotic. The best resolution values were obtained for α-methoxyphenylacetic acid, which is explained by the peculiarities of its structure: each of the substituents (C_6H_5–, –COOH, CH_3O–) is in its plane [21].

Macrocyclic antibiotics are widely used to separate the optical isomers of various pharmaceutical preparations and to assess their optical purity [22, 23]. The enantioseparation of profens and β-blockers has been studied in detail. Profens, that is, nonsteroidal anti-inflammatory preparations, are commonly used in the form of a racemic mixture, but only one of the enantiomers has therapeutic activity. The enantioseparation of profens using vancomycin, eremomycin, eremosaminyl aglycone, and ristomycin A was studied in [24, 25]. Fenoprofen, ibuprofen, indoprofen, ketoprofen, and flurbiprofen were used as test compounds. The study was carried out in two chromatographic modes: reversed-phase (water–methanol mobile phases) and polar-organic (methanol with additions of triethylamine and acetic acid). Eremomycin exhibited higher enantioselectivity with respect to profens. The enantioseparation of β-blockers is possible using teicoplanin and vancomycin as chiral selectors [26, 27].

Recently, the number of chiral preparations, separated with the help of eremomycin, was increased. The enantiomers of pemetrexed, levalbuterol, *N*-acetyl-D,L-glutamic acid, 8-(4-((4-chlorobenzyl)oxy)phenyl)-3-(2,4-dimethoxyphenyl)-6-oxo-2,3,4,6,7,8-hexahydropyrido[2,1-b][1, 3, 5]thiadiazene-9-carbonitrile, and 5-(4-chlorophenyl)-*N*-((5-(hydroxymethyl)-4-methylthiazol-2-yl)-(piperidin-2-yl)methyl)-1*H*-pyrrole-2-carboxamide are separated in a Nautilus-E column packed with silica, modified with eremomycin [21, 28–30].

Pemetrexed (*N*-[4-[2-(2-amino-4,7-dihydro-4-oxo-1*H*-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamicacid acid) is a preparation that belongs to the pharmacological group of antimetabolites. It is prescribed for malignant pleural mesothelioma and for non-small-cell lung cancer. The active substance of the preparation is the L-isomer of pemetrexed; therefore, its D-isomer should be separated. The enantioseparation of this preparation was achieved by reversed-phase chromatography [28]. A mixture of ammonium dihydrogen phosphate and organic solvents (methanol, acetonitrile) was used as the mobile phase. It was found by varying the nature of the organic solvent that partial or complete replacement of methanol with acetonitrile in the mobile phase in the

reversed-phase mode leads to a slight decrease in the resolution of the peaks but strongly affects the retention of substances, which enables optimizing the duration of the analysis, thus obtaining chromatograms with a good resolution of the peaks. It is known that the enantioselectivity of eremomycin is higher at low pH values; therefore, a solution of ammonium dihydrogen phosphate with pH 2.5–4.5 was used as an aqueous component of the mobile phase. As the retention of the substance increases with increasing pH, buffer solutions with pH 2.5 were used, at which an acceptable resolution (~2.5) is reached in less than 15 min. The best separation of the optical isomers was obtained by elution with a mobile phase of methanol–acetonitrile–50 mM $NH_4H_2PO_4$ with pH 2.5 (55 : 15 : 30 vol), with $R_S = 2.7$ and the analysis time not exceeding 10 min.

The limit of detection of the compound, calculated from the signal-to-noise ratio = 3 : 1, was 0.0003 mg/mL, which corresponds to 0.12% of the D-form from the total amount of the preparation. The results helped to determine the enantiomeric purity of the pemetrexed substance.

Levalbuterol is an R-isomer of albuterol and is used as a bronchodilator. The possibility of the separation of the enantiomers of albuterol was studied in the polar-organic and reversed-phase modes [29]. Successful separation was attained in the polar-organic mode. The mobile phases contained organic solvents (methanol, acetonitrile) with additions of acids (acetic, formic, or trifluoroacetic) and bases (triethylamine (**TEA**), tributylamine (**TBA**), or diethylamine (**DEA**)]) The effect of the methanol-to-acetonitrile ratio in the mobile phase on the enantioseparation was investigated; the maximum resolution of the enantiomeric peaks was observed at the methanol-to-acetonitrile volume ratio of 20 : 80. The addition of an acid and a base to the mobile phase increases the selectivity of separation of the enantiomers; the best peak separation is achieved using TEA and acetic acid. The best resolution of the peaks ($R_S = 0.9$) of the albuterol enantiomers using the adsorbent containing eremomycin was obtained for the mobile phase acetonitrile–methanol–TEA–acetic acid (80 : 20 : 0.075 : 0.025 vol) and a flow rate of 0.5 mL/min. The adsorbent modified with eremomycin did not provide the necessary resolution of the albuterol peaks ($R_S \geq 1.5$), according to the requirements of the Pharmacopoeia. For the complete separation of the albuterol enantiomers, a commercial Chirobiotic Tag column was used upon elution with a mobile phase of acetonitrile–methanol–TEA–acetic acid (90 : 10 : 0.05 : 0.05 vol) at a flow rate of 1 mL/min.

An urgent problem of modern medicine is the creation and study of new neurotropic preparations that can adequately protect the brain. A new preparation based on the *oxypyridinium-N-acetyl-L-glutamate derivative* was developed. The enantioseparation of *N*-acetyl-D,L-glutamic acid was carried out using a Nautilus-E column in the reversed-phase mode; the L-isomer was eluted first. To select the separation conditions, the nature and concentration of the organic solvent (20–40 vol %), the concentration of a NaH_2PO_4 solution (0.04–0.10 M), the pH of the solution (2.5–4), and the flow rate of the mobile phase were varied. The enantiomers were separated with a resolution higher than 2.5 for 16 min upon elution with the mobile phase of acetonitrile–0.10 M NaH_2PO_4 (pH 3) (30 : 70 vol). The limit of detection for the compound (the signal-to-noise ratio = 3 : 1) was 0.0003 mg/mL, which corresponds to 0.14 wt % of the D-form from the total amount of the preparation (the solution concentration, 2 mg/mL). According to the regulations, the concentration of the D-form the preparation should not exceed 0.2 wt %.

Pyridothiadiazine [8-(4-((4-chlorobenzyl)oxy)-phenyl)-3-(2,4-dimethoxyphenyl)-6-oxo-2,3,4,6,7,8-hexahydropyrido[2,1-b] [1, 3, 5]thiadiazene-9-carbonitrile] is a new preparation against tick-borne encephalitis virus. The enantioseparation of this preparation was achieved by reversed-phase chromatography [21]. Mixtures of an aqueous solution of ammonium dihydrogen phosphate or acetic acid with methanol were used as mobile phases; the better separation was obtained with a methanol concentration of 30 vol %. Replacing the ammonium dihydrogen phosphate solution with a 0.5 M acetic acid solution (pH 2.5) resulted in an improvement in the resolution of the peaks. The best resolution ($R_S = 0.73$) was obtained with a mobile phase of methanol–0.5 M acetic acid (pH 3.5) (70 : 30 vol).

5-(4-Chlorophenyl)-N-((5-(hydroxymethyl)-4-methylthiazol-2-yl)-(piperidin-2-yl)methyl)-1H-pyrrole-2-carboxamide is a new preparation for the treatment of systemic diseases. The R,R- and S,S-isomers of this substance were separated using as a chiral selector of eremomycin in the polar-organic mode upon elution with a mixture of methanol and isopropanol with a TEA addition [30]. The complete separation of the isomers was achieved in the case of the mobile phase of methanol–isopropanol–TEA (70 : 30 : 0.2 vol).

It was noted previously [17] that a chiral adsorbent with eremomycin enables the separation of the enantiomers of nonderivatized amino acids but is not selective for β-blockers. At the same time, a chiral adsorbent with vancomycin (a commercial Chiroboitic V column) [25] is used to separate the enantiomers of β-blockers but does not separate isomers of amino acids. It can be assumed that a chiral adsorbent with two macrocyclic antibiotics—vancomycin and eremomycin—offers the separation of the enantiomers of both β-blockers and amino acids using a single column. A mixed chiral adsorbent with vancomycin and eremomycin was synthesized [21, 31], and the separation of the enantiomers of some β-blockers (metoprolol, pindolol, alpenolol, oxprenolol, labetolol, and atenolol) and amino acids (tryptophan, phenylalanine, 3,4-dihydroxyphenylalanine, methionine,

acetylglutamic acid, alanine, norvaline, valine, lysine, arginine, and serine) was studied. Acetonitrile, methanol, isopropanol, and a buffer solution containing acetic acid and TEA were used as components of the mobile phase.

β-Blockers were eluted with a mixture of acetonitrile, methanol, and a triethylamine acetate (**TEAA**) buffer solution. The volume fraction of the buffer solution was varied in the range of 1–5 vol %, and its concentration was changed within 0.01–0.20 vol %; the volume methanol-to-acetonitrile ratio in the mobile phase was from 10 : 90 to 20 : 80. The mobile phase of methanol–TEAA (0.5%, pH 5) (90 : 10 vol) proved to be the most successful for the commercial column [32], while for the adsorbent with two antibiotics, the mobile phase methanol–acetonitrile–TEAA (0.1%, pH 4.5) (19 : 78 : 3 vol) is the most suitable. Under these conditions, the analyte retention factor for the synthesized adsorbent with two antibiotics is smaller, which decreases the analysis time. The enantioseparation factor of β-blockers for the new adsorbent is also higher (1.10–1.20); the separation of the metoprolol enantiomers (R_S = 1.2) turned out to be the best.

The amino acids were eluted with a mixture of organic modifiers (acetonitrile, methanol, isopropanol) and solutions of acetic acid or potassium dihydrogen phosphate. The volume fraction of the organic modifier varied from 0.5 to 10 vol % when potassium dihydrogen phosphate was used as the buffer solution, and from 1 to 20 vol % when using a solution of acetic acid. A proper resolution of the peaks ($R_S > 2$) was obtained for tryptophan and phenylalanine containing the indole and benzene rings, respectively, and for methionine containing a sulfur atom. Unlike the column packed with silica modified only with eremomycin, it was not possible to separate the enantiomers of amino acids that do not contain aromatic fragments, such as alanine, valine, serine, lysine, arginine, and norvaline. They were practically not retained in the new column, which may be due to a smaller contribution of electrostatic interactions to the retention and separation of enantiomers on the adsorbent with two chiral selectors [31].

A binary adsorbent containing eremomycin and bovine serum albumin (**BSA**) was synthesized. Its properties were studied by the example of amino acid derivatives, profens, and benzoin under the conditions of RP HPLC [21, 33]. Mixtures of methanol with a phosphate buffer solution were used as the eluent. Polar-organic chromatography was not used because of the possibility of denaturing the protein on the surface of the adsorbent under such conditions. The pH, concentration of the phosphate buffer solution, and volume fraction of methanol in the eluent were varied to select the conditions for the separation of the enantiomers. The enantiomers of profens and amino acid derivatives can be separated using the binary chiral adsorbent with eremomycin and BSA under the same conditions as for the silica-eremomycin adsorbent; the patterns of change in the chromatographic parameters depending on the composition of the mobile phase for both adsorbents are similar. The presence of a protein on the surface of the mixed adsorbent slightly affects the resolution of the peaks of enantiomers, decreasing the retention of substances. Probably, eremomycin plays the crucial role in enantiorecognition. This is probably because the protein, unlike eremomycin, is fixed on the surface of the adsorbent by means of physical adsorption, and its concentration is smaller. However, BSA plays an important role in the determination of enantiomers in biological fluids. The size of BSA molecules exceeds the pore size of silica , which makes the pores inaccessible for the penetration of other large molecules. It can be assumed that large molecules (for example, other proteins) in the solution being analyzed do not interfere with the determination of enantiomers when using such an adsorbent. Using a column with the mixed chiral adsorbent, the separation of the racemic mixture of ketoprofen and a marker protein was studied. All proteins with a molecular weight ranging 13.7–669 kDa were weakly retained in the column and did not interfere with the separation of the ketoprofen enantiomers (Fig. 1).

Use of macrocyclic antibiotics for the separation of enantiomers by capillary zone electrophoresis. Macrocyclic antibiotics are widely used as chiral selectors and in capillary electrophoresis (**CE**) [34–36]. In a number of studies, antibiotics have proved to be useful for the separation of enantiomers of various substances, including pharmaceutical preparations [37–39]. The main factors affecting the enantioseparation in CE are the composition and pH of the buffer solution, the concentration of added chiral selector, the addition of an organic modifier, and the effect of micelle formation when surfactants are added to the supporting electrolyte.

Depending on the pH of the solution, antibiotics (eremomycin, vancomycin, etc.) can be positively or negatively charged. At pH < 6–7, they are positively charged and adsorbed on the surface of the quartz capillary, changing its charge. In the case of enantioseparations involving glycopeptide antibiotics, the reversal of the electroosmotic flow (**EOF**) and the change in the direction of movement of the analytes take place due to adsorption interactions with the capillary walls, causing the low efficiency of separation. Several approaches can be proposed to achieve enantioseparation under the conditions of the strong adsorption of the chiral selector. The most straightforward method is to perform separation by applying external pressure. Dynamic or covalent surface modification with hydrophilic compounds, including a chiral selector, can also be a solution to the problem.

The scientists the Department of Chemistry of Moscow State University were the first who used ere-

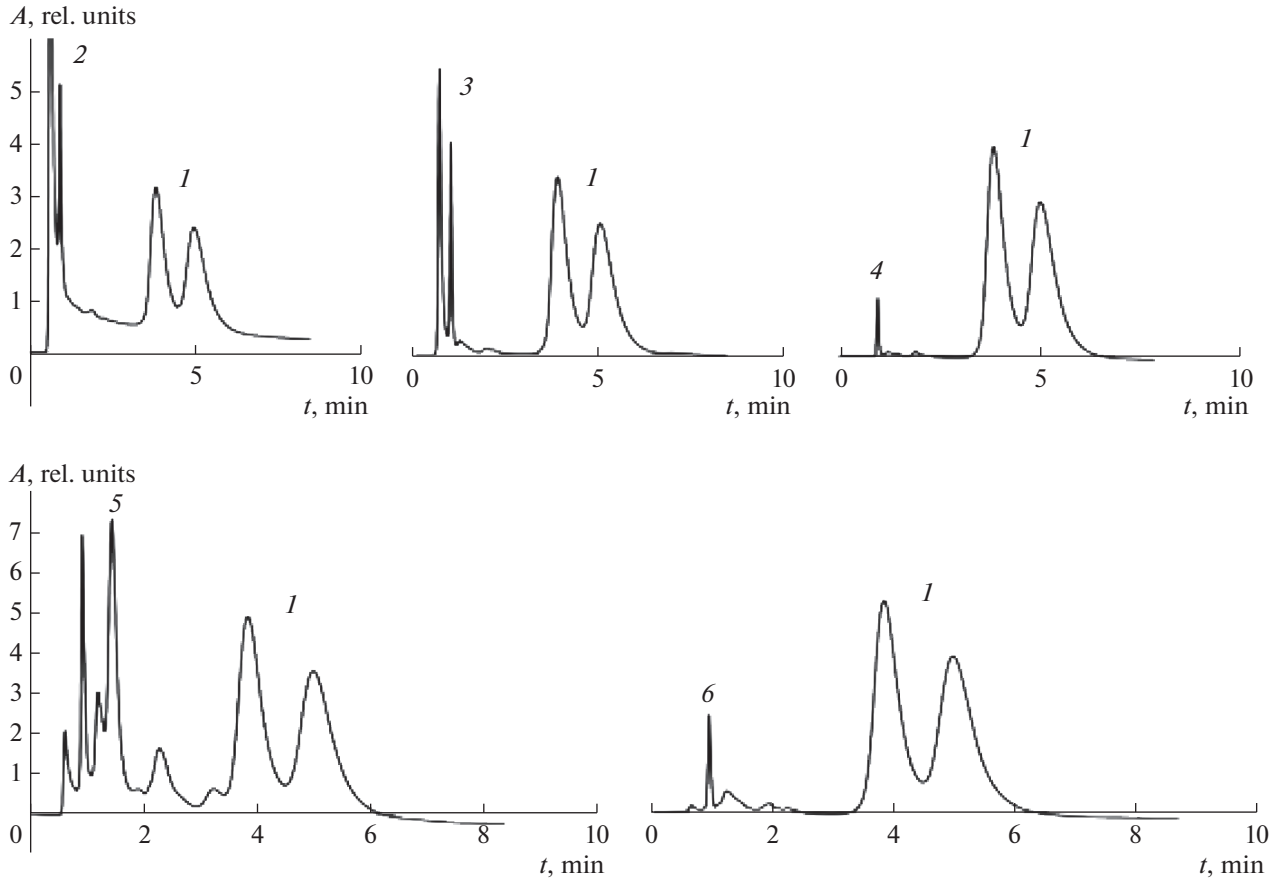


Fig. 1. Separation of ketoprofen enantiomers using silica modified with eremomycin and BSA in the presence of proteins [48]: (*1*) ketoprofen, (*2*) ribonuclease, (*3*) ovalbumin, (*4*) aldolase, (*5*) ferritin, and (*6*) thyroglobulin.

momycin as a chiral selector in capillary electrophoresis [40–42]. According to chiral HPLC, eremomycin has high enantioselectivity to α-amino acids and their derivatives and carboxylic acids. Enantioseparation with an applied external pressure (10 mbar) was studied using ibuprofen, indoprofen, ketoprofen, fenoprofen, flurbiprofen; dansyl derivatives of threonine, leucine, and phenylalanine; and CBZ derivatives of aspartic acid and alanine. It is shown that the optimal pH value for profens and amino acid derivatives is different, that is, 6.2 and 7.1, respectively, and the optimal applied voltage is 10 kV The best separation of enantiomers is achieved with an eremomycin concentration of 2.5 mM in 100 mM (for dansyl amino acids) or 50 mM (for profens) in a phosphate buffer solution; the separation of CBZ amino acids occurs at a concentration of 1 mM of eremomycin. The addition of various organic modifiers (methanol, acetonitrile, 18-crown-6) to the supporting electrolyte does not improve the separation. Unfortunately, the duration of the analysis in CE under pressure is long (up to 60 min), and the efficiency does not exceed 20000 theoretical plates per meter [42].

Enantiomers of mandelic, α-methoxyphenylacetic, 2-phenylpropionic, and 3-phenylbutanoic acids and profens were separated in capillaries dynamically modified with eremomycin [43]. For this purpose, the quartz capillary was washed with a 50 mM phosphate buffer solution (pH 6.1) containing 2.5–5.0 mM of eremomycin for 30 min. As the direction of the EOF changes, the separation was carried out under reverse polarity, applying a voltage of 10–15 kV. Buffers prepared from potassium hydrogen phosphate, 2-(*N*-morpholino)ethanesulfonic acid, tris(hydroxymethyl)aminomethane (TRIS) and containing 5 mM of eremomycin were used as the supporting electrolytes. The best reproducibility of the EOF is observed in a TRIS–phosphate buffer solution (RSD = 0.8%). This supporting electrolyte offers high efficiency and excellent separation (R_S > 2.3) of a larger number of compounds in less than 15 min. Unlike the CE separation under pressure, in a capillary that is dynamically modified with eremomycin, the enantiomers of several profens, for example, flurbiprofen and ketoprofen, can be simultaneously separated [41].

The dynamic modification of the quartz capillary surface with chitosan made it possible to create a positive charge on it by the protonation of amine groups of the modifier. To enhance the stability of the coating, two layers of chitosan were crosslinked with glutaraldehyde. Analytes (aromatic carboxylic acids and profens) were separated in 20 mM buffer solutions with pH 4.5–6.0 at a voltage of –10 kV. Compared with the separation in a quartz capillary under pressure, the modification of the capillary with crosslinked chitosan enabled the use of more dilute supporting electrolytes with a much smaller addition of chiral selector. Only 0.75 mM of eremomycin in the supporting electrolyte was sufficient to separate the enantiomers of indoprofen and flurbiprofen to the baseline, while the resolution of the ketoprofen enantiomers was 1.4. An increase in the concentration of eremomycin in the supporting electrolyte from 0.75 to 1.6 mM led to an increase in the migration times. The absence of external pressure and, consequently, a flatter flow in the capillary decreased the broadening of the zone and led to higher efficiency. The reversal of the EOF enabled the separation of the enantiomers of the substances under study in 7–8 min [44, 45].

Covalently immobilized coatings prepared using 3-aminopropyltrimethoxysilane and 3-glycidoxypropyltriethoxysilane [46] are more stable than dynamic coatings. The capillary walls were modified with 3-aminopropyltrimethoxysilane similarly to the amination of silica using aqueous organic media at room temperature. To increase the density and stability of the coating, the procedure was repeated twice; the amino acid layers were crosslinked with glutaraldehyde. Direct covalent immobilization of eremomycin on the capillary surface is impossible; therefore, 3-glycidoxypropyltriethoxysilane was used as a crosslinking agent. When the capillary is modified, the surface charge does not change. In the separation of the enantiomers of aromatic carboxylic acids and profens, a 20 mM acetate buffer solution containing (0.25–0.75) mM of eremomycin served as a supporting electrolyte; the applied voltage was –20 kV. Comparison of the used capillaries shows that modification of the surface significantly decreases the migration times of the analytes. Under these conditions, the enantiomers of mandelic and 3-phenylbutanoic acids could not be separated, but a good separation of the enantiomers of all profens studied was obtained. The selectivity of the separation of enantiomers is maximal with a capillary covalently modified by 3-aminopropyltrimethoxysilane with immobilized eremomycin (Fig. 2). Modification of the quartz capillary surface increased the efficiency, which offered the possibility of working with short (35 cm) capillaries and separating the enantiomers in a short time (less than 15 min). The advantages of such capillaries also include a higher reproducibility of migration times. Unfortunately, the amount of epoxysilane-immobilized eremomycin was not sufficient to separate the enantiomers of the compounds studied without the addition of the chiral selector to the background electrolyte, but an addition of 0.10–0.75 mM of the chiral selector into the supporting electrolyte is enough for the enantioseparation of profens.

The application of the developed approaches for the separation of enantiomers and the determination of biologically active compounds in CE under pressure using a capillary dynamically modified with eremomycin and in capillary electrochromatography with capillaries covalently modified with 3-aminopropyltrimethoxysilane with immobilized eremomycin was demonstrated by the example of the determination of the active components of the pharmaceutical preparations, that is, Ibuprofen, Strepfen, and Warfarin tablets and Bystrum-gel preparation (Table 3) [46].

The use of an organic solvent in the supporting electrolyte decreases adsorption by decreasing the dissociation of the silanol groups of the quartz surface and the selector molecules [41, 47]. The separation of profens in the presence of eremomycin in water–methanol supporting electrolytes with the mixtures of methanol and a 50 mM phosphate buffer solution (pH 4.8, 5.8, and 7.3) with various additions of eremomycin (0.5–2.0 mM) was studied. The methanol concentration in the supporting electrolyte was varied in the range of 0–70 vol %. The addition of more than 70 vol % of methanol into the supporting electrolyte is not possible due to the limited solubility of eremomycin. For all profens studied, enantioseparation to baseline was achieved with the eremomycin concentration of 2 mM. The resolution of the peaks of enantiomers decreases in the series of flurbiprofen > indoprofen > ketoprofen > fenoprofen. Even with a selector concentration of 0.5 mM, the separation of the enantiomers of flurbiprofen and indoprofen is achieved almost to the baseline. The advantages of the supporting electrolytes with the addition of methanol are a smaller generated current and, as a result, a smaller amount of Joule heat released (which increases the stability of chiral selector), less adsorption of eremomycin (which improves the reproducibility of the migration times and peak areas), and stability of the baseline. Under optimal conditions, the analysis time does not exceed 17 min [47].

In nonaqueous CE, macrolides (erythromycin, clarithromycin, and azithromycin) were also studied as chiral selector for the separation of enantiomers of various types (basic, acidic, neutral). In the case of these antibiotics, enantioseparation is achieved only for the basic compounds, that is, amines and amino alcohols. In nonaqueous supporting electrolytes, better results were obtained than in those containing water. Methanol, acetonitrile, and their mixture are selected as solvents because they have an excellent dissolving power and a low transparency limit in the UV region. In the study, the enantiomers of alprenolol,

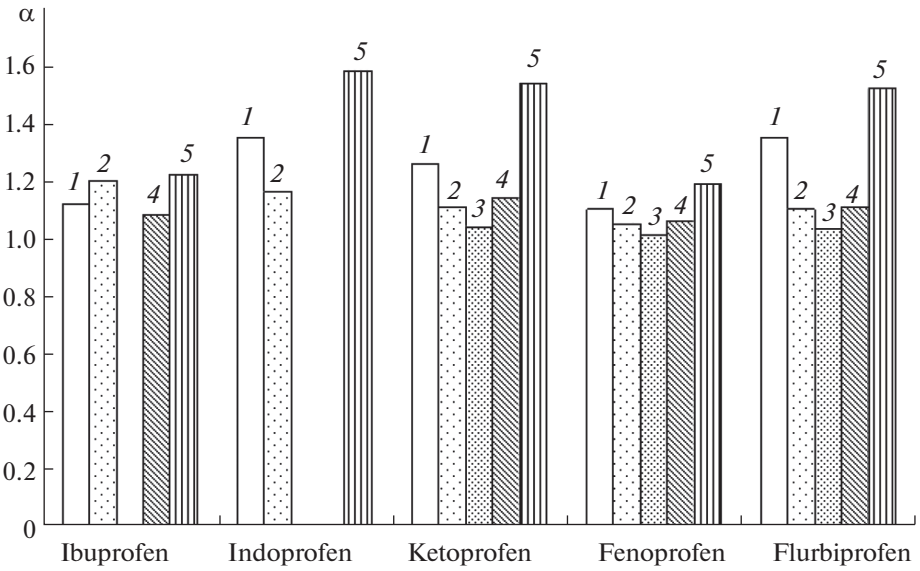


Fig. 2. Comparison of the selectivity of profen separation using various capillary electrophoresis modes: (*1*) unmodified capillary, (*2*) capillary modified with crosslinked chitosan, (*3*) aminated capillary, (*4*) capillary dynamically modified with eremomycin, and (*5*) capillary with immobilized eremomycin.

amphetamine, atenolol, clenbuterol, labetalol, methoxyphenamine, metoprolol, normetanephrine, pindolol, propranolol, oxprenolol, octopamine, *p*-hydroxynorhephedrine, *p*-chloramphetamine, synephrine, sotalol, tetrahydrozoline, tryptophanol, fenoterol, and ephedrine were separated [48, 49].

The exceptional role of boric acid in enantioseparations with macrolides as a chiral selector was revealed. For all antibiotics under study (azithromycin, clarithromycin, and erythromycin), the presence of boric acid in the supporting electrolyte in the absence of water is a necessary condition for achieving enantioseparation [48–51]. As the concentration of boric acid in the supporting electrolyte increases, the resolution of the enantiomeric peaks is improved. The example of clarithromycin [51] shows that with increasing boric acid concentration up to 240 mM, enantioseparation is improved, and then remains practically unchanged. All investigated antibiotics are vicinal diols, which form complexes with boric acid (Fig. 3). Apparently, the enantioseparation is due to the electrostatic interactions of the negatively charged

Table 3. Results of the determination of anionic compounds in pharmaceutical compositions using eremomycin as a chiral selector ($P = 0.95$, $n = 3$) [46]

Compound (dosage form)	CS concentration, mM	Analytical range, mg/L	Migration times, min[1]	c_{min}, mg/mL	Found
Ibuprofen (tablets, 200 mg/tablet)	2.5[2]	0.2–1	26.5 ± 0.5 30.7 ± 0.2	6.0×10^{-2}	201 ± 2 mg/tablet
Flurbiprofen (tablets, 8.75 mg/tablet)	5[3]	0.005–0.5	11.7 ± 0.2 17.0 ± 0.4	5.7×10^{-5}	8.6 ± 0.9 mg/tablet
	0.25[4]	–	9.31± 0.03 9.70 ± 0.03	–	
Warfarin (tablets, 2.5 mg/tablet)	5[3]	0.002–0.15	9.5 ± 0.1 10.1 ± 0.3	6.5×10^{-7}	2.3 ± 0.3 mg/tablet
Ketoprofen (gel, 2.5%)	0.25[5]	0.035–0.2	10.60 ± 0.04 11.20 ± 0.04	1.4×10^{-2}	23 ± 7 mg/g

[1] The migration times of the first and second enantiomers, respectively;
[2] quartz capillary;
[3] capillary dynamically modified with eremomycin;
[4] capillary modified with glycidoxypropyltriethoxysilane and eremomycin;
[5] capillary modified with 3-aminopropyltrimethoxysilane.

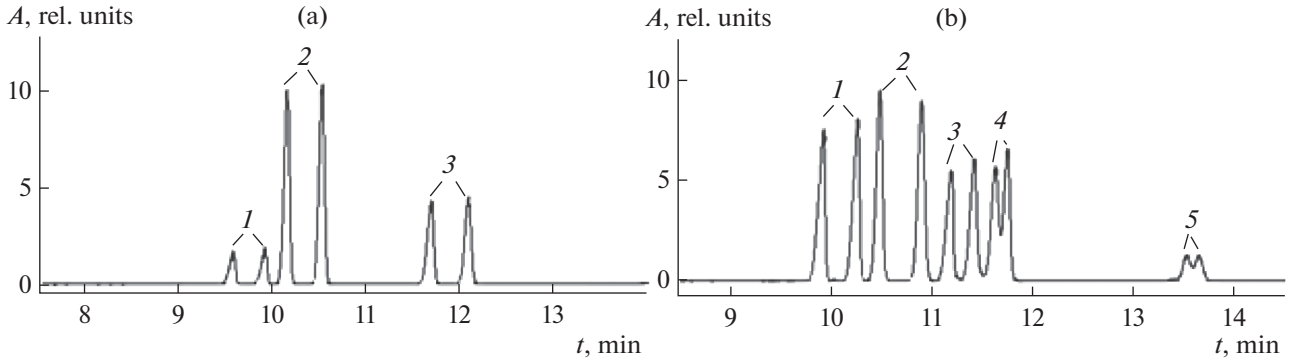
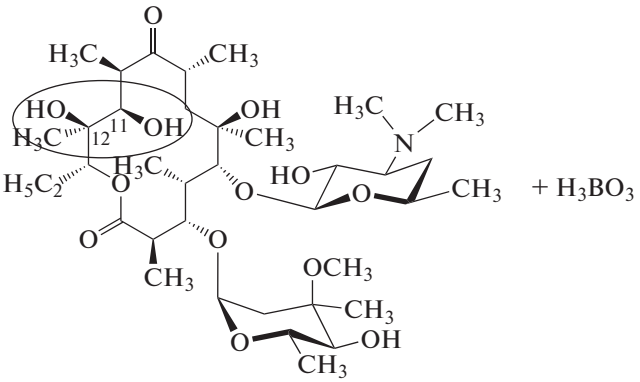


Fig. 3. Electropherogram of the racemate mixtures of (a) (*1*) alprenolol, (*2*) propranolol, and (*3*) atenolol and (b) (*1*) pindolol, (*2*) propranolol, (*3*) synephrine, (*4*) sotalol, and (*5*) fenoterol. Separation conditions: supporting electrolyte, 75 mM clarithromycin, 100 mM citric acid, 10 mM NaOH, and 240 mM H_3BO_3 in methanol; + 20 kV; 225 nm.

antibiotic complex with boric acid (see the mechanism below) and positively charged amine compounds.



Formation of a complex of erythromycin (**ERTRM**) with boric acid.

The nature and concentration of the additions of the base and acid and their ratio in the supporting electrolyte also affect the enantioseparation in the presence of erythromycin, azithromycin, and clarithromycin. The nature of the base most significantly affects the selectivity of the separation in the presence of azithromycin; it increases in the series of TRIS < TEA < TBA [50]. When using erythromycin, enantiorecognition proceeds better with the additives of DEA and TRIS [48]. The best supporting electrolyte in the use of clarithromycin is methanol with the additions of citric acid, base (NaOH) for dissolving the chiral selector and maintaining a certain acidity of the mixture, and boric acid to form a complex of clarithromycin–boric acid. The best separation is observed when using a citrate buffer solution with a concentration of 120 mM [51].

The concentration of the chiral selector affects the separation of enantiomers. In most cases, with an increase in the concentration of macrolides in the supporting electrolyte, the resolution of the enantiomeric peaks is improved, and the migration times of the analytes are increased, which indicates the formation of diastereomeric complexes between the selector and the enantiomers of the analyte. The use of too high concentrations of the chiral selector is limited by the solubility of antibiotics and sometimes by a critical increase in viscosity, at which the enantioseparation for some compounds becomes worse. The optimal concentration of the chiral selector depends on the nature of the analyte and the selector. With the use of clarithromycin as a chiral selector, the best R_S values for the analytes were achieved in the concentration range of 75–90 mM [48, 50, 51].

A comparison of the ability of macrolides to recognize enantiomers shows that erythromycin and clarithromycin are enantioselective with respect to compounds of the basic nature, that is, amines and amino alcohols [48]. Azithromycin showed enantioselectivity only to tetrahydrozoline. Table 4 lists the compounds, the enantiomers of which are separated in the presence of erythromycin and clarithromycin in the supporting electrolyte. The lower ability of azithromycin to separate enantiomers in comparison with erythromycin and clarithromycin can be explained by the fact that the hydroxyl groups in the azithromycin molecule are in the *trans* position rather than in the *cis* one and are less prone to reaction with boric acid to form a complex. The enantioseparation is caused by the electrostatic interactions of the negatively charged antibiotic complex with boric acid and positively charged amine compounds. In this regard, macrolides are not enantioselective to compounds of an acidic nature.

In addition to the separation of the enantiomers of the compounds, mixtures of substances are also separated. Figures 3a, 3b, and 4 show the electropherograms of artificial mixtures. Alprenolol, propranolol and atenolol; pindolol, propranolol, synephrine, sotalol, and fenoterol; and ephedrine, *p*-chloramphetamine, tryptophanol, and normetanephrine are separated.

Table 4. Separation of enantiomers of test compounds in the presence of erythromycin and clarithromycin in nonaqueous systems [48]

Supporting electrolyte	Erythromycin	Clarithromycin
Boric acid and base in methanol	*Complete separation*: amphetamine, ephedrine, synephrine, tryptophanol, *p*-chloramphetamine, tetrahydrozoline, methoxyphenamine *Partial separation*: metoprolol, oxprenolol, atenolol, alprenolol, pindolol, octopamine, *p*-hydroxynorephedrine, normetanephrine	*Complete separation*: clenbuterol, methoxyphenamine *Partial separation*: metoprolol
Citric acid and base in methanol (boric acid)	*Partial separation*: tetrahydrozoline	*Complete separation*: atenolol, alprenolol, metoprolol, pindolol, propranolol *Partial separation*: labetalol, synephrine, sotalol, fenoterol

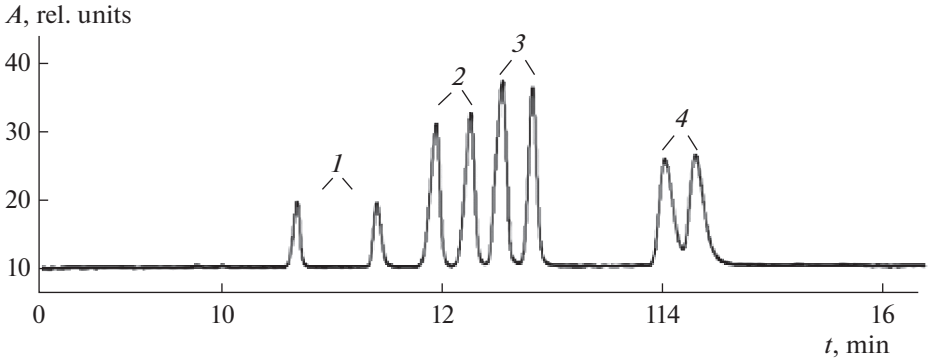


Fig. 4. Electropherogram of the racemate mixtures of (*1*) ephedrine, (*2*) *p*-chloramphetamine, (*3*) tryptophanol, and (*4*) normetanephrine. Separation conditions: supporting electrolyte, 100 mM erythromycin, 66 mM TRIS, and 39 mM H_3BO_3 in methanol; +30 kV; 214 nm.

CONCLUSIONS

Thus, silica modified with macrocyclic antibiotics (vancomycin, teicoplanin, teicoplanin aglycone, and eremomycin) is successfully used to separate the enantiomers of amino acids, various amino acid derivatives, α-phenylcarboxylic acids, β-blockers, and some pharmaceutical compounds by HPLC in the reversed-phase and polar-organic modes. The most interesting results were obtained for eremomycin. Mixed chiral adsorbents (eremomycin–vancomycin, eremomycin–BSA) are synthesized, which combine the properties of two selectors. Eremomycin and macrolides (azithromycin, erythromycin, and clarithromycin) are used as chiral selectors in aqueous and nonaqueous CE to separate the enantiomers of substances of different classes.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research, project no. 18-03-00742.

REFERENCES

1. Ilisz, I., Aranyi, A., Pataj, Z., and Péter, A., *Methods Mol. Biol.*, 2013, vol. 970, p. 137.
2. Scriba, G.K.E., *J. Chromatogr. A*, 2016, vol. 1467, p. 56.
3. Berthod, A., *Compr. Chirality*, 2012, vol. 8, p. 227.
4. Scriba, G.K.E. and Schiller, F., Chiral separations in capillary electrophoresis, in *Reference Module in Chemistry, Molecular Sciences and Chemical Engineering*, 2015, p. 1.
5. Ilisz, I., Aranyi, A., Pataj, Z., and Péter, A., *J. Pharm. Biomed. Anal.*, 2012, vol. 69, p. 28.
6. Ilisz, I., Aranyi, A., Pataj, Z., and Péter, A., *J. Chromatogr. A*, 2012, vol. 1269, p. 94.
7. Ilisz, I., Berkecz, R., and Péter, A., *J. Chromatogr. A*, 2009, vol. 1216, p. 1845.
8. Asnin, L., *J. Chromatogr. A*, 2012, vol. 1269, p. 3.
9. Armstrong, D.W., Tang, Y.B., Chen, S.S., Zhou, Y.W., Bagwill, C., and Chen, J.R., *Anal. Chem.*, 1994, vol. 66, p. 1473.
10. Armstrong, D.W., Rundlett, U., and Chen, J.R., *Chirality*, 1994, vol. 6, p. 496.
11. Armstrong, D.W., Liu, Y., and Ekborg-Ott, K.H., *Chirality*, 1995, vol. 18, no. 8, p. 1495.

12. Anan'eva, I.A., *Cand. Sci. (Chem.) Dissertation*, Moscow: Moscow State Univ., 2001.
13. Anan'eva, I.A., Shapovalova, E.N., Shpigun, O.A., and Armstrong, D.W., *Vestn. Mosk. Univ., Ser. 2: Khim.*, 2001, vol. 42, no. 4, p. 278.
14. Berthod, A., Liu, Y., Bagwill, C., and Armstrong, D.W., *J. Chromatogr. A*, 1996, vol. 731, p. 123.
15. Berdnikova, T.F., Lomakina, N.N., Olsuf'eva, E.N., Aleksandrova, L.I., Potapova, N.P., Rozynov, B.V., Malkova, I.V., and Orlova, G.I., *Antibiot. Khimioter.*, 1991, vol. 36, no. 6, p. 28.
16. Staroverov, S.M., Kuznetsov, M.A., Nesterenko, P.N., Vasiarov, G.G., Katrukha, G.S., and Fedorova, G.B., *J. Chromatogr. A*, 2006, vol. 1108, p. 263.
17. Kuznetsov, M.A., Nesterenko, P.N., Vasiyarov, G.G., and Staroverov, S.M., *J. Anal. Chem.*, 2008, vol. 63, no. 1, p. 57.
18. Natykan, A.A., Sycheva, K.Yu., Chernobrovkin, M.G., Shapovalova, E.N., and Shpigun, O.A., *Zavod. Lab., Diagn. Mater.*, 2011, vol. 77, no. 3, p. 18.
19. Kuznetsov, M.A., *Cand. Sci. (Chem.) Dissertation*, Moscow: Moscow State Univ., 2008.
20. Fedorova, I.A., Shapovalova, E.N., Staroverov, S.M., and Shpigun, O.A., *Sorbtsionnye Khromatogr. Protsessy*, 2015, vol. 15, no. 6, p. 769.
21. Fedorova, I.A., *Cand. Sci. (Chem.) Dissertation*, Moscow: Moscow State Univ., 2017.
22. D'Acquarica, I., Gasparrini, F., Misiti, D., Pierini, M., and Villani, C., *Adv. Chromatogr.*, 2007, vol. 46, p. 109.
23. Ribeiro, A.R., Maia, A.S., Cass, Q.B., and Tiritan, M.E., *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2014, vol. 968, p. 8.
24. Kuznetsov, M.A., Nesterenko, P.N., Vasiyarov, G.G., and Staroverov, S.M., *Appl. Biochem. Microbiol.*, 2006, vol. 42, no. 6, p. 536.
25. Anan'eva, I.A., Chernobrovkin, M.G., Shapovalova, E.N., and Shpigun, O.A., *Zavod. Lab., Diagn. Mater.*, 2003, vol. 69, no. 12, p. 3.
26. Anan'eva, I.A., Shapovalova, E.N., Shpigun, O.A., and Armstrong, D.W., *J. Anal. Chem.*, 2003, vol. 58, no. 7, p. 663.
27. Bosakova, Z., Curınova, E., and Tesarova, E., *J. Chromatogr. A*, 2005, vol. 1088, p. 94.
28. Shapovalova, E.N., Fedorova, I.A., Priporova, A.A., Anan'eva, I.A., and Shpigun, O.A., *Analitika Kontrol'*, 2017, vol. 20, no. 2, p.168.
29. Shapovalova, E.N., Fedorova, I.A., Priporova, A.A., Anan'eva, I.A., and Shpigun, O.A., *Moscow Univ. Chem. Bull. (Engl. Transl.)*, 2017, vol. 72, no. 1, p. 56.
30. Shapovalova, E.N., Fedorova, I.V., Golubova, A.D., and Komaiko, A.I., Abstracts of Papers, *Tretii s"ezda analitikov Rossii* (Third Meeting of Russian Analytical Chemists), Moscow, 2017, p. 235.
31. Fedorova, I.A., Shapovalova, E.N., and Shpigun, O.A., *J. Anal. Chem.*, 2017, vol. 72, no. 1, p. 76.
32. Petrusevska, K., Kuznetsov, M.A., Gedicke, K., Meshko, V., Staroverov, S.M., and Seidel-Morgenstern, A., *J. Sep. Sci.*, 2006, vol. 29, p. 1447.
33. Fedorova, I.A., Shapovalova, E.N., Shpigun, O.A., and Staroverov, S.M., *J. Food Drug Anal.*, 2016, vol. 24, no. 4, p. 848.
34. Ward, T.J. and Oswald, T.M., *J. Chromatogr. A*, 1997, vol. 792, p. 309.
35. Armstrong, D.W. and Nair, U.B., *Electrophoresis*, 1997, vol. 18, p. 2331.
36. Ali, I., Al-Othman, Z.A., Al-Warthan, A., Asnin, L., and Chudinov, A., *J. Sep. Sci.*, 2014, vol. 37, no. 18, p. 2447.
37. Ha, P., Hoogmartens, J., and Van Schepdael, A., *J. Pharm. Biomed. Anal.*, 2006, vol. 41, p. 1.
38. Prokhorova, A.F., Shapovalova, E.N., and Shpigun, O.A., *J. Pharm. Biomed. Anal.*, 2010, vol. 53, p. 1170.
39. Prokhorova, A.F., Budanova, N.Yu., Shapovalova, E.N., and Shpigun, O.A., *Zavod. Lab., Diagn. Mater.*, 2010, vol. 76, no. 11, p. 7.
40. Prokhorova, A.F., Shapovalova, E.N., Shpak, A.V., Staroverov, S.M., and Shpigun, O.A., *J. Chromatogr. A*, 2009, vol. 1216, p. 3674.
41. Lebedeva, M.V., Prokhorova, A.F., Shapovalova, E.N., Shpigun, O.A., Staroverov, S.M., and Kuznetsov, M.A., *Sorbtsionnye Khromatogr. Protsessy*, 2011, vol. 11, no. 5, p. 589.
42. Prokhorova, A.F., Kuznetsov, M.A., Shapovalova, E.N., Staroverov, S.M., and Shpigun, O.A., *Moscow Univ. Chem. Bull. (Engl. Transl.)*, 2010, vol. 65, no. 5, p. 295.
43. Prokhorova, A.F., Larin, A.V., Mikhalyuk, A.N., Staroverov, S.M., Shapovalova, E.N., and Shpigun, O.A., *Electrophoresis*, 2011, vol. 32, p. 2663.
44. Prokhorova, A.F., Kuznetsov, M.A., Shapovalova, E.N., Staroverov, S.M., and Shpigun, O.A., *Procedia Chem.*, 2010, vol. 2, no. 1, p. 9.
45. Prokhorova, A.F., Shapovalova, E.N., and Shpigun, O.A., *J. Pharm. Biomed. Anal.*, 2010, vol. 53, no. 5, p. 1170.
46. Prokhorova, A.F., *Cand. Sci. (Chem.) Dissertation*, Moscow: Moscow State Univ., 2010.
47. Lebedeva, M.V., Prokhorova, A.F., Shapovalova, E.N., and Shpigun, O.A., *Moscow Univ. Chem. Bull. (Engl. Transl.)*, 2013, vol. 68, no. 5, p. 215.
48. Lebedeva, M.V., *Cand. Sci. (Chem.) Dissertation*, Moscow: Moscow State Univ., 2014.
49. Lebedeva, M.V., Bulgakova, G.A., Prokhorova, A.F., Shapovalova, E.N., Chernobrovkin, M.G., and Shpigun, O.A., *Chromatographia*, 2013, vol. 76, nos. 7–8, p. 375.
50. Bulgakova, G.A. and Lebedeva, M.V., Abstracts of Papers, *XX Mezhdunar. nauchn. konfio studentov, aspirantov i molodykh uchenykh "Lomonosov-2013"* (XX Int. Sci. Conf. of Students, Graduate Students, and Young Scientists "Lomonosov-2013"), Moscow, 2013. https://lomonosov-msu.ru/archive/Lomonosov_2013/structure_31_2341.htm. Accessed February 23, 2018.
51. Lebedeva, M.V., Prokhorova, A.F., Shapovalova, E.N., and Shpigun, O.A., *Electrophoresis*, 2014, vol. 35, no. 19, p. 2759.

SPELL: 1. OK