PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY

Chemistry and Biology 2023, **57**(2), p. 110–117

Chemistry

CHIRAL SEPARATION OF (2*S*,9*S*)-DIAMINO-2,9-DIBENZYLDECA-4,6-DIYNEDIOIC ACID AND (2*R*,9*R*)-DIAMINO-2,9-DIBENZYLDECA-4,6-DIYNEDIOIC ACID

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The enantiomers of the chiral compound (2*S*, 9*S*)-diamino-2,9-dibenzyldeca-4,6-diynedioic acid and (2*R*, 9*R*)-diamino-2,9-dibenzyldeca-4,6-diynedioic acid were separated by a chiral high-performance liquid chromatography (HPLC) method. The separation of α-amino acids was performed using a 5μ 4.0×250 *mm* column, which was obtained on the basis of a sorbent with eremomycin. The sorbent is silica gel (with 11 *nm* pore diameter and a surface area of 300 m^2/g) with epoxy groups grafted on them with antibiotic eremomycin.

Chiral HPLC is one of the most common methods for separating enantiomers of chiral compounds. Synthesis of new pharmaceutical substances, which are one of the isomers of a substance, obtaining of new drugs based on them, as well as the need to control the presence of the second isomer in such drugs, impose new requirements on the modern chromat-ographic separation of isomers.

https://doi.org/10.46991/PYSU:B/2023.57.2.110

***Keywords*:** non-protein amino acids, chiral separation, antibiotic eremomycin as a chiral selector.

Introduction. Over the past decade, chiral analysis has become increasingly important in the separation sciences. The diverse functions and properties of enantiomeric amino acids and peptides make their separation necessary as research progresses in many applicable fields of science and technology. Basic enantiomeric purity of the building blocks for peptides and proteins is essential for the final quality of the products of the future, whether for diagnosis or treatment [1]. The development of methods for separating enantiomers has attracted great interest, since it has become evident that potential biological or pharmacological applications are mostly restricted to one of the enantiomers. The important analytical task of the separation of isomers is achieved mainly by chromatographic and electrophoretic methods [2].

Macrocyclic antibiotics have proved to be an exceptionally useful class of chiral selectors for the separation of enantiomers of biological and pharmacological importance. Enantioseparation may be possible via several different mechanisms,

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including inclusion in a hydrophobic pocket, π-π-complex formation, dipole stacking, hydrogen bonding, electrostatic, short-range van der Waals interactions, steric/rigidity effects, or combinations thereof. Possible interactions strictly depend on how the enantiomers fit into the aglycone cavity, which is determined by the structural geometry [3, 4].

The first works on the use of glycopeptide antibiotics for chiral separation belong to Armstrong and date back to 1994 [5, 6]. They describe the use of three macrocyclic antibiotics – vancomycin, rifamycin B and thiostrepton, covalently bonded to stationary phase silica gel in chiral high-performance liquid chromatography (HPLC). These antibiotics have shown high enantioselectivity of various compounds in both reversed-phase and normal phase chromatography modes [5, 6]. Armstrong et al. [7] applied teicoplanin, teicoplanin aglycone and ristocetin A as selectors for the separation of forty-two polymorphic and epimeric peptides (up to 13 amino acids in length) and teicoplanin and its aglycone analogue [8] as stationary phases for even larger peptides (up to 36 amino acids in length) with mass-spectrometry (MS) detection [9, 10].

As of today, macrocyclic antibiotics are one of the most widely used chiral selectors, which make it possible to separate enantiomers of various classes of substances [11–15]. Macrocyclic glycopeptide antibiotics, which include eremomycin, have proved the validity of their use as chiral selectors in HPLC due to their structural characteristics. Usually, their structure 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 numerous interactions of an antibiotic with a chiral analyte through hydrogen bonds, π-π-interactions, as well as many dipole, electrostatic, and hydrophobic interactions [16, 17].

The closest analogue of eremomycin is the macrocyclic antibiotic vancomycin. The difference between the structure of eremomycin and vancomycin is that the eremomycin molecule contains only one chlorine atom, while the vancomycin molecule contains two. Eremomycin also has an additional carbohydrate residue with a primary amino group – eremosamine (4-epi-vancosamine), which differs from the similar vancomycin residue in the disaccharide branch with the reverse configuration of C4 carbon [18, 19].

The hydrophobic aromatic rings of the antibiotic are localized within the molecule, forming hydrophobic “pockets”, the sizes of which are optimal for amino acids with an ethyl substituent in the side chain (α-aminobutyric acid). A further increase in the size of the side substituent leads to steric hindrances of its penetration into such a “pocket”, which leads to a decrease in the retention of D-isomers and enantioselectivity.

In the mechanism of separation of amino acids with eremomycin as a chiral selector, interactions both nonspecific (hydrophobic) and specific (hydrogen bonds, dipole-dipole interactions, ion-exchange, donor-acceptor, π-π-interactions) play an essential role. Moreover, the selectivity of separation of amino acid enantiomers decreases in the series: cyclic amino acids – aromatic and hydrophobic amino acids – amino acids with acidic and basic functional groups in the side chain [19].

Materials and Methods. HPLC method for α-amino acids was developed using Waters Separations module 2695 (USA) series instrument equipped with a

column oven and a UV detector; the data were processed using Empower 2 software. α-Amino acids were separated using Nautilus-E 5μ 4.0×250 *mm* column ("BioChimMac ST Company", Moscow, Russia).

The separation of α-amino acids was done in isocratic mode at 30°C. The mobile phase was comprised of acetonitrile and monosodium phosphate buffer (25 *mmol*/*L*) with composition of 20:80 v/v at a flow rate of 0.5 *mL*/*min*. Detection was carried out at a wavelength of 200 *nm* and the injection volume was 10 μL.

All α-amino acids were synthesized by the laboratory of Asymmetric Catalysis (SPC "Armbiotechnology" NAS RA).

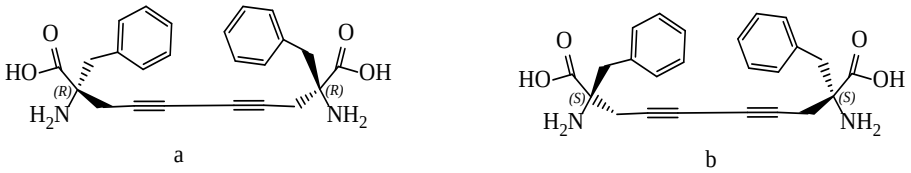


Fig. 1. (2*R*, 9*R*)-diamino-2,9-dibenzyldeca-4,6-diynedioic acid (a) and (2*S*, 9*S*)-diamino-2,9-dibenzyldeca-4,6-diynedioic acid (b).

Fig. 1 shows the chemical structure of enantiomers of non-protein amino acids. HPLC grade acetonitrile and monosodium phosphate buffer (≥ 99%) were procured from "Sigma-Aldrich" Chemicals Company (USA). HPLC grade water was obtained from water deionization system PURELAB® Option-Q ("ELGA Labwater", United Kingdom). The solvents and buffers prepared were suitably filtered through 0.45 μm Whatman® membrane filter ("Merck", Germany). All samples were weighed on an analytical scale (Sartorius cp2p, Italy).

Results and Discussion. Chiral separation of enantiomers of non-protein α-amino acids on the Nautilus-E C18 column is presented in Fig. 2. A good performance of the assay was obtained for all newly synthesized non-protein amino acids.

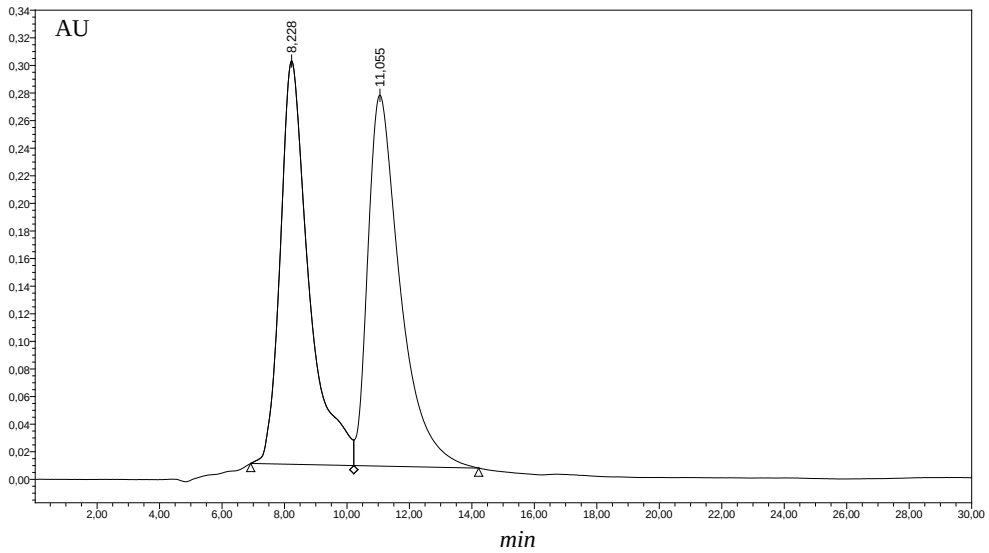


Fig. 2. Chromatogram of chiral separation of (2*S*, 9*S*)-diamino-2,9-dibenzyldeca-4,6-diynedioic acid (8.228 *min*) and (2*R*, 9*R*)-diamino-2,9-dibenzyldeca-4,6-diynedioic acid (11.055 *min*).

Specificity. Tab. 1 shows that the relative standard deviation for the peak area is no more than 0.56% and for the retention time is no more than 1.17%.

Table 1

The results of the indicator evaluation of specificity

α-Amino acids, 0.5 *mg*/*mL*	(2*S*,9*S*)-Diamino-2,9-dibenzyldeca--4,6-diynedioic acid		(2*R*,9*R*)-Diamino-2,9-dibenzyldeca--4,6-diynedioic acid	
	retention time, *min*	area, AU	retention time, *min*	area, AU
Sample 1	8.217	33350244	11.081	35228426
Sample 2	8.228	33450034	11.072	35235671
Sample 3	8.201	33398001	11.067	35364537
Sample 4	8.224	33360780	11.076	35246456
Sample 5	8.198	33353201	11.059	35398642
Sample 6	8.207	33403784	11.055	35274582
Average value	8.210	33386007	11.07	35291386
SD	0.0124	38861	0.0099	72421
RSD, %	0.150	0.12	0.1	0.205

Precision. There were 6 consecutive injections of the standard solution. The results are presented in Tab. 2. As it can be seen from Tab. 2, our results do not exceed the permissible limit.

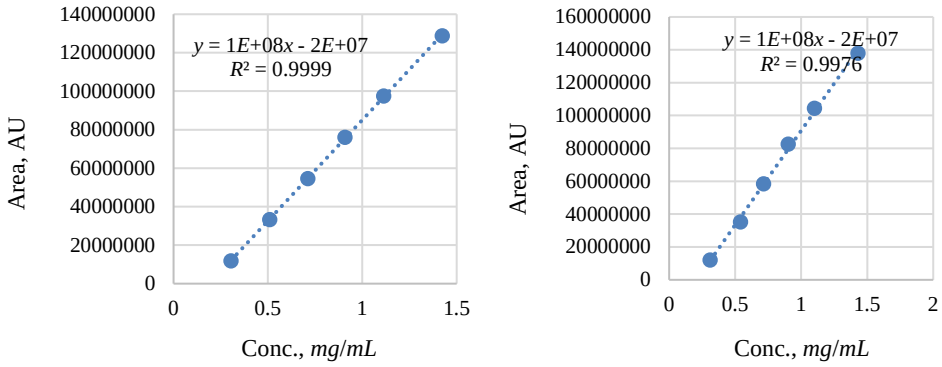
Table 2

The results of the accuracy measurement evaluation

Samples	(2*S*,9*S*)-Diamino-2,9-dibenzyldeca--4,6-diynedioic acid		(2*R*,9*R*)-Diamino-2,9-dibenzyldeca--4,6-diynedioic acid	
	first day	second day	first day	second day
Sample 1	33350244	33275364	35228426	35276982
Sample 2	33450034	33309224	35235671	35230567
Sample 3	33398001	33267435	35364537	35242764
Sample 4	33360780	33330678	35246456	35278452
Sample 5	33353201	33290432	35398642	35290619
Sample 6	33403784	33302816	35274582	35245634
Average value	33386007	33295992	35291386	35260836
RSD, %	0.12	0.07	0.205	0.07

Linearity and Linearity Range. The analytical curve was found to be linear over a wide concentration range (0.3–1.4 *mg*/*mL*) for (*S*)- and (*R*)-amino acids with a correlation coefficient of 0.9999 and 0.9976 (Fig. 3 and Tab. 3).

Thus, this HPLC method can be considered to show adequate linearity in the concentration range (0.3–1.1 *mg*/*mL*) for quantitative analysis of aminoacids (2*S*,9*S*)-diamino-2,9-dibenzyldeca-4,6-diynedioic and (2*R*,9*R*)-diamino-2,9-dibenzyl-deca-4,6-diynedioic acids under the experimental conditions described.



a b

Fig. 3. Graph of linearity of method for (2*R*,9*R*)-diamino-2,9-dibenzyldeca-4,6-diynedioic acid (a) and (2*S*,9*S*)-diamino-2,9-dibenzyldeca-4,6-diynedioic acid (b).

Table 3

The results of linearity and linearity range

Sample code	(2*S*,9*S*)-Diamino-2,9-dibenzyldeca--4,6-diynedioic acid		(2*R*,9*R*)-Diamino-2,9-dibenzyldeca--4,6-diynedioic acid	
	concentration, *mg*/*mL*	area, AU	concentration, *mg*/*mL*	area, AU
Calib. 1	0.304	11985008	0.311	12084325
Calib. 2	0.51	33350244	0.511	35228426
Calib. 3	0.712	54715480	0.716	58388237
Calib. 4	0.908	76080775	0.923	82528048
Calib. 5	1.114	97554011	1.102	104377859
Calib. 6	1.424	128919247	1.430	137928769
Correlation coefficient, *r*	0.9999		0.9976	

Minimum limit of detection (LOD) and limit of quantification (LOQ) for amino acids. Signal-to-noise ratios of 3 : 1 and 10 : 1 were obtained for the LOD and LOQ, respectively. The smaller the LOD and LOQ, the higher the sensitivity of the method. The LOD and LOQ were found to be 0.043 *mg*/*mL* and 0.1 *mg*/*mL* for (2*S*, 9*S*)-diamino-2,9-dibenzyldeca-4,6-diynedioic and 0.13 *mg*/*mL* and 0.293 *mg*/*mL* for (2*R*,9*R*)-diamino-2,9-dibenzyldeca-4,6-diynedioic acids.

Accuracy. The results presented in Tabs. 4 and 5 refer to the average of three assays for each concentration.

Table 4

The results of the accuracy evaluation of (2S, 9S)-diamino-2,9-dibenzyldeca-4,6-diynedioic acid

(S)-Benzyl propargylglycine	QCL 0.220 *mg*/*mL*		QCM 0.600 *mg*/*mL*		QCH 1.202 *mg*/*mL*	
	1	2	1	2	1	2
Found concentration, *mg*/*mL*	0.224	0.221	0.5969	0.5905	1.226	1.235
RE, %	101.7	105	99.5	98.4	102	102.78
RE average, %	103.4		98.96		102.4	

Table 5

The results of the accuracy evaluation of (2R,9R)-diamino-2,9-dibenzyldeca-4,6-diynedioic acid

(*R*)-Benzyl propargylglycine	QCL 0.231 *mg*/*mL*		QCM 0.616 *mg*/*mL*		QCH 1.203 *mg*/*mL*	
	1	2	1	2	1	2
Found concentration, *mg*/*mL*	0.2259	0.2257	0.620	0.619	1.204	1.2039
RE, %	97.8	97.7	100.65	100.62	100.09	100.08
RE average, %	97.77		100.64		100.1	

Note: QCL – limit of quantification low concentrations), QCM – limit of quantification medium concentrations, QCH – limit of quantification high concentrations, RE – reconcentration; 1, 2 – amounts of injections.

In Tabs. 4 and 5 it is seen, that our results range from 98% to 100%, which are acceptable (British pharmacopoeia standard 1998; ANVISA 2003).

Conclusion. A chiral high-performance liquid chromatography method was developed to separate the enantiomers (2*S*, 9*S*)-diamino-2,9-dibenzyldeca-4,6-diynedioic acid and (2*R*, 9*R*)-diamino-2,9-dibenzyldeca-4,6-diynedioic acid on the basis of a chiral column filled with a sorbent with an antibiotic of the vancomycin group – eremomycin, i.e. the antibiotic was used as a chiral selector.

Table 6

Assay validation

Parameters	(2*S*,9*S*)-Diamino-2,9--dibenzyldeca-4,6-diynedioic acid	(2*R*,9*R*)-Diamino-2,9--dibenzyldeca-4,6-diynedioic acid
	value	
Accuracy	99.940104±0.5146968	99.829575±1.1428897
Slope	104882891.7	113368622.3
Intercept	–19812162.1	–22585644.5
Linearity range, *mg*/*mL*	0.220–1.202	0.231–1.203
Correlation coefficient, *r*	0.9999	0.9976
SE of intercept	553486.2	1355527.3
SD of intercept	1356041	3321042
LOD	0.043	0.1
LOQ	0.13	0.293

The validation method performed has shown (Tab. 6), that the developed method meets the current requirements for the above mentioned technical indicators and can be employed for the assay of new synthesized non-protein α-amino acids.

Received 14.04.2023
Reviewed 24.05.2023
Accepted 06.06.2023

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https://doi.org/10.1016/j.chroma.2006.01.073

Ա. Հ. ԾԱՏՈՒՐՅԱՆ

(2*S*, 9*S*)-ԵՐԿԱՄԻՆՈ-2,9-ԵՐԿԲԵՆԶԻԼԴԵԿԱ-4,6-ԵՐԿԻՆ-ԴԻՈԱԹԹՎԻ ԵՎ (2*R*, 9*R*)-ԵՐԿԱՄԻՆՈ-2,9-ԵՐԿԲԵՆԶԻԼԴԵԿԱ-4,6-ԵՐԿԻՆ-ԴԻՈԱԹԹՎԻ ՔԻՐԱԼԱՅԻՆ ԲԱԺԱՆՈՒՄ

(2*S*, 9*S*)-երկամինո-2,9-երկբենզիլդեկա-4,6-երկին-դիոաթթվի և (2*R*, 9*R*)-երկամինո-2,9-երկբենզիլդեկա-4,6-երկին-դիոաթթվի քիրալային միացության էնանտիոմերներն բաժանվել են քիրալային բարձր արդյունավետության հեղուկ քրոմատոգրաֆիայի (ԲԱՀՔ) մեթոդով: Սորբենտն իրենից ներկայացնում է սիլիկաժել (11 *նմ* ծակոտի տրամագծով և 300 $մ^2/գ$ մակերեսով) էպօքսիդային խմբերով, որոնք պատվաստված են հակաբիոտիկ էրեմոմիցինով:

Քիրալային ԲԱՀՔ քիրալային միացությունների էնանտիոմերների բաժանման ամենատարածված մեթոդներից մեկն է: Հետևյալ գործոնները, ինչպիսիք են նոր դեղագործական նյութերի սինթեզը, որոնք հանդիսանում են նյութի իզոմերներից մեկը, դրանց հիման վրա նոր դեղամիջոցների ստացումը, ինչպես նաև նման դեղամիջոցներում երկրորդ իզոմերի առկայությունը վերահսկելու անհրաժեշտությունը, պարտադրում են իզոմերների ժամանակակից քրոմատոգրաֆիկ բաժանման նոր պահանջներ:

А. О. ЦАТУРЯН

ХИРАЛЬНОЕ РАЗДЕЛЕНИЕ (2*S*, 9*S*)-ДИАМИНО-2,9-ДИБЕНЗИЛДЕКА-4,6-ДИИНДИЕВОЙ И (2*R*, 9*R*)-ДИАМИНО-2,9-ДИБЕНЗИЛДЕКА-4,6-ДИИНДИЕВОЙ КИСЛОТ

Энантиомеры хирального соединения (2*S*, 9*S*)-диамино-2,9-дибензилдека-4,6-дииндиевой и (2*R*, 9*R*)-диамино-2,9-дибензилдека-4,6-дииндиевой кислот были разделены методом хиральной высокоэффективной жидкостной хроматографии (ВЭЖХ). Сорбент представляет собой силикагель (диаметром пор 11 *нм*, площадью поверхности 300 $м^2/г$) с эпоксидными группами, привитыми антибиотиком эремомицином.

Хиральная ВЭЖХ – один из наиболее распространенных методов разделения энантиомеров хиральных соединений. Синтез новых фармацевтических субстанций, которые являются одними из изомеров вещества, получение на их основе новых препаратов, а также необходимость контроля наличия второго изомера в таких лекарственных средствах предъявляют новые требования к современному хроматографическому разделению изомеров.