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**ANALYSIS**

# IMPACT OF STRESS FACTORS ON OPTICAL ISOMERISM OF BENAZEPRIL HYDROCHLORIDE

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**Abstract:** Benazepril hydrochloride contains two stereogenic centers, but is currently available as single enantiomer (S,S configuration) for the treatment of hypertension. Its enantiomer (R,R configuration) and the diastereoisomeric pair (R,S and S,R) can be regarded as impurities. Stereochemical stability of S,S isomer of benazepril hydrochloride and its potential susceptibility to conversion in the active substance and in Lisonid tablets were examinated. The separation with the use of the TLC method with the following system: chromatographic plates Chiralplate and a mobile phase: methanol – acetonitrile – 1 mM copper(II) acetate (4 : 2 : 4, v/v/v) with saturation of glacial acetic acid for 1 h and the HPLC method system: Chiral AGP column (150 × 4.0 mm × 5 μm) and a mobile phase: phosphate buffer pH = 6.0 – methanol (80 : 20, v/v) were obtained. Active substance – benazepril hydrochloride and Lisonid tablets 20 mg were subjected to the impact of different stress factors. Samples were examined after 1 and 6 weeks. It was found that none of the applied stress factors caused the transformation of the S,S enantiomer of benazepril hydrochloride in the substance and tablets to other identified stereoisomers – only the compound decomposition has occurred.

**Keywords:** hypertension, angiotensin converting enzyme inhibitors (ACE inhibitors), optical isomerism, HPLC, TLC, benazepril hydrochloride

Chemical compounds containing chiral centers in their molecules show optical isomerism. As a result, they may be in form of enantiomers or diastereoisomers. Enantiomers have the same dimensions and shapes as well as the same physical and chemical properties. Diastereoisomers, on the other hand, differ in physical and chemical properties. Having the present state of knowledge, it is significant to define the activity direction of enantiomers existing as medicinal products. It is possible that all identified enantiomers have identical bioactivity; it is a different situation when only one isomer is bioactive, others are inactive. It is important to define an optical purity in chiral medicines and develop analytical methods aimed at dividing enantiomers (1, 2).

Angiotensin converting enzyme inhibitors (ACE inhibitors) used in arterial hypertension treatment form a large group of medical substances containing chiral centers.

Hypertension is one of the most common diseases of the circulatory system. Due to the fact that this disorder is very widespread, it is a factor in artherosclerosis and its clinical forms: coronary disease, cardiac arrest, apoplexy, it is perceived as a social disease.

ACE inhibitors are currently a group of "first choice drugs" used in the therapy of arterial hypertension. They may be applied in treating ischemic disease and cardiac insufficiency. Their active metabolites created as a result of hydrolysis, cause the decrease in ACE activity (and related reduction of angiotensin II biosynthesis), the decrease of the release of aldosterone (as a result of lowering the concentration of angiotensin II), the increase in the concentration of vasodialysic kinin and prostaglandin as well as an indirect decrease in the synthesis of catecholamines and overall sympathetic activity. The effect of this action is the relaxation of

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vessels and diminution of the volume of circulating blood, which leads to a decrease of pressure and peripheral resistance (3, 4).

This group of medicines include such compounds as: benazepril, chinapril, cilazapril, enalapril, imidapril, fosinopril, captopril, lisinopril, moexipril, perindopril, ramipril, spirapril, trandolapril and zofenopril, in form of bases and salts of various acids.

From the recent literature on the subject, it can be concluded that several methods were used for the separation and determining enantiomers of chiral compounds, belonging to the group of ACE inhibitors in pharmaceutical products.

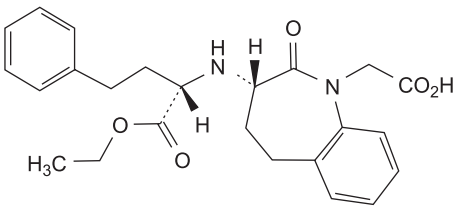
The authors applied mostly electrochemical methods along with the use of enanotioselective membrane electrodes. In this manner there determined enantiomers of: captopril (5, 6), perindopril (7, 8) as well as enalapril and ramipril (8).

The other method applied to separate isomers was the method of HPLC (9, 10). Diastereoisomers of captopril were determined in the column Chirobiotic T by Aster with application of detectors DAD or MS (9).

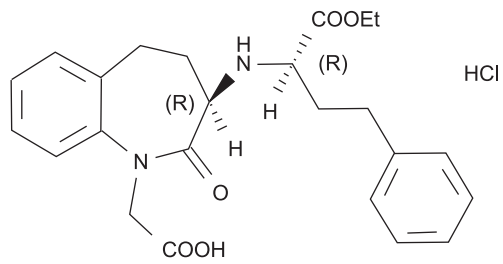
In the available literature, there was only one work on the separation of enantiomers of benazepril. The determination was performed applying a HPLC method wth the column Chiral AGP and the use of DAD detector (10).

The subject of the studies is benazepril hydrochloride, belonging to the group of ACE inhibitors. This compound contains two stereogenic centers, but is currently available as a single enantiomer (S,S configuration) for the treatment of hypertension. Its enantiomer (R,R configuration) and the diastereoisomeric pair (R,S and S,R) can be regarded as impurities. Chemical names are listed below.

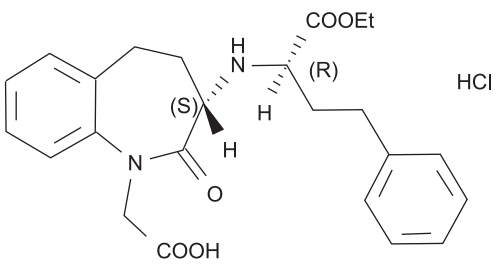
Enantiomers may differ in biological activity as well as therapeutic, pharmacological and pharmacokinetic effects. It is significant to acquire knowledge on susceptibility of chiral compounds to activity of factors which cause enantiomerization. The aim of this study was to estimate stereochemical stability of S,S isomer of benazepril hydrochloride as well as to examine its potential susceptibility to conversion in the active substance and in Lisonid tablets.



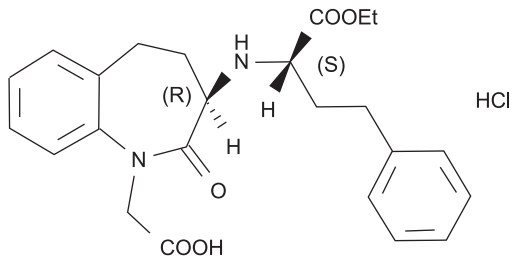

BENAZEPRIL HYDROCHLORIDE – S,S
[(3S)-3-[[(1S)-1-(ethoxycarbonyl)-3-phenylpropyl] amino]-2-oxo-2,3,4,5-tetrahydro-1H-1-benzazepin-1-yl] acetic acid hydrochloride




BENAZEPRIL HYDROCHLORIDE – R,R
[(3R)-3-[[(1R)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-2-oxo-2,3,4,5-tetrahydro-1H-1-benzazepin-1-yl] acetic acid hydrochloride




BENAZEPRIL HYDROCHLORIDE – S,R
[(3S)-3-[[(1R)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-2-oxo-2,3,4,5-tetrahydro-1H-1-benzazepin-1-yl] acetic acid hydrochloride




BENAZEPRIL HYDROCHLORIDE – R,S
[(3R)-3-[[(1R)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]-2-oxo-2,3,4,5-tetrahydro-1H-1-benzazepin-1-yl] acetic acid hydrochloride

## EXPERIMENTAL

### Materials for analysis and instrumentation

Analytically pure and high purity reagents for HPLC by Rathburn. Modifiers: sodium salt of sulfobutylether β-cyclodextrin (SBE β-CNNa), tetrabutylammonium bisulfate ($TBAHSO_4$).

Liquid chromatograph Dionex *Ultimate 3000* with a spectrophotometric detector. Liquid chromatograph Shimadzu with a spectrophotometric detector. UV lamp produced by Hanau. 1000 W UV lamp. Drier produced by Memmert.

Chromatographic plates: silica gel 60 $F_{254}$ by Merck, Silica gel RP 18 $F_{254S}$ by Merck, Chiralplate to separate enantiomers (Silica gel RP with $Cu^{2+}$) by Macherey-Nagel.

HPLC columns by Daicel Chemical Industries: Chiralcel OD (250 × 4.6 mm × 10 μm), Chiralcel OC (250 × 4.6 mm × 5 μm), Chiralcel OJ (250 × 4.6 mm × 5 μm), Chiraspher NT (250 × 4.0 mm × 5 μm), Chiral AGP (150 × 4.0 mm × 5 μm).

### Reference materials

Benazepril hydrochloride (S,S enantiomer) Ref. St. s. 959CEE, CGP 42454 (R,S diastereoisomer) Ref. St. s. CGP 42454-A/II, CGP 42456 (R,R enantiomer) Ref. St. s. CGP 42456-A/II, CGS 14829 (S,R diastereoisomer) Ref. St. s. CGS 14829-A/II.

### Active substance

Benazepril hydrochloride s. 08/94 (Novartis).

### Medicinal product

Lisonid – coated tablets 20 mg s.117885 (Pharma Arzneimittel GmbH).

### Methods of separation and determination of enantiomers of benazepril hydrochloride

### Thin layer chromatography (TLC)

At the first stage of the studies, optimal systems for TLC, providing separation of enantiomers of the studied compound were searched.

The following standard solutions were prepared: benazepril hydrochloride – S,S enantiomer, CGP 42454 – R,S diastereoisomer, CGP 42456 – R,R enantiomer and CGS 14829 – S,R diastereoisomer with concentration of 1 mg/mL in methanol and the mixture of all isomers with concentration of 1 mg/mL each.

On chromatographic plates: silica gel 60 $F_{254}$, silica gel RP 18 $F_{254S}$ and Chiralplate (2 cm from the edge and 2 cm from the bottom) there was transferred 10 μg of each of the isomers separately and their mixture. The plates were developed in various mobile phases, both with modifiers and without them:

I. chromatographic plates silica gel 60 $F_{254}$ and mobile phase (with modifier): acetonitrile – 0.5% SBE β-CNNa – 0.02 M $TBAHSO_4$ (8 : 1 : 1, v/v/v);
II. chromatographic plates silica gel 60 $F_{254}$ and mobile phase (with modifier): acetonitrile – 0.5% SBE β-CNNa (8 : 2, v/v);
III. chromatographic plates silica gel RP 18 $F_{254S}$ and mobile phase (with a modifier): acetonitrile – 0.5% SBE β-CNNa (1 : 1, v/v);
IV. chromatographic plates silica gel RP 18 $F_{254S}$ and mobile phase (with a modifier): acetonitrile – 0.5% SBE β-CNNa – triethylamine (20 : 20 : 0.1, v/v/v);
V. chromatographic plates silica gel RP 18 $F_{254S}$ and mobile phase (with a modifier): acetonitrile – 0.5% SBE β-CNNa – trifluoroacetic acid (20 : 20 : 0.1, v/v/v);
VI. chromatographic plates Chiralplate and mobile phase: methanol – acetonitrile – water (2 : 3 : 5, v/v/v);

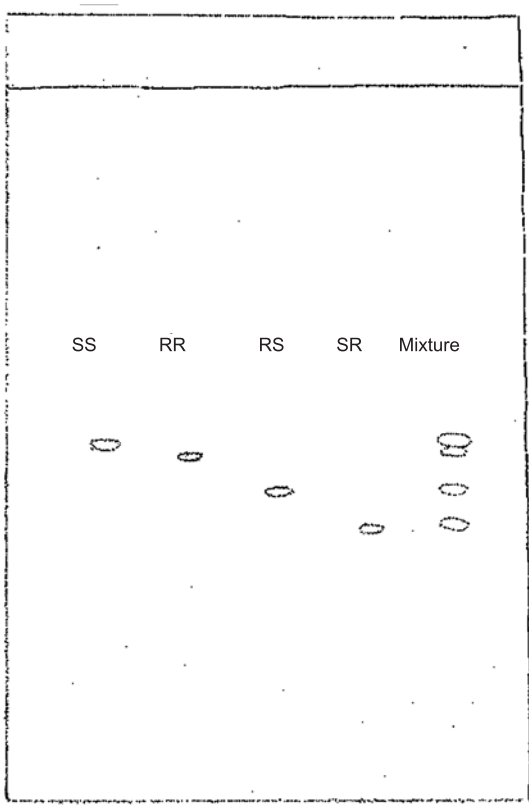



Figure 1. Chromatogram (TLC) of benazepril hydrochloride enantiomers (System IX)

Table 1. Rf values for tested enantiomers of benazepril hydrochloride in selected chromatographic systems.

| Benazepril hydrochloride enantiomers | System I Rf | System II Rf | System III Rf | System IV Rf | System V Rf | System VI Rf | System VII Rf | System VIII Rf | System IX Rf |
|---|---|---|---|---|---|---|---|---|---|
| S,S enantiomer | 0.80 | 0.80 | 0.26 | 0.29 | 0.30 | 0.39 | 0.57 | 0.42 | 0.44 |
| R,R enantiomer | 0.79 | 0.79 | 0.25 | 0.30 | 0.33 | 0.36 | 0.55 | 0.38 | 0.42 |
| S,R diastereoisomer | 0.84 | 0.84 | 0.25 | 0.26 | 0.33 | 0.34 | 0.50 | 0.33 | 0.36 |
| R,S diastereoisomer | 0.84 | 0.83 | 0.26 | 0.26 | 0.34 | 0.29 | 0.50 | 0.29 | 0.31 |

Chromatographic systems: I. plates silica gel 60 $F_{254}$ and mobile phase (with modifier): acetonitrile – 0.5% SBE β-CNNa – 0.02 M $TBAHSO_4$ (8 : 1 : 1, v/v/v); II. plates silica gel 60 $F_{254}$ and mobile phase (with modifier): acetonitrile – 0.5% SBE β-CNNa (8 : 2, v/v); III. plates silica gel RP 18 $F_{254S}$ and mobile phase (with a modifier): acetonitrile – 0.5% SBE β-CNNa (1 : 1, v/v); IV. plates silica gel RP 18 $F_{254S}$ and mobile phase (with a modifier): acetonitrile – 0.5% SBE β-CNNa – triethylamine (20 : 20 : 0.1, v/v/v); V. plates silica gel RP 18 $F_{254S}$ and mobile phase (with a modifier): acetonitrile – 0.5% SBE β-CNNa – trifluoroacetic acid (20 : 20 : 0.1, v/v/v); VI. plates Chiralplate and mobile phase: methanol – acetonitrile – water (2 : 3 : 5, v/v/v); VII. plates Chiralplate and mobile phase: methanol – acetonitrile – water – triethylamine (20 : 30 : 50 : 0.5, v/v/v/v); VIII. plates Chiralplate and mobile phase: methanol – acetonitrile – water – trifluoroacetic acid (20 : 30 : 50 : 0.5, v/v/v/v); IX. plates Chiralplate and mobile phase: methanol – acetonitrile – water – 1 mM copper(II) acetate (4 : 2 : 4, v/v/v) with saturation with glacial acetic acid for one hour.

VII. chromatographic plates Chiralplate and mobile phase: methanol – acetonitrile – water – triethylamine (20 : 30 : 50 : 0.5, v/v/v/v);

VIII. chromatographic plates Chiralplate and mobile phase: methanol – acetonitrile – water – trifluoroacetic acid (20 : 30 : 50 : 0.5, v/v/v/v);

IX. chromatographic plates Chiralplate and mobile phase: methanol – acetonitrile – water – 1 mM copper(II) acetate (4 : 2 : 4, v/v/v) with saturation with glacial acetic acid for one hour.

After air drying of the plates, the spots positions were determined in 254 nm UV light.

TLC chromatogram of separating enantiomers of benazepril hydrochloride in the system IX is presented in Figure 1.

The obtained Rf values for the studied enantiomers are presented in Table 1.

**High-performance liquid chromatography (HPLC)**

At the next stage of the studies, an optimal system providing the separation of enantiomers of benazepril hydrochloride with HPLC was sought.

Standard solutions were prepared: benazepril hydrochloride (S,S enantiomer), CGP 42454 (R,R enantiomer), CGP 42454 (R,S diastereoisomer) and CGS 14829 (S,R diastereoisomer) with concentration of 1 mg/mL in methanol.

On chromatographic columns: Chiralcel OC, Chiralcel OD, Chiralcel OJ, Chiraspher NT and Chiral AGP 10 μL of each of prepared methanol solutions of enantiomers and diastereoisomers was transferred, using various mobile phases.

The separation (partial or total) of particular enantiomers was obtained in the following three HPLC systems:

I. Chiralcel OD column (250 × 4.6 mm × 10 μm), mobile phase: hexane – isopropanol – trifluoroacetic acid (850 : 150 : 1, v/v/v), column temperature: 30°C, flow rate: 1 mL/min, wavelength λ = 254 nm;

II. Chiralcel OC column (250 × 4.6 mm × 5 μm), mobile phase: hexane – isopropanol – trifluoroacetic acid (850 : 150 : 1, v/v/v), column temperature: 30°C, flow rate: 0.8 mL/min, wavelength λ = 254 nm;

III. Chiral AGP (150 × 4.0 mm × 5 μm), mobile phase: phosphate buffer pH = 6.0 – methanol (80 : 20, v/v), column temperature: 30°C, flow rate: 0.9 mL/min, wavelength λ = 240 nm.

The obtained retention times for particular enantiomers are presented in Table 2.

Total separation of studied enantiomers was obtained in system III (Fig. 2) for a mixture of all enantiomers of benazepril hydrochloride: S,S (con-

Table 2. Retention times of benazepril hydrochloride enantiomers in selected HPLC systems.

| Benazepril hydrochloride enantiomers | Retention times in HPLC System I [min] | Retention times in HPLC System II [min] | Retention times in HPLC System III [min] |
|---|---|---|---|
| S,S enantiomer | 21.0 | 9.7 | 6.9 |
| R,R enantiomer | 15.5 | 7.8 | 13.0 |
| S,R diastereoisomer | 12.5 | 10.5 | 15.3 |
| R,S diastereoisomer | 15.0 | 9.6 | 25.4 |

Systems: I. Chiralcel OD column (250 × 4.6 mm × 10 µm), mobile phase: hexane – isopropanol – trifluoroacetic acid (850 : 150 : 1, v/v/v), column temp. 30°C, flow rate 1 mL/min, λ = 254 nm; II. Chiralcel OC column (250 × 4.6 mm × 5 µm), mobile phase: hexane – isopropanol – trifluoroacetic acid (850 : 150 : 1, v/v/v), column temp. 30°C, flow rate 0.8 mL/min, λ = 254 nm; III. Chiral AGP (150 × 4.0 mm × 5 µm), mobile phase: phosphate buffer pH = 6.0 – methanol (80 : 20, v/v), column temp. 30°C, flow rate: 0.9 mL/min, λ = 240 nm.

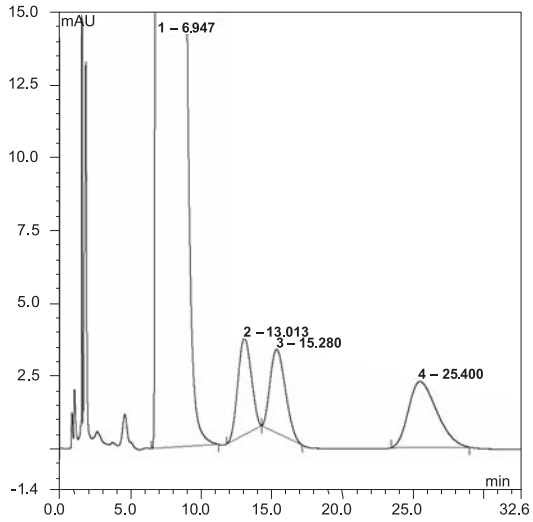



Figure 2. Chromatogram (HPLC) of benazepril hydrochloride enantiomers (System III)

centration of 1 mg/mL) and R,R, R,S and S,R (concentration of 0.01 mg/mL) on the column Chiral AGP.

In the systems I and II only partial separation of enantiomers was obtained.

**Susceptibility of benazepril hydrochloride to stereoconversion for active substances and in tablets**

The examination included the active substance of benazepril hydrochloride and coated tablets Lisonid 20 mg.

***Preparation of samples***

Forty milligrams of benazepril hydrochloride and amount of mass tablet of Lisonid, adequate to 40 mg of active substance, (mass of ca. 2 tablets) were subjected to the impact of different stress factors:

1. 1 M sodium hydroxide solution (10 mL) and temperature 40°C;
2. 0.05 M sodium hydroxide solution (10 mL) and temperature 40°C;
3. 1 M hydrochloric acid solution (10 mL) and temperature 40°C;
4. 6% hydrogen peroxide solution (10 mL) and temperature 40°C;
5. temperature 40°C;
6. 1000 W UV light, 3 times for 10 min.

Susceptibility of benazepril hydrochloride to stereoisomerism in conditions no. 3, 4 and 5 was defined after 1 and 6 weeks; in conditions no. 1 after 1 week and in conditions no 2 after 6 weeks. Samples in conditions no. 6 were examined directly after having been exposed to UV light.

***Preparation of solutions of samples and reference materials***

Prepration of the mixture of enantiomers of benazepril hydrochloride

Solutions of enantiomers of benazepril hydrochloride: R,R, R,S and S,R with concentration of 0.5 mg/mL in methanol were prepared.

To a volumetric flask, 50 mg of S,S enantiomer of benazepril hydrochloride was weighed, filled with 1 mL of methanol solutions of enantiomers: R,R, R,S and S,R, 10 mL methanol were added and then filled with the mobile phase to 50.0 mL.

Final concentration of S,S benazepril hydrochloride enantiomer was 1 mg/mL, those of enantiomers R,R, R,S and S,R were 0.01 mg/mL.

Preparation of examined samples

The samples after conditions no. 1–3 were filtered through membrane filters (0.45 µm) and were

adjusted to pH ca. 5.0. Samples (2.5 mL) of solutions were transferred to volumetric flasks and filled with mobile phase to 10 mL. Initial concentration of S,S benazepril hydrochloride enantiomer was 1 mg/mL.

The samples after conditions no. 4 were filtered with membrane filters (0.45 μm). Samples (2.5 mL) of solutions were transferred to volumetric flasks and filled with a mobile phase to 10 mL. Initial concentration of S,S benazepril hydrochloride enantiomer was 1 mg/mL.

From the samples after conditions no. 5 and 6, 10 mg of substance or the amount of mass tablet equivalent to 10 mg of active substance was weighed to volumetric flasks and 1 mL of methanol was added. Samples were shaken for 5 mintes on ultrasonic bath and filled with a mobile phase to 10 mL. The samples were filtered with membrane filters (0.45 μm). Initial concentration of S,S benazepril hydrochloride enantiomer was 1 mg/mL.

For examination of the prepared solutions the conditions of system III were applied: column: Chiral AGP (150 × 4.0 mm × 5 μm); mobile phase: phosphate buffer pH = 6.0 – methanol (80 : 20, v/v); wavelength: λ = 240 nm; column temperature: 30°C; flow rate: 0.9 mL/min; injection volume: 20 μL.

## DISCUSSION OF RESULTS

Knowledge on the configurational stability of substances is of utmost importance in case of chiral medicines. It is significant to estimate the stereochemical durability of enantiomers and to examine their potential susceptibility to conversion under various factors.

As a result, there was made an attempt to examine the stereochemical durability of S,S benazepril hydrochloride enantiomer in an active substance and medicinal product – coated tablets Lisonid 20 mg.

At the first stage of the study, the chromatographic methods of separation of examined enantiomers were explored applying TLC and HPLC methods.

The best separation with the use of the TLC method, was obtained with the following system: chromatographic plates Chiralplate and mobile phase: methanol – acetronitrile – 1 mM copper(II) acetate (4 : 2 : 4, v/v/v) with saturation of glacial acetic acid for 1 h.

The Rf values amounted to: S,S enantiomer – 0.44, R,R enantiomer – 0.42, S,R diastereoisomer – 0.36, R,S diastereoisomer – 0.31, respectively. (Table 1, Fig. 1).

For separation with HPLC method, several chiral columns as well as mobile phases were tested. The system, described in Ph. Eur., proved to be the best one: Chiral AGP (150 × 4.0 mm × 5 μm) and mobile phase: phosphate buffer pH = 6.0 – methanol (80 : 20, v/v), column temperature: 30°C, flow rate: 0.9 mL/min, wavelength λ = 240 nm. The obtained retention times were: S,S enantiomer – 6.9 min, R,R enantiomer – 13.0 min, S,R diastereoisomer – 15.3 min, R,S diastereoisomer – 25.4 min (Table 2, Fig. 2).

The TLC system no. IX gave only identification of enantiomers and could be used in hight-speed test of stereochemical stability research of S,S enantiomer of benazepril hydrochloride.

Method that gave the highest resolution of benazepril hydrochloride enantiomers was HPLC with Chiral AGP column. This is a validated, precise, sensitive and accurate method, that seems to be the best for identification and determination for all studied compounds.

This system was subsequently used for examination of stereochemical stability of benazepril hydrochloride in the substance and Lisonid – coated tablets 20 mg.

Active substance – benazepril hydrochloride and coated tablets Lisonid 20 mg were subjected to the impact of different stress factors named above.

In 1 M sodium hydroxide solution, at 40°C, there was a total decomposition of benazepril hydrochloride in the substance and tablets to unknown contaminants within one week. There was no transfer of S,S enantiomer into other stereoisomers.

In 0.05 M sodium hydroxide solution, at 40°C, there was a decomposition of benazepril hydrochloride to unknown contaminants, in the substance in 100% and in tablets in 95% within one week.

Benazepril hydrochloride in 1 M hydrochloric acid solution and temperature of 40°C, both in the substance and tablets underwent decomposition to unknown contaminants in 20% after one week and in 90% after 6 weeks.

The influence of 6% hydrogen peroxide solution and temperature of 40°C on the compound, resulted in the decomposition to unknown contaminants in 26% of the substance and in 95% in tablets after 1 week. After 6 weeks there was a decomposition of 50% in the substance. In tablets it remained at the same level (95%).

Benazepril hydrochloride in the substance and medicinal product at the temperature of 40°C after 1 week and after 6 weeks was stable and did not change.

Treatment with 1000 W UV light (3 × 10 min) on benazepril hydrochloride did not cause any

changes in the substance, while in tablets, there was a decomposition of 98% to unknown contaminants.

It was found that none of the applied stress factors caused the transformation of the S,S enantiomer of benazepril hydrochloride in the substance and tablets to other identified stereoisomers – only the compound decomposition occurred.

## CONCLUSIONS

1. Conditions of separation of benazepril hydrochloride enantiomers were developed using TLC and HPLC.
2. The HPLC method could be used for identification and separation of isomers in the active substance and medicinal product.
3. Stability examination of benazepril hydrochloride in substance and Lisonid tablets as well as separation of stereoisomers by means of the HPLC method were performed. In the applied conditions, S,S benazepril hydrochloride enantiomer did not transform to other identified stereoisomers – only the compound decomposition occurred.

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