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EXAMINATION OF THE OPTICAL PURITY OF VORICONAZOLE ON THE BRUSH-TYPE STATIONARY PHASE

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ABSTRACT. The purpose of this research is to develop an HPLC method for determining the content of voriconazole enantiomers in powder for solution for infusion. Chromatographic analysis was conducted using a brush-type (Pirkle-type) stationary phase with isocratic elution. The mobile phase comprised a triethylamine-formate buffer system at pH 5.0/methanol/acetonitrile in an 80/15/5 (v/v/v) ratio, flowing at 1.0 mL/min. The temperature of the column was maintained at 30°C, and UV detection was carried out at 256 nm. The method was validated in accordance with ICH guidelines. After the selectivity was confirmed, a satisfactory resolution of the voriconazole enantiomers in the powder for solution for infusion was obtained. Order of the elution of the enantiomers was verified by comparing the retention times of the enantiomers in the sample with the corresponding standard of pure voriconazole isomer (impurity D). Linearity for voriconazole impurity D was assessed within a concentration range from 0.600 µg/mL to 1.500 µg/mL (correlation coefficient > 0.9999). Method precision was confirmed through intra-assay precision and inter-assay precision (RSD < 2%). The accuracy of the method was tested by analyzing three different concentrations, with analytical yields ranging from 93.75% to 102.27%. The validated method proved to be suitable for routine analysis in testing optical purity of voriconazole powder for solution for infusion.

Keywords: voriconazole, enantiomer, optical purity, HPLC, brush-type stationary phase.

INTRODUCTION

Voriconazole is a triazole antifungal drug. This drug inhibits cytochrome P-450-dependent demethylation of fungal 14α-lanosterol, which is a key step in fungal ergosterol

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biosynthesis. It shows more selectivity towards fungal cytochrome P-450 enzymes than mammalian cytochrome P-450 enzyme.

Voriconazole is a powerful antifungal medication indicated for use in both adults and children aged two years and older. It is primarily used for the treatment of:

- invasive aspergillosis;
- candidiasis in non-neutropenic patients;
- fluconazole-resistant, severe, invasive *Candida* infections;
- severe fungal infections caused by *Scedosporium spp*. and *Fusarium spp*.;

Voriconazole is a critical medication in the management of these serious fungal infections, providing an essential option for patients who may not respond to other treatments (RANG *et al.*, 2005; VARAGIĆ and MILOŠEVIĆ, 2012; NOVAKOVIĆ, 2011; BEALE, 2011).

Voriconazole is a white or off-white powder. It has poor aqueous solubility and it is easily soluble in acetone and in dichloromethane. It contains two chiral centers and four optical isomers. Voriconazole (Fig. 1A), which correspond to 2R, 3S stereoisomer has therapeutic effect and its enantiomer is classified as impurity D (Fig. 1B). Voriconazole has a monograph in Ph. Eur. 11 (EUROPEAN PHARMACOPOEIA, 2025) and USP 36 (UNITED STATES PHARMACOPEIA, 2024). Its enantiomeric purity is controlled by liquid chromatography (LC) (EUROPEAN PHARMACOPOEIA, 2025).

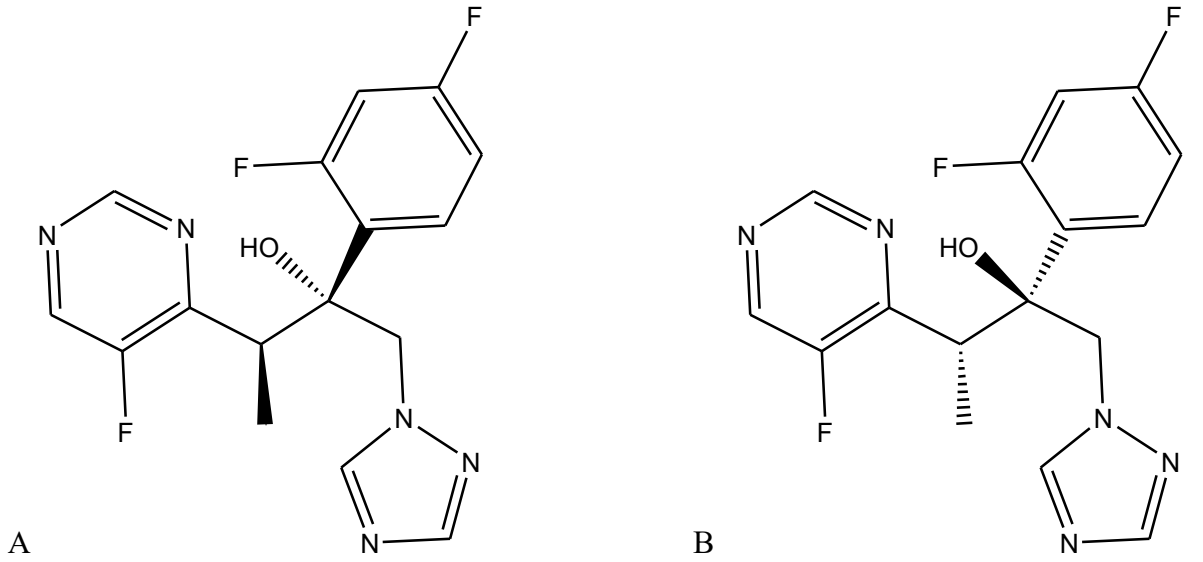


Figure 1. Chemical structure of A) voriconazole and B) voriconazole impurity D (voriconazole enantiomer).

Chiral high performance liquid chromatography (HPLC) is widely used for analyzing enantiomers due to its chiral nature. It is a separation technique based on the different distribution of substances between two phases: stationary and mobile (WAHAB *et al.*, 2018). Chiral HPLC can be direct or indirect. Direct chromatographic analysis can involve incorporating a chiral selector into the mobile phase or utilizing a chiral stationary phase. The chiral selector builds covalent or non-covalent bonds with the enantiomers of the racemic mixture, giving diastereoisomeric compounds or diastereoisomeric aggregates that differ from each other in physicochemical properties. The use of chiral stationary phases for the separation of enantiomers is the most common method in enantiomer analysis today. The stationary phase consists of an optically pure substance that can be chemically bonded or physically applied to the carrier. Indirect chromatographic analysis involves derivatization of the sample with a chiral derivatization agent (optically pure reagent), and then separation of the obtained diastereoisomers by classical chromatography. For the detection of enantiomers,

a UV/VIS detector is most oftenly used (NIKOLIN and ŠOBER, 2003; IVKOVIĆ, 2004; MALENOVIĆ and STOJANOVIĆ, 2010).

A wide range of chiral stationary phases, based on cyclodextrins, proteins, antibiotics, polysaccharide derivatives, chiral acrylamides and brush-type stationary phases have been used to address a greater range of chiral separation problems.

Brush-type stationary phases, also referred to as Pirkle-type or donor-acceptor stationary phases, are commonly used in chromatography. The first synthesized and most widely used is (R)-3,5-dinitrobenzoylphenylglycine (DNBPG) stationary phase whose structure is presented in the Figure 2 (PIRKLE *et al.*, 1980; PIRKLE *et al.*, 1981).

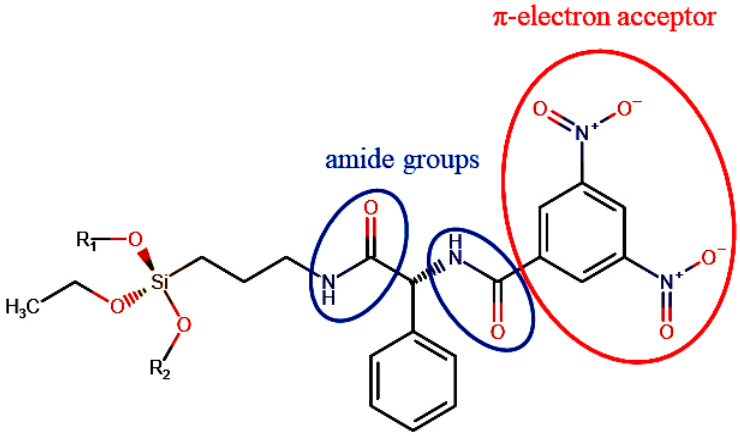


Figure 2. Structure of 3,5-dinitrobenzoylphenylglycine (DNBPG) stationary phase

This phase is a π-electron acceptor and is used to separate enantiomers that contain π-electron donor groups in their structure (aromatic systems). The presence of two amide groups enables the stationary phase to form hydrogen bonds with enantiomers as well as dipole-dipole interactions. Pirkle-type stationary phases where the chiral selector is a π-electron donating group (naphthyl, phenyl) are usually used for the separation of enantiomers that are structurally amines, aminoalcohols, amino acids, alcohols, thiols, carbamates or carbamides (PIRKLE *et al.*, 1980).

Hybrid Pirkle-type phases are mixed π-electron acceptor - π-electron donor and can be considered universal. Enantiomer recognition on these stationary phases takes place via at least three interaction sites, one of which is stereochemically dependent. Diastereoisomeric complexes are formed through a combination of π-π interactions, hydrogen bonds, steric interactions, and dipole-dipole interactions. Among these, two interactions must be attractive, while the third can be either attractive or repulsive. In normal phase chromatography, a mixture of non-polar organic solvents and polar modifiers (such as lower alcohols) can serve as the mobile phase. Conversely, Pirkle-type stationary phases are also suitable for reverse-phase systems, where the mobile phase consists of a mixture of buffers and polar organic solvents. In order to increase the enantioselectivity on Pirkle-type stationary phases, it is possible to derivatize the tested mixture with an achiral reagent (FERNANDES *et al.*, 2013; PIRKLE and POCHAPSKY, 1989).

In the literature there are several papers dealing with the problem of testing the optical purity of voriconazole (SERVAIS *et al.*, 2014; OWENS *et al.*, 1999; LIU and ZOU, 2008; SHAIKH and PATIL, 2012). Optimization of the conditions and validation of the HPLC method for testing optical purity in the stability studies of voriconazole solutions were described in a paper of Servais *et al.* (SERVAIS *et al.*, 2014). As a chiral selector, the authors used 4-chloro-3-methylphenylcarbamate in a solvent mixture of acetonitrile/methanol/diethylamine/trifluoroacetic acid linked to the stationary phase. Owens et al. used several different chiral stationary phases (α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, hydroxypropyl-beta-

cyclodextrin, and hydroxyethyl-beta-cyclodextrin), but none of these chiral stationary phases proved selective enough for separation of four stereoisomers of voriconazole (OWENS *et al.*, 1999). Liu Y and Zou H used derivatized cellulose coated with 3,5-dimethylphenylcarbamate to separate the enantiomers of voriconazole. The prepared chiral stationary phase showed good ability to recognize and separate enantiomers when compared to corresponding commercial columns (LIU and ZOU, 2008). Reverse-phase HPLC methods have been designed to quantitatively analyze voriconazole, along with its degradation products and diastereoisomeric impurities in tablet formulations. The chromatographic separation was performed on an Inertsil ODS 3V column (150 x 4.6 mm, 5 µm). The mobile phase was composed of 0.05 M sodium dihydrogen phosphate and a mixture of acetonitrile and methanol in a 90:10 (v/v) ratio, with a flow rate of 1.2 mL/min, and detection was carried out at 256 nm (SHAIKH and PATIL, 2012).

Given that voriconazole structurally meets the criteria for enantioseparation on a Pirkle-type (or brush-type) stationary phase, this phase was selected for the experimental investigation. The objective of this research was to determine the chromatographic parameters necessary for the separation of voriconazole enantiomers using a brush-type stationary phase. Additionally, the study sought to validate the HPLC method for determining the content of voriconazole enantiomers, ensuring specificity, linearity, accuracy, and precision. This validated method was then applied to test the optical purity of commercially available voriconazole lyophilized powder for solution for infusion.

MATERIALS AND METHODS

Equipment and reagents

Voriconazole and its impurity D (enantiomer) were provided by LGC Limited (UK, Teddington). Voriconazole powder for solution for infusion, strength 200mg (one milliliter of solution after reconstitution contains 10 mg of voriconazole) was supplied by Alvogen, Inc. (USA, Pine Brook).

Triethylamine (TEA) with a purity of ≥ 99.5%, acetonitrile (ACN) of HPLC grade, methanol (MeOH) of HPLC grade, and formic acid with a purity of ≥ 99.5% were all sourced from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). Sodium chloride, citric acid, sodium citrate (all pro analysis grade), and 35% hydrochloric acid (pro analysis grade) were obtained from Merck (Darmstadt, Germany). Purified water was produced using a TKA GenPure water purification system from Thermo Electron LED GmbH, Germany.

Chromatographic conditions

The analysis was conducted using a Dionex UltiMate 3000 UHPLC system (Thermo Fisher Scientific, USA) equipped with a quaternary pump, autosampler, and DAD detector, using Chromeleon 7 as control software software. For the separation, a ChiraSpher® NT chromatographic column (MilliporeSigma, USA), 250 x 4 mm, with a particle size of 4.5 µm, was utilized. The mobile phase comprised 80% triethylamine-formate (buffer (pH 5.0), 5% ACN, and 15% MeOH (v/v/v), and the column temperature was maintained at 30 °C. The flow rate was set to 1.0 mL/min, with an injection volume of 15 µl. UV detection was carried out at 256 nm.

Preparation of solutions

Preparation of TEAF pH 5.0 buffer solution

To prepare the solution, 250 mL of water was transferred to a 1000 mL volumetric flask, 1 mL of TEA was added, and the flask was then filled to the mark with water. The pH of the prepared solution was adjusted to 5 using formic acid.

Preparation of solutions for the system suitability
Sample solution
The lyophilized powder for solution for infusion in the vial was dissolved into 20 mL of water.

Stock solution of voriconazole impurity D standard
The 5 mg of impurity D was accurately transferred to a 50 mL volumetric flask, where it was dissolved in 25 mL of the mobile phase and then topped up to the mark with the same mobile phase (resulting in a concentration of 0.1 mg/mL).

Solution a) 2.5 mL of the prepared solution was transferred into a 10 mL volumetric flask and diluted to the mark with the mobile phase.
Solution b) 2.5 mL of the sample solution was transferred into 10 mL volumetric flask, and 200 µL of the Stock solution of impurity D was added and diluted to the mark with the mobile phase.

Preparation of the solution for the specificity of the method
A mixture of sodium chloride, sodium citrate and citric acid whose pH was adjusted to 7.4 with a 1M solution of hydrochloric acid.

Preparation of impurity D standard solution for the linearity of the method
Stock solution of voriconazole impurity D standard
The 5 mg of impurity D was accurately transferred to a 50 mL volumetric flask, dissolved in 25 mL of the mobile phase, and then brought to volume with the mobile phase to achieve a concentration of 0.1 mg/mL.

Preparation of working standard solutions for the preparation of the calibration curve
Volumes of 60 µL, 80 µL, 100 µL, 120 µL, and 150 µL of the impurity D stock solution were transferred into five 10 mL volumetric flasks, each of which was then filled to the mark with the mobile phase. Concentrations of working solutions ranged from 0.6 µg/mL to 1.5 µg/mL.

Preparation of solutions for the precision of the method
Preparation of solutions for reproducibility assessment
A volume of 100 µL from the stock solution of the impurity D standard was transferred into a 10 mL volumetric flask and then topped up to the mark with the mobile phase, resulting in a concentration of 1 µg/mL.

Preparation of solutions for reproducibility assessment at limit of quantification (LOQ)
A volume of 60 µL from the stock solution of the impurity D standard was transferred into a 10 mL volumetric flask and diluted to the mark with the mobile phase, resulting in a concentration of 0.6 µg/mL.

Preparation of sample solution for inter-assay precision assessment
Another analyst, on the second day, prepared a new set of sample solutions according to the procedure described in the paragraph *Preparation of solutions for reproducibility assessment* and *Preparation of solutions for reproducibility assessment at limit of quantification (LOQ)*.

Preparation of solutions for the accuracy of the method
Stock solution of voriconazole impurity D standard
The preparation procedure was described in the paragraph *Preparation of impurity D standard solutions for the linearity of the method.*

Preparation of solution for analysis of lyophilized voriconazole
Finished product Voriconazole powder for solution for infusion, strength 200mg was dissolved in 20 mL of purified water (c = 10 mg/mL).

Preparation of test solutions for the accuracy of the method
2.5 mL of the voriconazole lyophilizate solution under analysis was transferred into three separate 10 mL volumetric flasks. To each flask, 60 µL, 100 µL, and 120 µL of the impurity D stock solution were added, and then each flask was filled to the mark with the mobile phase.

Preparation of standard solutions for the accuracy of the method
Volumes of 60 µL, 100 µL, and 120 µL from the stock solution of impurity D standard were added to three separate 10 mL volumetric flasks, which were then filled to the mark with the mobile phase.

Preparation of solutions for determining LOQ of voriconazole impurity D
60 µL of the stock solution of impurity D standard was transferred to a 10 mL volumetric flask (paragraph *Preparation of impurity D standard solutions for the linearity of the method*) and diluted to the mark with the mobile phase.

Preparation of solutions for determining LOD of voriconazole impurity D
50 µL of the stock solution of impurity D standard was transferred to a 10 mL volumetric flask (paragraph *Preparation of impurity D standard solution for the linearity of the method*) and diluted to the mark with the mobile phase.

Preparation of solutions for determination of voriconazole impurity D content
Tested sample solution
The lyophilized voriconazole powder for solution for infusion was dissolved in 20 mL of water. From this solution, 2.5 mL was transferred into each of three 10 mL volumetric flasks, and each flask was filled to the mark with the mobile phase.

Solutions were filtered through a 0.45 µm PTFE membrane filter before the injection into the HPLC system.

Carrying out the chromatographic procedure

Under the described chromatographic conditions, the column is conditioned with the selected mobile phase (TEAF buffer pH 5/methanol/acetonitrile 60/40/0.1 (v/v/v), 70:30:0.1 (v/v/v) and 80:20:0.1 (v/v/v)) until a stable base line is reached. Among the chromatographic parameters, the retention time (retention parameter) and the resolution factor as a separation parameter were monitored.

RESULTS AND DISCUSSION

The separation of voriconazole and impurity D on cyclodextrin chiral phases is detailed in the literature and pharmacopoeial methods, and is achieved through the formation of inclusion complexes (SERVAIS *et al*., 2014; OWENS *et al.*, 1999; LIU and ZOU, 2008; SHAIKH and PATIL, 2012; EUROPEAN PHARMACOPOEIA, 2025; UNITED STATES PHARMACOPEIA, 2024). Our work deals with setting up a method for testing the optical purity

of voriconazole, so we validated a method for determining the content of voriconazole enantiomers and not voriconazole.

We opted for donor-acceptor stationary phases, specifically brush-type stationary phases, also referred to as Pirkle-type stationary phases, due to the presence of aromatic systems (Fig. 1) such as benzene, pyrimidine, and 1,2,4 triazole in voriconazole and its enantiomeric impurity. These stationary phases facilitate separation based on the π-π interactions of the aromatic systems of the compounds and 3,5-dinitrobenzoylphenylglycine on the surface of the stationary phase. This paper presents a direct chiral HPLC method for assessing the optical purity of voriconazole in lyophilized powder for solution for infusion. In our research we described and validated method, which utilized a brush-type (Pirkle-type) stationary phase consisting of silica gel coated with poly-(N-acryloyl-(S)-phenylalanine ethyl ester), and an achiral mobile phase composed of 80% TEAF buffer pH 5.0, 15% MeOH, and 5% ACN (v/v/v) as a polar mobile phase.

System Suitability

The parameter that defines the suitability of the chromatographic system for chiral separation is the resolution factor (*R*s). *R*s should be in the range from 1.5 to 3 (MALENOVIĆ and STOJANOVIĆ, 2010). Based on the obtained result for *R*s = 2.1, it can be concluded that the requirement for the system suitability has been met (Fig. 3).

Specificity/selectivity of the method

In order to assess the specificity of the method, a placebo mixture solution containing voriconazole lyophilizate and a solution of the voriconazole impurity D standard were introduced into the chromatographic system. The chromatogram of the voriconazole impurity D standard solution reveals a retention time of 10.72 min for voriconazole impurity D. Conversely, the chromatogram of the placebo mixture does not exhibit any peaks with retention times matching that of voriconazole impurity D, indicating that the proposed method is indeed specific for voriconazole impurity D (Fig. 4).

Linearity of the method

To assess the linearity of the method, a calibration curve was constructed using the least squares method. The calibration curve illustrates how the concentration of the voriconazole impurity D standard solution (consisting of five solutions injected three times into the chromatographic system) correlates with the calculated average peak areas of the voriconazole impurity D standard. The results obtained are detailed in Table 1 and Figure 5.

Based on the obtained data for the correlation coefficient r ($0.9999 \geq 0.9990$) and the significance of the segment on the ordinate ($t_b < t_{tab}$), it can be concluded that the degree of scattering of points around the ideal line is small, i.e. the method is linear for a given range of concentrations.

Method Precision

Method precision was evaluated at two levels:

1. Repeatability (intra-assay precision),
2. Intermediate precision (inter-assay precision).

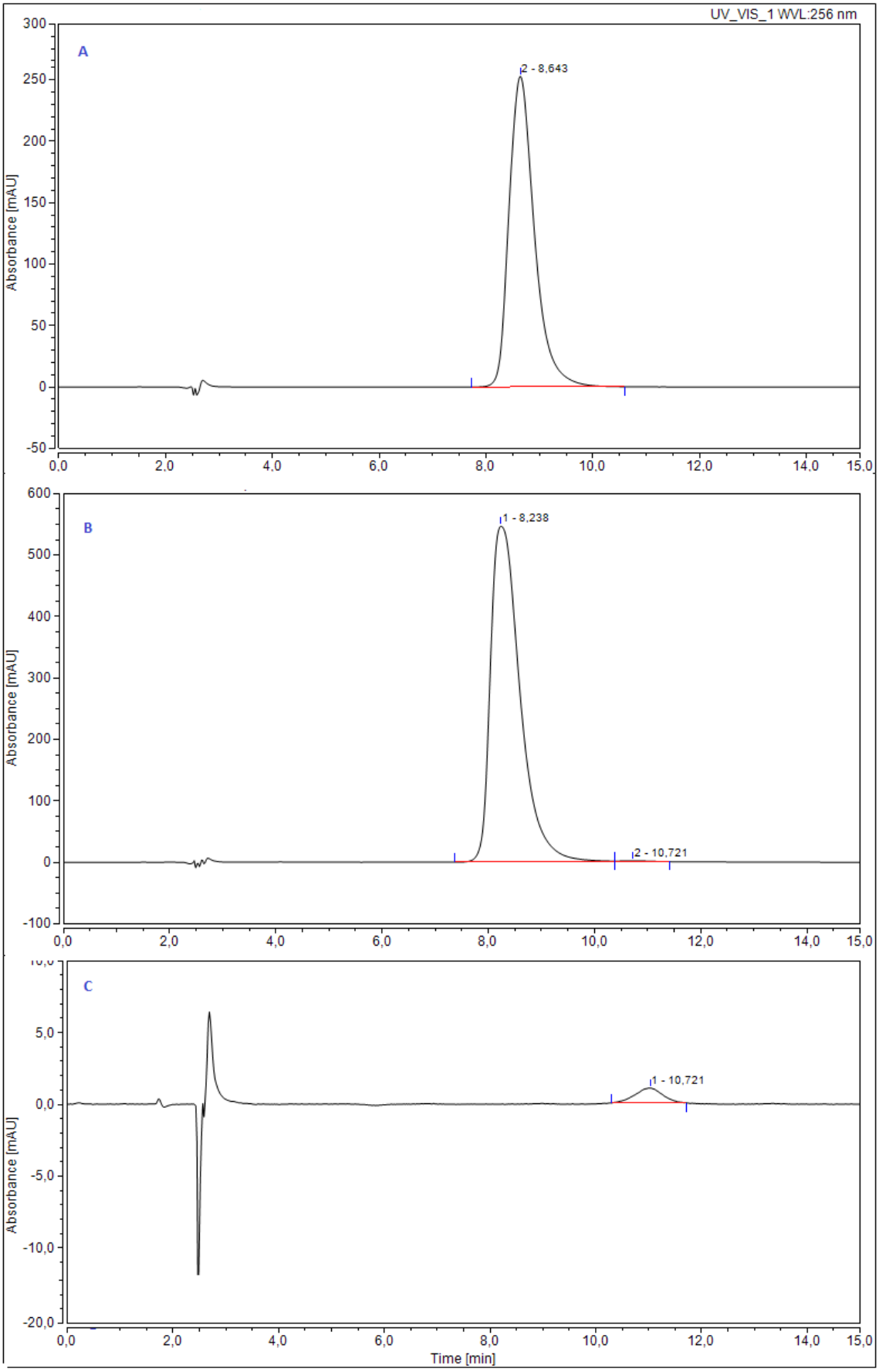
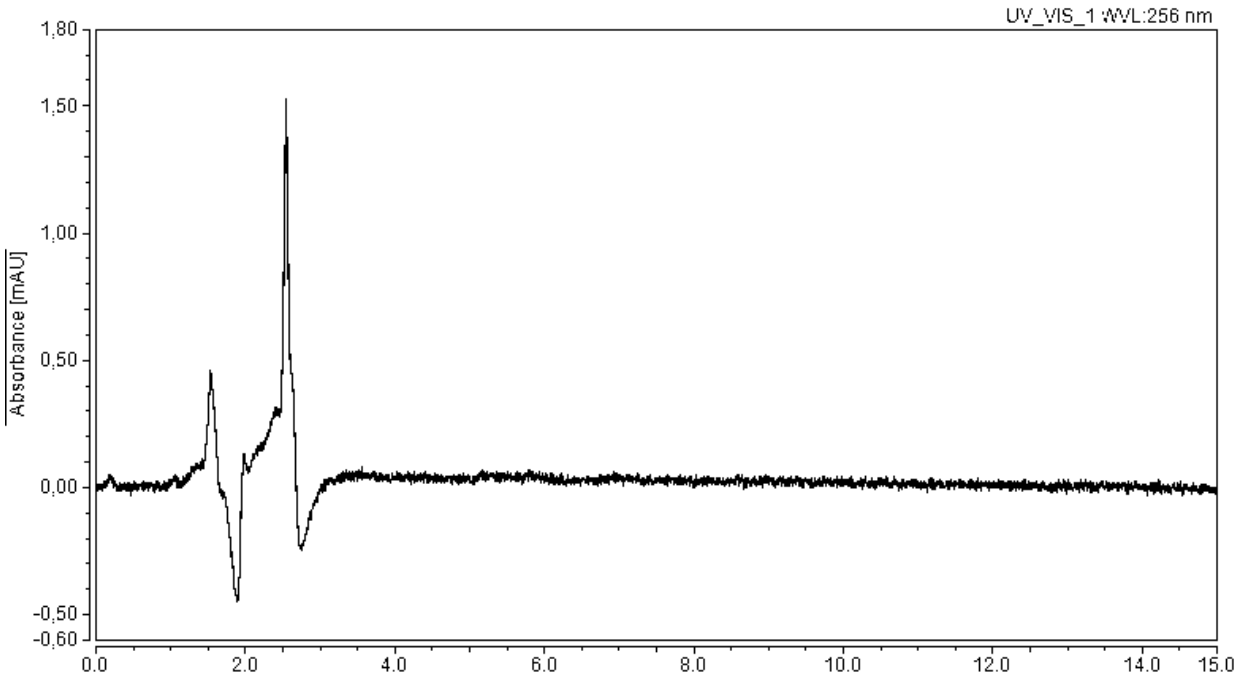


Figure 3. Chromatograms of A) the analyzed solution of voriconazole, B) the spiked solution of voriconazole with impurity D standard and C) the solution of impurity D standard



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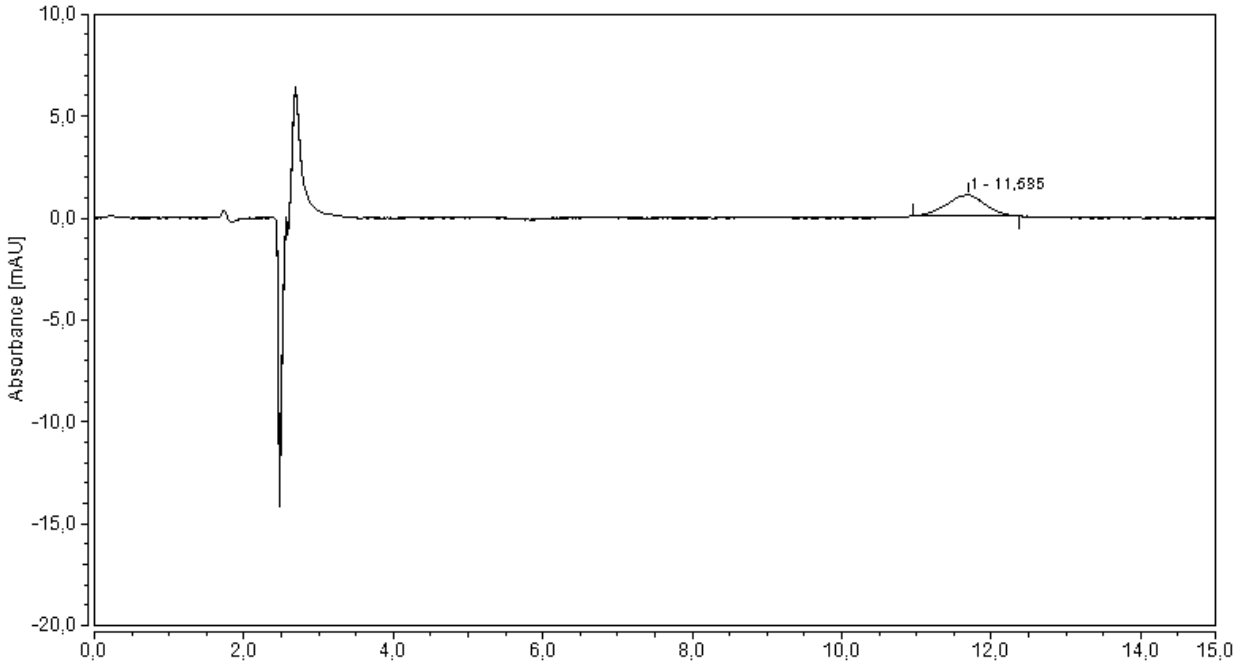

B

Figure 4. Chromatograms of A) placebo mixtures and B) voriconazole impurity D standard

Table 1. Data for calculating the calibration curve

Cimpurity D standard **(µg/mL)**	**As*** **(mAU*s)**
0.600	0.388
0.800	0.508
1.000	0.636
1.200	0.761
1.500	0.954

* mean peak area of voriconazole impurity D standard

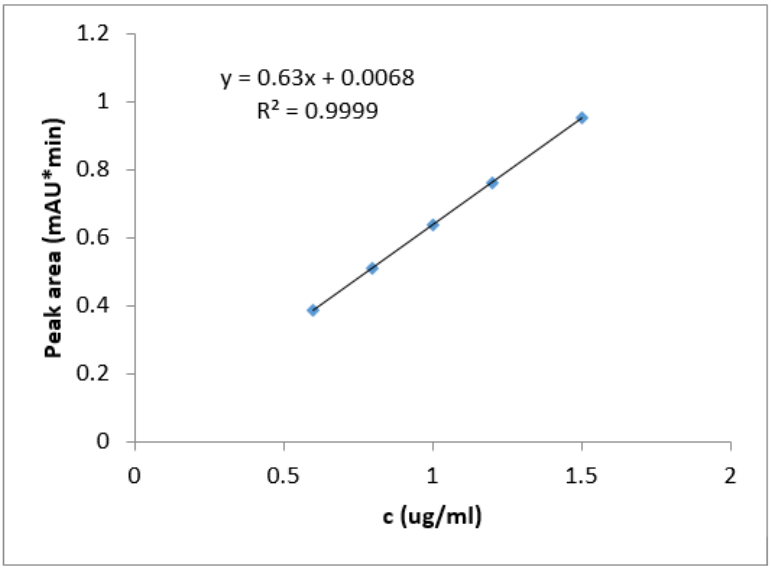

Figure 5. Calibration curve for evaluating the linearity of the method

The parameters for the regression equation are shown in Table 2.

Table 2. Parameters of the regression analysis

Concentration range of voriconazole impurity D (µg/mL)	**y = ax + b**	***R***	***R²***	***t Stat***	***P-value***
0.600-1.500	y = 0.6300 x + 0.0068	0.9999	0.9999	***1.480***	***0.230***

P-value ≥0.05

Table 3. Data for evaluation of the repeatability of the method

Injection concentration of voriconazole impurity D (µg/mL)	**Number of injections**	**As* (mAU*s)**
1.000	1	0.644
	2	0.631
	3	0.632
	4	0.636
	5	0.617
	6	0.625
Mean value		**0.631**
Sd		**0.008**
RSD		**1.309**

*standard peak area

Repeatability

To assess the level of precision of this method, six solutions containing impurity D standard (c = 1 µg/mL) were individually prepared and introduced into the calibrated HPLC

system. Based on the obtained values of the peak areas of voriconazole impurity D, using the straight line equation (paragraph *Linearity of the method*), concentrations of voriconazole impurity D in the tested solutions were calculated for each individual injection. The results are shown in Table 3. Based on the obtained values for RSD < 2%, it can be concluded that the method meets the requirements for reproducibility.

Intermediate precision

Intermediate precision was assessed by a second analyst on a different day who independently prepared six voriconazole sample solutions and injected and analyzed them under the specified chromatographic conditions The results obtained are detailed in Table 4.

Table 4. Data for calculating the intermediate precision (inter-assay precision)

Injection concentration of voriconazole impurity D (µg/mL)	Number of injections	As* (mAU*s)
1.000	1	0.614
	2	0.631
	3	0.622
	4	0.658
	5	0.637
	6	0.649
Mean value		**0.633**
Sd		**0.013**
RSD		**2.042**

* standard peak area

The obtained value for RSD (%) for twelve injections is 2.042, which is less than 3%, thereby fulfilling the requirement for medium precision of the method.

Method accuracy

Three different concentrations of impurity D standard solution were added to prepared voriconazole solutions in order to test the method's accuracy: LOQ, 100%, and 120% relative to the working concentration of 1µg/mL. The solutions were then analyzed to assess the accuracy of the method. The solutions were injected three times into the conditioned chromatography system. The outcomes are presented as a percentage of the calculated yield (recovery) for a specified quantity of the analyzed substance (Tab. 5). The obtained recovery values for all three tested concentrations range from 93.753% to 102.272%, which meets the requirements for the accuracy of the method intended for testing (70-130%).

Limit of quantification and detection

The concentration of impurity D that can be determined/detected by the selected method with reliable precision and accuracy was determined experimentally, using the signal-to-noise method. The limit of quantification was determined to be 0.600µg/mL, while the detection limit was found to be 0.500µg/mL.

Table 5. Results of testing the accuracy of the method

Concentration of voriconazole impurity D (µg/mL)	As* (mAU*s)	Aeks** (mAU*s)	Recovery (%)
		0.279	96.871
0.600	0.288	0.270	93.753
		0.277	96.183
		0.654	96.031
1.000	0.681	0.674	98.972
		0.644	94.574
		0.774	97.853
1.200	0.791	0.809	102.272
		0.781	98.741
	mean value		**97.250**

* peak area of voriconazole D impurity standard

** peak area of voriconazole D impurity from test solution voriconazole powder for solution for infusion

Determination of voriconazole impurity D content in powder for solution for infusion

A validated chromatographic method was employed to quantify the content of voriconazole impurity D in the lyophilized powder intended for solution for infusion. By analyzing the chromatogram of the prepared voriconazole lyophilizate solution, it can be concluded that impurity D was not detected in the tested solution (Fig. 6).

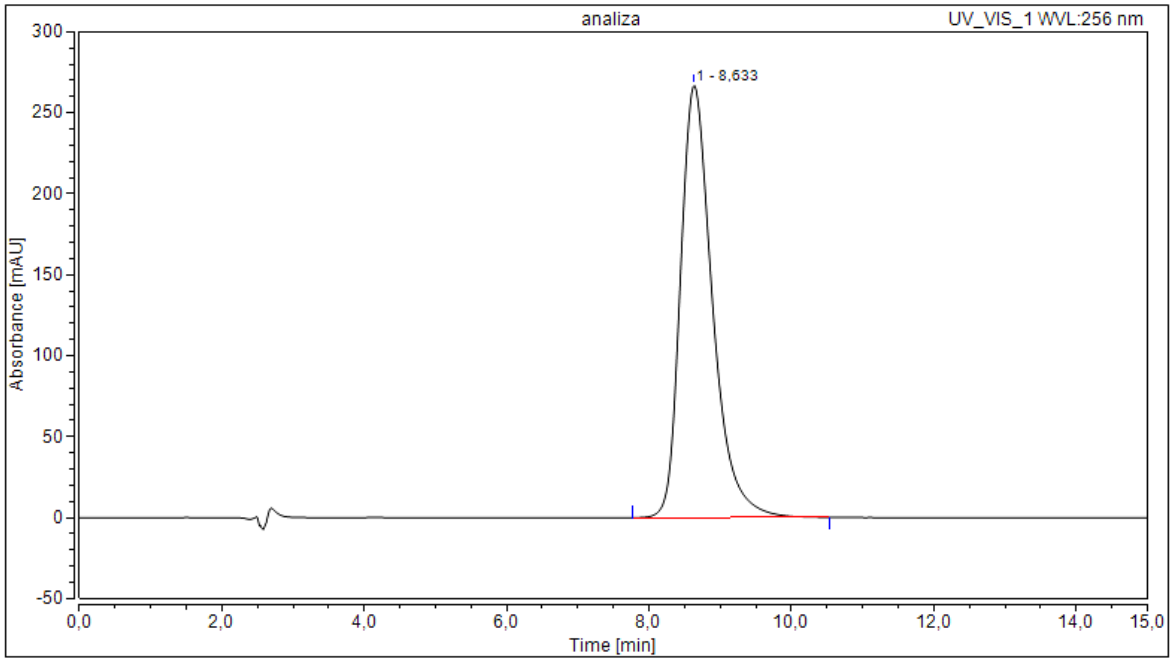

Figure 6. Chromatograms of voriconazole impurity D standard and voriconazole impurity D from analyzed solution of powder for solution for infusion

CONCLUSION

This article outlines the chromatographic parameters that are appropriate for separating voriconazole enantiomers, as well as a verified technique for quantifying the levels of voriconazole enantiomers in powder for infusion solutions. The examination was carried out using the RP HPLC method on a Pirkle-type (or brush-type) stationary phase, with the mobile phase composed of TEAF buffer pH 5/MeOH/ACN in a ratio of 80/15/5 (v/v/v). The technique exhibits linearity within the concentration range of 0.600 µg/mL to 1.500 µg/mL, allowing for accurate and precise determination. Based on the obtained values for RSD, which are for repeatability <2%, intermediate precision <3%, it is concluded that the method meets the requirements for the precision of the method. The requirements for the accuracy of the method are met, which is evaluated by the value of Recovery, which should be in the interval 70-130% (for impurities whose limit is 0.1-0.5%). The results of experimental work (93.753% -102.272 %) fall within the limits of the interval. The method was validated in accordance with International council for harmonisation of technical requirements for pharmaceuticals for human use (ICH) guidelines (ICH, 2023). Unlike other methods that require expensive chiral stationary phases, the method based on Pirkle-type stationary phase is far more economical and favorable for determining the content of impurity D in the investigated powder for solution for parenteral preparations of voriconazole.

References:

[1] BEALE, J.M., (2011): Anti-infective Agents. Chapter 6. *In:* Beale, J., Block, J. (eds) *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry*. Lippincott Williams & Wilkins, Philadelphia: 191-206 pp.

[2] EUROPEAN PHARMACOPOEIA (PH. EUR.) (2025): Voriconazole monograph (reference 01/2024:2576). 11th ed. Council of Europe, Strasburg, Codex: 5296 p.

[3] FERNANDES, C., TIRITAN, M.E., PINTO, M. (2013): Small Molecules as Chromatographic Tools for HPLC Enantiomeric Resolution: Pirkle-Type Chiral Stationary Phases Evolution. *Chromatographia* **76**: 871–897. doi: 10.1007/s10337-013-2469-8

[4] ICH (2023): ICH harmonised guideline. Validation of analytical procedures Q2(R2). https://database.ich.org/sites/default/files/ICH_Q2%28R2%29_Guideline_2023_1130.pdf

[5] IVKOVIĆ, B. (2004): Application of different chiral selectors in the separation of metoprolol-tartrate enantiomers by planar and high performance liquid chromatography. *Master thesis*, University of Belgrade, Faculty of Pharmacy, Belgrade. [in Serbian]

[6] LIU, Y., ZOU, H. (2008): High-performance liquid chromatographic evaluation of a coated cellulose tris(3,5-dimethylphenylcarbamate) chiral stationary phase having a small-pore silica support. *Journal of Chromatography A* **1178** (1-2): 118-25. doi: 10.1016/j.chroma.2007.11.055

[7] MALENOVIĆ, A., STOJANOVIĆ, B. (2010): Pharmaceutical analysis: practicum. University of Belgrade, Faculty of Pharmacy, Belgrade: 19-34 pp. [in Serbian]

[8] NIKOLIN, B., ŠOBER, M. (2003): Drug analysis. Publishing, Belgrade, pp.215-230. [in Serbian]

[9] NOVAKOVIĆ, A. (2011): Pharmacotherapy of fungal infections. *In:* Ugrešić, N., Stepanović-Petrović, R., Savić, M. (eds) *Pharmacotherapy for pharmacists*. University of Belgrade, Faculty of Pharmacy, Belgrade: 609-29 pp. [in Serbian]

[10] OWENS, P.K., FELL, A.F., COLEMAN, M.W., BERRIDGE, J.C. (1999): Separation of the voriconazole stereoisomers by capillary electrophoresis and liquid chromatography. *Enantiomer* **4** (2): 79-90. PMID: 10483712

[11] PIRKLE, W.H., FINN, J.M., SCHREINER, J.L., HAMPER, B.C. (1981): A widely useful chiral stationary phase for the high-performance liquid chromatography separation of enantiomers. *Journal of the American Chemical Society* **103** (13): 3964–3966. doi: 10.1 021/ja00403a076

[12] PIRKLE, W.H., HOUSE, D.W. (1979): Chiral high-performance liquid chromatographic stationary phases. 1. Separation of the enantiomers of sulfoxides, amines, amino acids, alcohols, hydroxy acids, lactones, and mercaptans. *The Journal of Organic Chemistry* **44** (12): 1957–1960. doi:10.1021/jo01326a014

[13] PIRKLE, W.H., HOUSE, D.W., FINN, J.M. (1980): Broad spectrum resolution of optical isomers using chiral high-performance liquid chromatographic bonded phases. *Journal of Chromatography A* **192** (1): 143–158. doi: 10.1016/S0021-9673(00)81849-X

[14] PIRKLE, W.H., POCHAPSKY, T.C. (1989): Considerations of chiral recognition relevant to the liquid chromatographic separation of enantiomers. *Chemical Reviews* **89** (2): 347–362. doi: 10.1021/cr00092a006

[15] RANG, H.P., DALE, M.M., RITTER, J.M., MOORE, P.K. (2005): Antifungal drugs. Chapter 47. *In*: *Rang and Dale: Pharmacology*; Data status, Belgrade: 666-86 pp.

[16] SERVAIS, A.C., MOLDOVAN, R., FARCAS, E., CROMMEN, J., ROLAND, I., FILLET, M. (2014): Development and validation of a liquid chromatographic method for the stability study of a pharmaceutical formulation containing voriconazole using cellulose tris(4-chloro-3-methylphenylcarbamate) as chiral selector and polar organic mobile phases. *Journal of chromatography A* **1363**: 178-82. doi: 10.1016/j.chroma.2014.06.082

[17] SHAIKH, K.A., PATIL, A.T. (2012): A validated stability-indicating liquid chromatographic method for determination of degradation impurities and diastereomers in voriconazole tablets. *Scientia pharmaceutica* **80** (4): 879-88. doi: 10.3797/scipharm.1204-24

[18] UNITED STATES PHARMACOPEIA (USP) (2024): Voriconazole monograph (reference Volume No. PF 37(4)). The United States Pharmacopeial Convention, Inc. doi: 10.31003/USPNF_M1061_06_01

[19] VARAGIĆ, V., MILOŠEVIĆ, M. (2012): Antifungal and antiviral drugs. *In*: *Pharmacology*. Elit Medica, Belgrade, 604-11 pp. [in Serbian]

[20] WAHAB, M., WEATHERLY, C., PATIL, R., ARMSTRONG, W. (2018): Chiral Liquid Chromatography. Chapter 12. *In:* Polavarapu, P. (ed) *Chiral Analysis*. Elsevier, Amsterdam, Netherlands: 507-564 pp. doi:10.1016/b978-0-444-64027-7.00014-8