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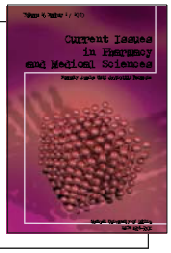

Photostability of triazole antifungal drugs in the solid state

RADOSŁAW J. EKIERT*, JAN KRZEK

Department of Inorganic and Analytical Chemistry, Pharmaceutical Faculty, Jagiellonian University Medical College, Cracow, Poland

ABSTRACT

The publication is devoted to photostability assessment of four triazole antifungal drugs: fluconazole, itraconazole, posaconazole and voriconazole. The compounds were exposed in the solid state using the whole spectrum of UV-Vis radiation. The analyses were performed using high performance thin layer chromatography (HPTLC) technique with densitometric detection. The results indicates considerable degradation of structurally similar itraconazole and posaconazole which could be clinically significant. After 72 hours of itraconazole irradiation there remain less than 25%, and 60% in case of posaconazole. To a lesser extent photodegradation concern two other compounds with a separate chemical structure: fluconazole and voriconazole. After 72 hours of irradiation there left 75% and 82% of these substances, respectively. The strict dependence between compound photostability and its chemical structure was observed.

Keywords: photostability, triazole antifungal drugs, high performance thin-layer chromatography, densitometry

INTRODUCTION

Triazole antifungal drugs, such as fluconazole, itraconazole, posaconazole and voriconazole (Fig. 1.), possess an important position in antifungal therapy. Because of the constantly rising number of mycoses, these medicines are in increasingly common usage. The efficacy and safety of each drug depends largely on its quality, among others photostability [3,5,9,12,15]. The hitherto researches in this field are very limited for triazole antifungal drugs. The investigations concerning fluconazole [13,14] and posaconazole [10] indicates their stability, but in the case of the second compound it is only a declaration of a marketing authorization holder. Itraconazole is considered as photostable drug but it caused hypersensitivity reaction

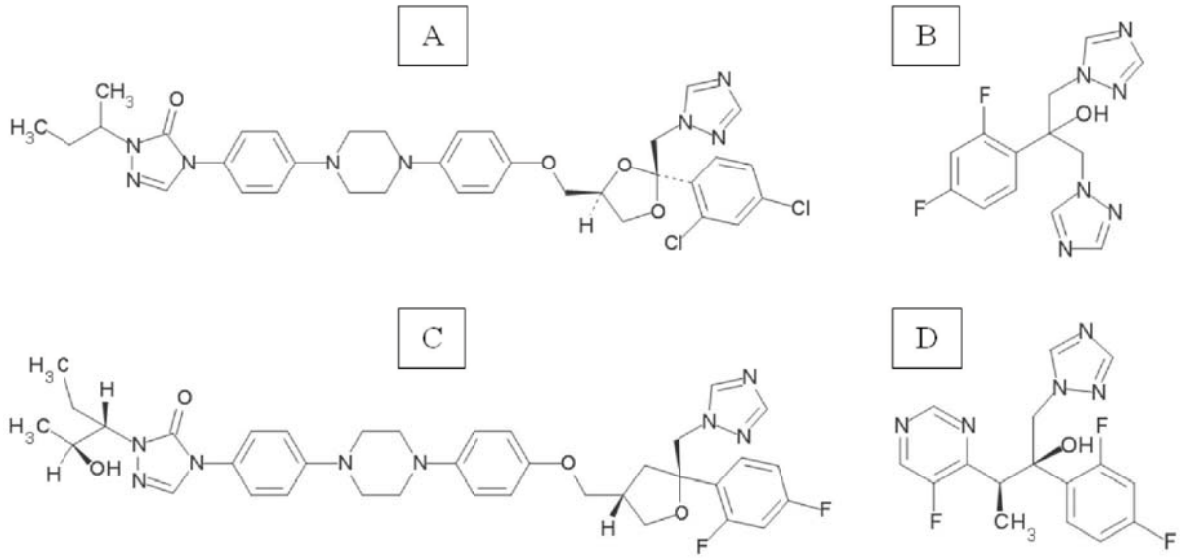

Fig. 1. Chemical structure of itraconazole (A), fluconazole (B), posaconazole (C) and voriconazole (D)

Corresponding author
* Department of Inorganic and Analytical Chemistry, Pharmaceutical Faculty, Jagiellonian University Medical College, 9 Medyczna St., 30-688 Cracow, Poland
e-mail: radoslaw.ekiert@uj.edu.pl

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when exposed to sunlight [2]. It was confirmed, that voriconazole is not stable while intensively irradiated and may be the cause of light sensitive reaction [4,8,11].

The presented project is a consequence of previous authors' analytical studies in this group of substances. The analytical conditions of qualitative and quantitative thin layer chromatographic procedure were already established [6]. In the next step a thorough review covering all chromatographic and electrophoretic techniques used in azole analyses were done [7] aiming identification of still omitted analytical matters. Authors conclude that further studies concerning azole photostability will be reasonable and valuable. The present investigations of photostability of four azole antifungal substances; fluconazole, itraconazole, posaconazole, voriconazole in uniform analytical conditions were performed using climatic chamber and high-performance TLC with densitometric detection.

MATERIAL AND METHODS

Chemicals. Fluconazole, itraconazole and voriconazole were purchased from Sigma-Aldrich (St.Louis, USA), whereas posaconazole comes from Sequoia Research Products (Pangbourne, UK). Standard solutions had concentration of 1 mg/ml. Itraconazole was dissolved in chloroform + methanol, 1:3 (v/v). Other three compounds were dissolved only in methanol. The standard and sample solutions were kept for up to 1 week in a refrigerator at approx. 4-8°C and protected from light.

Methanol, chloroform, hexane, ethyl acetate, glacial acetic acid were supplied by Chempur (Piekary Śląskie, Poland). All chemicals were of analytical grade. Bidistilled water comes from own laboratory distillery.

Apparatus. The climatic chamber KBF-ICH 240 APT.line™ manufactured by Binder (Tuttlingen, Germany) was used. The source of UV-Vis radiation conform with ICH requirements.

The Linomat 5 band applying module and Scanner 3 densitometer were used. Additionally, a fluorescence detector at wavelength of 254 nm was utilized in order to observe quenching of fluorescence by the investigated substances. All the three pieces of equipment came from Camag (Muttenz, Switzerland). Normal chromatographic chamber with dimensions: 18 cm x 9 cm x 18 cm manufactured by Sigma Aldrich (St. Louis, USA) was used.

Irradiation method parameters. The standard solutions were applied on Petri plates in 1 ml volume, evaporated on water bath in temperature approx. 50°C. Substance layer applied on the plate do not exceed 1 mm in thickness. The irradiation in whole UV-Vis range was performed in climatic chamber Binder with ICH compliant illumination source. The standard conditions were set as follows: T= 20°C, relative humidity= 60%. The distance from lamp to plate was approximately 20 cm. The exposition time equaled 72 hours. The solid substances after irradiation were turned into solutions with initial concentrations of 1 mg/ml.

In order to exclude the influence of temperature itself, control test on water bath and dark control in chamber was carried out. The plates wrapped in aluminum foil were put in to the chamber for the same period of time. The influence of factors other than radiation was clearly excluded.

Chromatographic method parameters. The standard solutions and the sample solutions were applied onto HPTLC silica gel 60 plates of 10 cm x 10 cm in size with F254 fluorescence factor (Merck, Darmstadt, Germany). The solutions were applied with Linomat module with rate 200 nl/s. The bands were formed 10 mm from the bottom of the plate edge, while the front of chromatograms was fixed at 5 mm from the upper edge of the plate so the development distance was 85 mm. There were four tracks on each plate. Band width was 10 mm and space between bands was 10 mm. The first and last tracks were situated 12 mm from the side edges of the plates. The standard solutions were applied on chromatographic plate in volumes of 10 µl. The determination procedure required application of 50 µl of the sample solutions.

The plates were developed using mobile phase consisted of hexane – ethyl acetate – methanol – water – glacial acetic acid (42:40:15:2:1, v/v/v/v/v), what is justified by authors earlier studies [6]. Each time development required preparation of 25 ml of mobile phase. The development of chromatograms took place in a closed chromatographic chamber. Plates were put into chamber after a few minutes of saturation. The development time was approximately 28 min.

In the next step the plates were scanned separately by using the Scanner 3 densitometer in the UV range 200-400 nm in the absorbance/reflectance mode. The slit dimension was defined as 6.0 mm x 0.6 mm. The scanning speed used for determination was 20 mm/s, while in order to record spectra speed equaled 100 nm/s. Scanning parameters were set using winCats Planar Chromato- graphy Manager computer program, v.1.3.4. The source of UV radiation was deuterium lamp. Scanning was performed at a wavelength of 260 nm, what is justified by authors' earlier studies [6].

RESULTS AND DISCUSSION

The identification of the triazole drugs was based on the received values of retention factor (R_F) and recorded UV spectra. The experimentally defined R_F values equaled: for fluconazole 0.37, posaconazole 0.60, voriconazole 0.73, while in case of itraconazole 0.97.

The analyses for all four drugs at all stages were deliberately conducted in the same conditions, in order to allow reliable comparison of results. Because of the possibility to execute direct determinations in UV range, there was

no necessity to stain the bands and perform analyses in visible light.

The irradiation study with subsequent analysis using high performance thin layer chromatography with densitometric detection revealed considerable degradation of itraconazole and posaconazole which could be clinically significant. The susceptibility to photodegradation is probably a result of ether bond occurrence in chemical structure. In case of other two drugs, that do not have ether bond degradation was relatively small. The detailed results of degradation process are presented in Table 1 and on Figures 2-5. Percentage share of remaining triazole compounds and degradation products were computed utilizing internal normalization method. Basing on results, an important conclusion rises; in stability and toxicity assessment we shouldn't judge the drugs from one therapeutic group similarly but the individual chemical structure is crucial. It is also worth to mention, that after irradiation of itraconazole authors observed the phenomenon of photochromism. The irradiation products had olive colour whereas itraconazole reference standard is white. The other samples do not change the tinge.

The presented researches concern structure stability relationship (SSR) and structure toxicity relationship (STR)

Table 1. Degradation degree of triazol antifungal drugs after 72 h of UV-Vis irradiation.

Triazole compound	Remaining amount [%]	Number of degradation products	Percentage share of degradation products [%]
Itraconazole	22.64	9	2.14, 2.06, 2.09, 2.09, 10.49, 22.37, 11.69, 10.93, 13.50
Posaconazole	57.02	8	0.31, 0.35, 1.40, 12.47, 9.12, 15.20, 3.16, 0.98
Fluconazole	75.23	2	10.17, 14.60
Voriconazole	81.91	6	0.37, 7.02, 0.50, 1.86, 2.18, 6.17

issues. In the group of triazole antifungal agents there were almost none researches concerning structure activity relationship (SAR). The only work coming into the area of SAR, describing mostly the quantitative structure retention relationship (QSRR) was carried out a decade ago [1]. That paper presents information about hydropho- bicity of bifonazole, clotrimazole, fenticonazole, fluconazole, ketoconazole, miconazole, metronidazole and itraconazole. The hydrophobicity was established from the linear relationships between the solute R_M values and the concentration of organic modifier. It was proven that, except itraconazole, the measurement $C_0 = R_M^0/m$ correlate with calculated log *P* values and can be a lipophilicity descriptor. Moreover the hydrophobicity was successfully

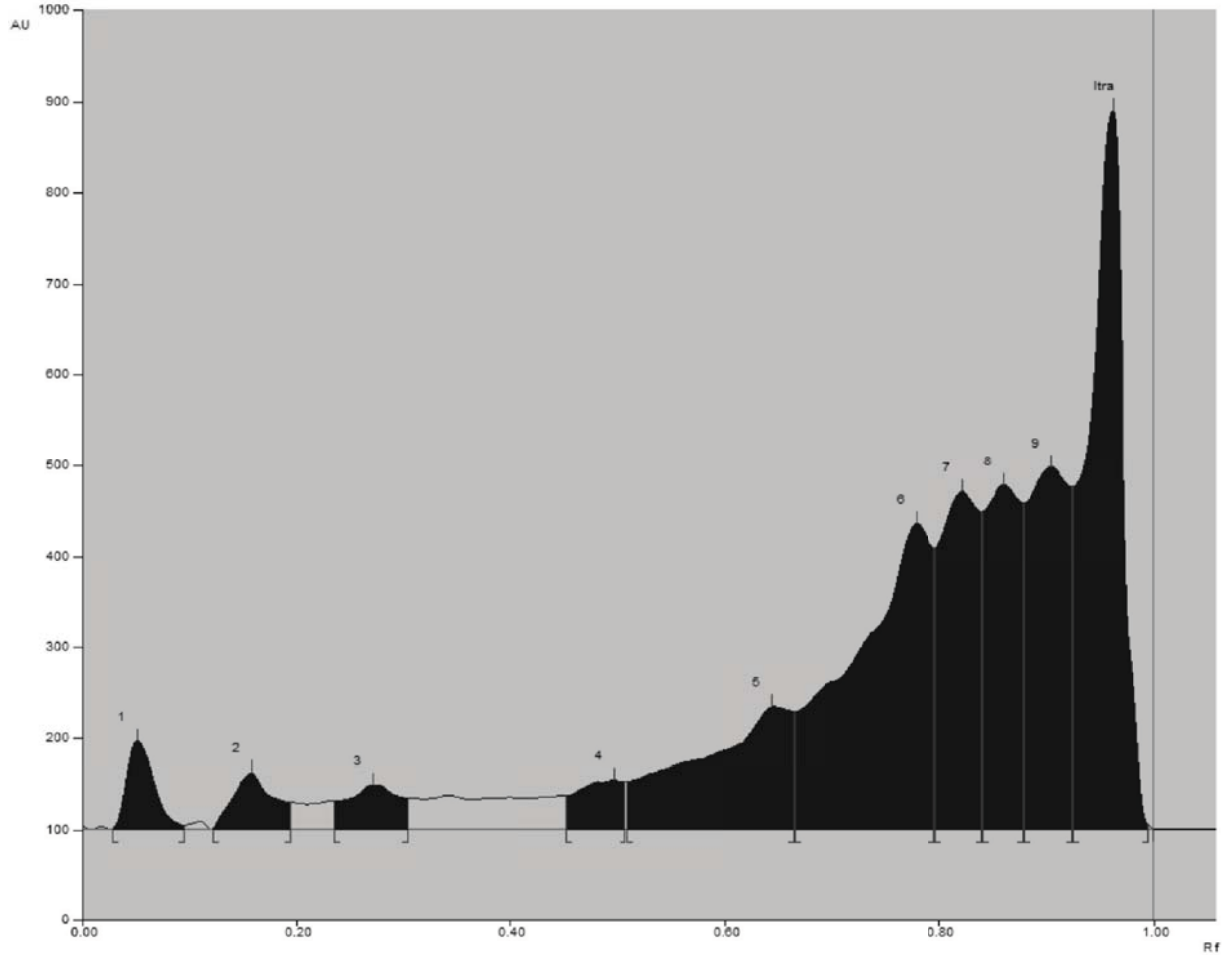

Fig. 2. Densitogram presenting degradation of itraconazole after 72 h of UV-Vis irradiation. Itra- itraconazole

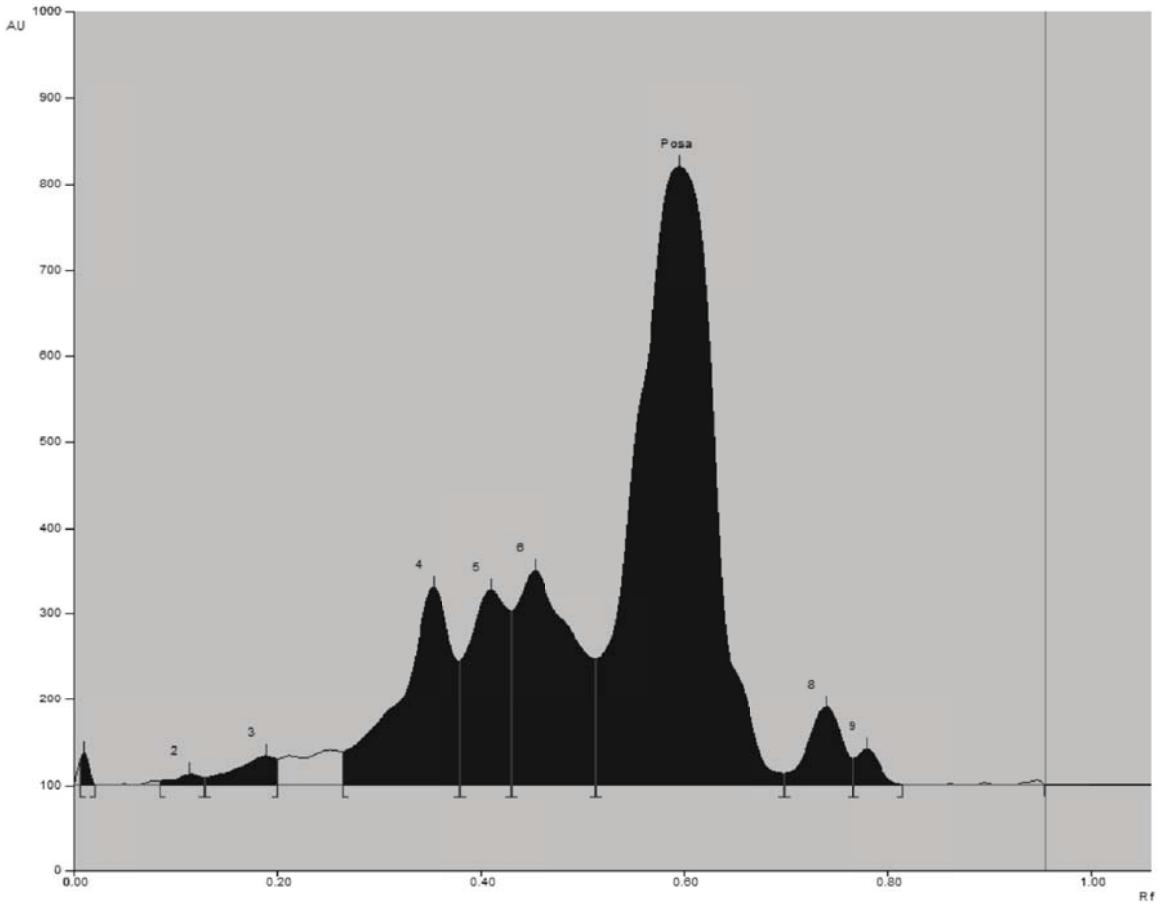

Fig. 3. Densitogram presenting degradation of posaconazole after 72 h of UV-Vis irradiation. Posa- posaconazole

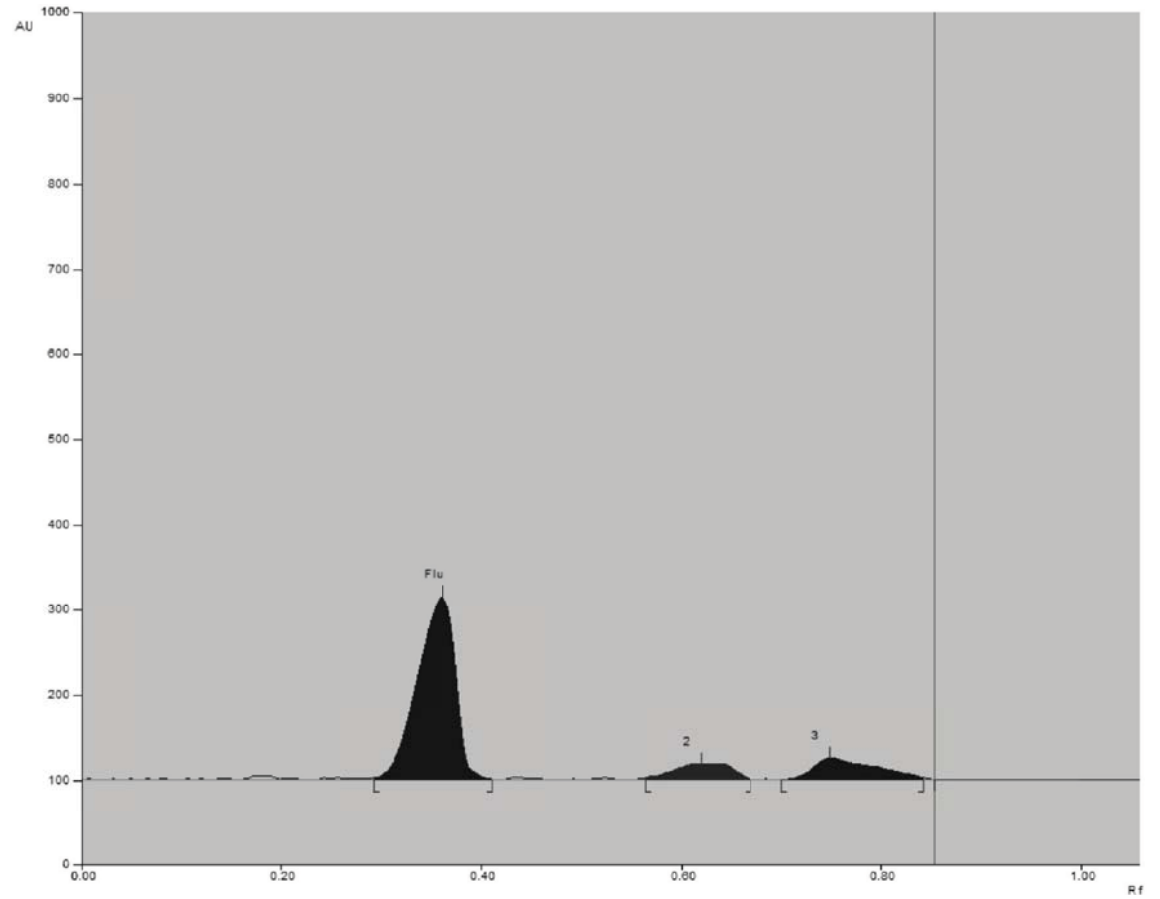

Fig. 4. Densitogram presenting degradation of fluconazole after 72 h of UV-Vis irradiation. Flu- fluconazole

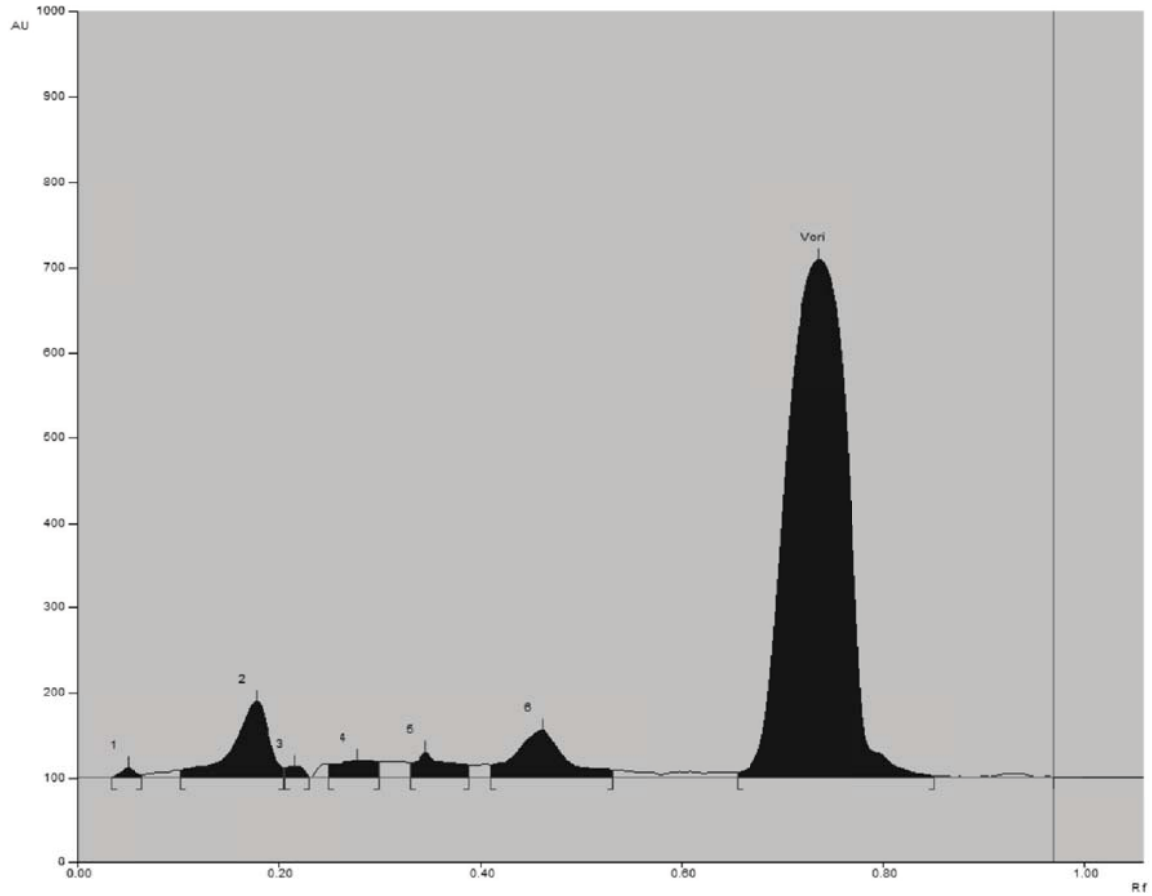

Fig. 5. Densitogram presenting degradation of voriconazole after 72 h of UV-Vis irradiation. Vori- voriconazole

correlated with antifungal activity expressed by MIC_{50} against dermatophytes. The lipophilicity is a simplified measure of penetration potential through biological barriers what affects pharmacological activity. The further studies in this area will be still welcomed.

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