Potency of Posaconazole Tablet Formulations Manufactured and Marketed in Bangladesh

SHRABONY MAZUMDER[1], RUPA AKTER[2], MD. RAFIQUZZAMAN[3]
[1, 2, 3] *Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh*

Abstract-

Background: Posaconazole is a widely used antifungal drug known for its potency against various fungal infections. The determination of its potency is crucial for ensuring the appropriate dosage and efficacy of the drug samples. UV spectroscopy is a widely accepted method for the quantitative analysis of pharmaceutical active ingredients of drug samples. There is no study available on determination of potency of posaconazole, marketed in Bangladesh, by UV spectroscopy.

Aims & Objectives: The present research work aims to determine the potency of posaconazole drug products of various pharmaceutical companies of Bangladesh using UV spectroscopic method and to discuss the findings.

Significant findings: The UV method involves measuring the absorbance of posaconazole. In this study lambda max (λ_{max}) was found at 264 nm for posaconazole in methanol . From the absorbance of the standard solutions at different concentrations, the regression equation was found as y = 0.0487x + 0.0088 and the coefficient of determination (R^2) was evaluated as 0.9997, which indicated topnotch linearity of the calibration curve and data fitting.

The absorbance of samples of four (4) different tablets solutions were measured and the concentrations were calculated using the regression equation. Concentrations were found 19.1752 µg/mL, 18.8542 µg/mL, 19.2806 µg/mL, 19.3094 µg/mL and potency of the samples were evaluated 95.87%, 94.27%, 96.40%, 96.55%, respectively. It was found that all of the drug samples complied with the acceptance level stated in US Pharmacopeia. The decreasing order of potencies of the samples is Sample-4 (of ACI)> Sample-3 (of Opsonin)> Sample-1 (of Square)> Sample-2 (of Square).

I. INTRODUCTION

Of the classes of antimycotics, compounds having azole moiety within the structure (conazoles) (Andes et al., 2011), posaconazole, a structural analogue of itraconazole, chemically 4-{4-[4-(4-{[(3R,5R)-5-(2,4-difluorophenyl)-5-(1H-1,2,4-triazol-1-ylmethyl)-tetrahydrofuran-3-yl]methoxy}phenyl)piperazin-1-yl]phenyl}-2-[(1S,2S)-1-ethyl-2-hydroxypropyl]-2,4-dihydro-3H-1,2,4-triazol-3-one (Fig. 1), is one of the most promising antifungals approved by Food and

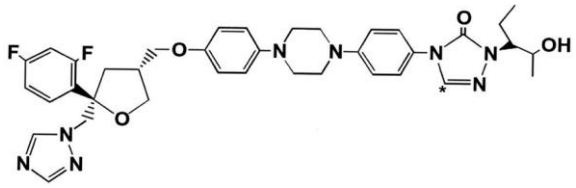

Figure 1: Chemical structure of posaconazole (Krieter et al., 2004)

Drug Administration (FDA) in September of 2006 (Moore et al., 2015; Kauffman et al., 2007) as well as in the E. U. (2005) for the treatment of aspergillosis, candidiasis, and other invasive fungal infections in immunocompromised patients older than 13 years (Kujawski et al., 2019). At present, Posaconazole is available in three authorized forms that include the oral suspension (40 mg/mL), intravenous injections (18 mg/mL) and delayed-release tablets (100 mg) (Moore et al., 2015; Kauffman et al., 2007; Farowski et al., 2007).

Posaconazole, a triazole antifungal drug, works against fungus by attaching to the heme co-factor on the cytochrome P-450 dependent enzyme sterol 14α-demethylase, thus blocking its function. As a result, the production of ergosterol, a crucial part of the fungal cell membrane, is inhibited and methylated sterol precursors accumulate and finally fungi are inhibited (USFDA, 2015).

Posaconazole exhibits a wider range of antimycotic activity than previous conazoles, demonstrating its ability to combat a wide variety of yeasts, filamentous fungi, and Candida species, including those that are resistant to fluconazole, such as C. glabata, C. krusei,

C. guilliermondii, C. dubliniensis, C. parapsilosis, and C. tropicalis. The spectrum of activity of Posaconazole also includes additional pathogenic fungi, such as Aspergillus species, Cryptococcus neoformans, Blastomyces dermatitidis, Trichsporon species, and Scedosporium species. Posaconazole also demonstrates antiviral action against Parechovirus A3, the virus that is responsible for the life-threatening cerebral infections in infants and is active against the protozoal parasites Trypanosoma and Leishmania in animal models (Peyton et al.,2015; Rhoden et al., 2018). Furthermore, according to the European Medicines Agency (2010), posaconazole is authorized for the treatment of oropharyngeal candidiasis, for the treatment of patients with IFD who are intolerant to first-line therapy, and as salvage treatment of IFD caused by rare pathogens such as fusariosis, chromoblastomycosis, mycetoma, and coccidioidomycosis. There is little evidence of hepatotoxicity or cardiotoxicity associated with posaconazole, and there is no discernible correlation between posaconazole exposure and treatment-related toxicity has been identified to date (Chen et al., 2020). There are various methods for determination or quantification of active ingredients of drugs. Such as UV-Vis spectroscopic method (Deepali & Elvis, 2010), HPLC (Yilmaz et al., 2022), Gas Chromatography (Suleman et al., 2015) etc. UV-Vis spectroscopic method is a cost effective, easy and rapid technique. The UV method is often employed for potency assay of posaconazole in tablets (Andressa et al., 2015) due to its effectiveness in quantifying the concentration of posaconazole active ingredient present in the tablet formulations. The UV method is widely accepted in pharmaceutical analysis (Deepali & Elvis, 2010), ensuring compatibility with regulatory requirements with potency assays. However, literature review showed that no study was conducted and reported to measure the potency of posaconazole in tablet formulations manufactured and marketed in Bangladesh, using UV spectroscopic method. Therefore, authors considered it is important to determine the potency of posaconazole tablets manufactured and marketed in Bangladesh. Accordingly, they aimed to measure potency of posaconazole tablet formulations manufactured and marketed in Bangladesh using UV spectroscopic method and discussed their findings in this paper.

II. EXPERIMENT

- Sample collection:

Four (4) different tablet samples of three (3) different pharmaceutical companies were collected from different areas of Dhaka. Each tablet costs 200 tk.

Table 1: Collected brands of Posaconazole tablet samples and relevant information

SL No.	Brand Name	Manufacturer	Batch No.	Mfg date	Exp Date
1.	Xpos	Square Pharmaceuticals	1J01531	SEP 2021	AUG 2023
2.	Xpos	Square Pharmaceuticals	2E00914	JUN 2022	MAY 2023
3.	Posanox	Opsonin Pharma	TKJ418	OCT 2022	OCT 2024
4.	Posac	ACI Pharmaceuticals	0M120	JUN 2022	JUN 2024

- Solvent

Analytical grade methanol (Merk KGaA, Germany) supplied to the Department of Pharmacy, Jahangirnagar University by the standard retail sellers or vendors in Bangladesh was used as solvent in the present research work.

- Posaconazole Standard Material

Posaconazole reference standard was obtained as a gift from a pharmacist of a renowned Pharmaceutical Company, Dhaka, Bangladesh, on personal communication.

- Glassware & apparatus

Pipette (5mL, 10mL), Volumetric flask (50mL, 10mL), Funnel, Beaker (200mL, 250mL), Quartz cells, Mortar & pestle, Spatula, Pipette filler etc. were used in the experiments.

- Instruments used

Electronic Balance (Shimadzu AUW220D), Analytic Jena - UV-Vis Spectrophotometer (Specord - 205) with 1cm path length available in Wazed Miah Science Research Center, Jahangirnagar University were used in the present study.

- Solutions Prepared

Preparation of Posaconazole Standard Solutions
Posaconazole Standard-1 (40 µg/mL)

A stock solution of posaconazole was prepared by dissolving 2 mg of pure posaconazole in 50 mL methanol in a volumetric flask to obtain a concentration of 40 µg/mL.

Posaconazole Standard-2 (20 µg/mL)
Five (5 mL) milliliters of the standard-1 solution was taken in a 10 mL volumetric flask and methanol was added up to the mark with well shaking.

Posaconazole Standard-3 (10 µg/mL)
Five (5 mL) milliliters of the standard-2 solution was taken in a 10mL volumetric flask and methanol was added up to the mark with well shaking.

Posaconazole Standard-4 (5 µg/mL)
Five (5 mL) milliliters of the standard-3 solution was taken in a 10 mL volumetric flask and methanol was added up to the mark with well shaking.

- Preparation of Posaconazole Sample Solutions

Sample-1 (Xpos - 1)
One tablet was taken and its coating was removed followed by weighing of the tablet. Total weight of the tablet was 679.6 mg. It was then taken into a mortar and was crushed into a fine powder using a pestle. Powder (6.796 mg) of the tablet was weighed and transferred in a 50 mL volumetric flask. Methanol was added gradually and dissolved the powder by mixing thoroughly. Methanol was added up to the mark to prepare the solution having concentration 20 µg/mL. Similarly, three (3) sample solutions were prepared from the same powder of the tablet of Xpos-1.

The same procedure was used to prepare the other sample solutions (sample-2, sample-3, and sample-4) with Xpos - 2, Posanox, and Posac, respectively except the amount of powder taken in each case as stated below.

Sample-2 (Xpos - 2)
Powder was prepared from a tablet of Xpos-2 for the sample-2 and 6.80 mg of the prepared powder was taken in a 50 mL volumetric flask and it was dissolved pouring methanol up to the mark at the neck of the flask.

Sample-3 (Posanox)
Powder was prepared from a tablet of Posanox for the sample-3 and 6.81 mg of the prepared powder was taken in a 50 mL volumetric flask and it was dissolved pouring methanol up to the mark at the neck of the flask.

Sample-4 (Posac)
Powder was prepared from a tablet of Posac for the sample-3 and 6.77 mg of the prepared powder was taken in a 50 mL volumetric flask and it was dissolved pouring methanol up to the mark at the neck of the flask.

- Calibration of UV-Vis Spectrophotometer

A blank solvent (methanol) was used as a reference to calibrate the instrument to zero absorbance from 200 nm to 800 nm and the spectrum did not show any reasonable absorbance in the mentioned range of wavelength except a shoulder or absorbance lower than 210 nm.

- Absorption Spectrum of Posaconazole

Standard solution (20 µg/mL) of Posaconazole was taken into a cuvette and it was placed into the designate spot of the UV machine. The absorption spectrum of the solution was taken from 200 nm to 800 nm. It showed a single absorption centering absorption maximum at 264 nm. Later the same measurement was carried out more carefully setting the wavelength 200 nm to 400 nm of UV range. The recorded spectrum is shown in the Fig. 2 under results and discussions section. It clearly shows that the absorption maximum of posaconazole in methanol is at 264 nm.

- Regression analysis with the posaconazole standard solutions

For regression analysis, absorbance of the prepared standard solutions of Posaconazole were measured at 264 nm. Absorbance of the standard posaconazole solutions having concentrations (µg/mL) 5, 10, 20, and 40 were found 0.2659, 0.4910, 0.9647 and 1.9635, respectively. Absorbance versus concentrations of posaconazole standard solutions resulted in regression

line as shown in the Fig. 3 under results and discussions part.

III. RESULTS AND DISCUSSIONS

Posaconazole is an antifungal medication used to treat various fungal infections. It is an INN drug. The UV method is widely accepted in pharmaceutical analysis (Deepali & Elvis, 2010), ensuring compatibility with regulatory requirements with potency assays. It is often employed for potency assay of posaconazole in tablets (Andressa et al., 2015). In the present study, authors aimed to evaluate potency of posaconazole tablet formulations manufactured and marketed in Bangladesh. Potency analysis of medicinal products helps the stakeholder to have an idea about quality of the manufactured and marketed drugs and it put pressure to the manufacturers as well as to the drug authority to ensure quality of drug products released in the market.

Posaconazole in methanol under the present experimental situation showed an absorption maximum (λ_{max}) at 264 nm (Fig. 2). In the literature, λ_{max} has been reported as 260 nm (Andressa et al., 2015). Variation of λ_{max} value about 2 to 4 nm for a substance in a given solvent measured in different instruments is usual and well accepted.

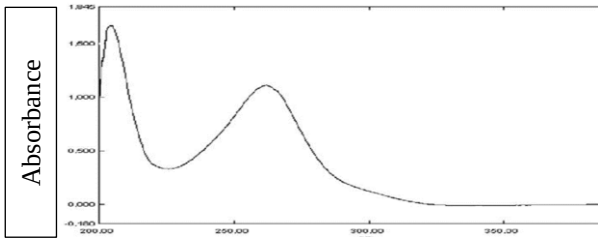

Figure 2: UV spectrum of posaconazole reference standard (20 µg/mL) in methanol

Regression analysis with the absorbance data of the prepared standard solutions of Posaconazole resulted in the following calibration straight line (Fig. 3). Regression equation was found as y = 0.0487x + 0.0088 with R^2 value 0.9997. Similar coefficient of determination (R^2) results were observed by others (Andressa et al., 2015; Deepali & Elvis, 2010) . Coefficient of determination value (R^2 = 0.9997) observed in the present study indicated excellent data fit and linearity of the obtained calibration line. Therefore, equation (y = 0.0487x + 0.0088) of the the calibration line was used with confidence to find the concentration of sample solutions of Posaconazole in this study.

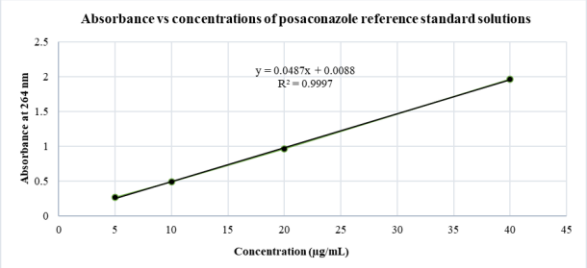

Figure 3: Calibration curve found with Posaconazole reference standard solutions

Concentration of each of the sample solutions was calculated from the measured absorbance at 264 nm data (Tables 2-5) of its solution using regression equation (y = 0.0487x + 0.0088) and the results obtained have been tabulated in Tables 2-5. The evaluated average concentration of the respective sample solution was used to back calculate the amount of Posaconazole present in its original tablet sample. The potency of each sample solution was found out from the calculated amount of Posaconazole and the claimed amount of posaconazole in the respective tablet dosage form. The observed results are presented in Table 2 to Table 5.

Table 2: Concentration and potency results of posaconazole tablet (Sample-1).

SL No.	Absorbance at 264 nm	Concentration found (µg/mL)	Average concentration (µg/mL)	Potency (%)
1	0.9413	19.1478	19.1752	95.87
2	0.9394	19.1088		
3	0.9472	19.2690		

Table 3: Concentration and potency results of posaconazole tablet (Sample-2).

SL No.	Absorbance at 264 nm	Concentration found (µg/mL)	Average concentration (µg/mL)	Potency (%)
1	0.917	18.6489	18.8542	94.27
2	0.926	18.8337		
3	0.938	19.0801		

Table 4: Concentration and potency results of posaconazole tablet (Sample-3).

SL No.	Absorbance at 264 nm	Concentration found (µg/mL)	Average concentration (µg/mL)	Potency (%)
1	0.9426	19.1745	19.2806	96.40
2	0.9478	19.2813		
3	0.9529	19.3860		

Table 5: Concentration and potency results of posaconazole tablet (Sample-4).

SL No.	Absorbance at 264 nm	Concentration found (µg/mL)	Average concentration (µg/mL)	Potency (%)
1	0.9512	19.3511	19.3094	96.55
2	0.9494	19.3142		
3	0.9469	19.2628		

The potency of the samples of posaconazole tablets, Sample-1, Sample-2, Sample-3, and Sample-4, were calculated and found as 95.87%, 94.27%, 96.40% and 96.55%, respectively (Tables 2-5). According to USP (2007) specification of potency for posaconazole tablets should be 100 ± 10%. Therefore, it was found that all of the posaconazole drug samples complied with the specifications stated in US Pharmacopeia. The drug samples available in the present study were of renowned and high capacity companies. As a result their potency were complied with the official specification of the drug. The result could have different if the sample size would be big enough and of companies of diverse capacities. It is therefore suggested to conduct potency determination of posaconazole tablet with a big sample size and with the inclusion of drug samples from companies of different capacities.

To have a vivid comparison of the determined potency, the results were plotted as a bar diagram

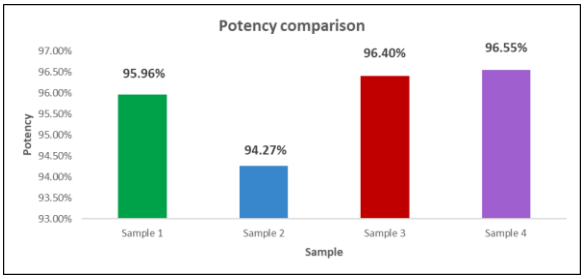

Figure 4: Potency of the different samples of posaconazole tablets.

(Fig. 4). It is clear from the graph that the decreasing order of potency of the samples is Sample-4 (of ACI)> Sample-3 (of Opsonin)> Sample-1 (of Square)> Sample-2 (of Square).

CONCLUSION

Posaconazole reference standard in methanol showed absorption maximum (λ_{max}) at 264 nm. The calibration curve obtained from the absorbance vs concentrations of posaconazole standard solutions demonstrated excellent linearity and data fit with high coefficient of determination (R^2 = 0.9997). The regression equation of the calibration straight line allowed quantification of posaconazole present in the respective posaconazole tablet samples and hence

their potency from the measured absorbance data of the solutions of the respective collected posaconazole drug sample. All of the assessed posaconazole drug samples contained active ingredients 94 % - 97 % of the claimed amount and thus complied with the specifications stated in US Pharmacopeia. The order of potencies found: Sample-4 (of ACI)> Sample-3 (of Opsonin)> Sample-1 (of Square)> Sample-2 (of Square). It is suggested to conduct potency determination of posaconazole tablet in future with a big sample size and with the inclusion of drug samples from companies of different capacities.

REFERENCES

[1] Andes, D.R., Dismukes,W.E., Azoles,in: Kauffman,C.A., Pappas, P.G., Sobel, J.D. (2011). Dismukes WE (Eds.). Essentials Clin. Mycol., 2nd ed., Springer-Verlag New York, New York, 61–93. doi:10.1007/978-1-4419-6640-7.

[2] Andressa,S.B., Sendy,S.O., Andreas,S.L.M., Cassia,V.G.(2015).UV Spectrophotometric method for determination of posaconazole: Comparison to HPLC. Revista de Ciências Farmacêutica Básica e Aplicadas - RCFBA, 36(4):491-495

[3] Chen, L., Krekels, E.H.J., Verweij, P.E., Buil, J.B., Knibbe, C.A.J., Brüggemann. R.J.M.(2020). Pharmacokinetics and Pharmacodynamics of Posaconazole, Drugs, 80:671–695 https://doi.org/10.1007/s40265-020-01306-y

[4] Deepali, G., & Elvis, M. (2010).UV Spectrophotometric Method for Assay of the Anti-Retroviral Agent Lamivudine in Active Pharmaceutical Ingredient and in its Tablet Formulation. J Young Pharm.,2(4): 417–419.

[5] European Medicines Agency, 2010. Summary of posaconazole characteristics. https://www.ema.europa.eu/en/documents/product-information/noxafil-epar-product-information_en.pdf. Accessed on 15 Apr 2020

[6] Farowski, F., Vehreschild, J.J., Cornely, O.A .(2007). Posaconazole: A next-generation triazole antifungal. Future Microbiol., 2: 231–243. doi:10.2217/17460913.2.3.231

[7] Kauffman,C.A., Malani, A.N., Easley,C., Kirkpatrick, P (2007). Posaconazole, Nat. Rev. Drug Discov., 6:183–184. doi:10.1038/nrd2270.

[8] Krieter, P., Flannery, B., Musick, T., Gohdes, M., Martinho, M., Courtney, R.(2004).Disposition of Posaconazole following Single-Dose Oral Administration in Healthy Subjects. ASM Journals., 48(9): 3543-3551.

[9] Kujawski, J., Czaja, K., Dettlaff, K., Żwawiak, J., Ratajczak,T., Bernard, M.K.(2019). Structural and spectroscopic properties of posaconazole – Experimental and theoretical studies, Journal of Molecular Structure., 1181: 179-189. doi: https://doi.org/10.1016/j.molstruc.2018.12.074.

[10] Moore,J.N., Healy, J.R., Kraft, W.K. (2015). HHS Public Access, Expert Rev. Clin. Pharmacol.,8: 321–334. doi:10.7205/MILMED-D-14-00168. Long-chain.

[11] Peyton, L.R., Gallagher, S., Hashemzadeh, M. (2015). Triazole antifungals: A review. Drugs of Today., 51(12): 705–718. doi:10.1358/dot.2015.51.12.2421058.

[12] Rhoden, E., Nix,W.A., Weldon,W.C., Selvarangan, R.(2018). Antifungal azoles itraconazole and posaconazole exhibit potent in vitro antiviral activity against clinical isolates of parechovirus A3 (Picornaviridae), Antiviral Res.,149: 75–77. doi:10.1016/j.antiviral.2017.11.011.

[13] Suleman, S., Verheust, Y., Dumoulin, A., Wynendaele, E. D'., Hondt, M., Vandercruyssen, K., Veryse, L., Duchateau, L., Spiegeleer, B.D. (2015). Gas chromatographic method for the determination of lumefantrine in antimalarial finished pharmaceutical products Journal of Food and Drug Analysis. 2015; 23(3): 552-559.

[14] USFDA, 2015. Noxafil instruction. https://www.accessdata.fda.gov/drugsatfda_docs/label/2014/205053s1lbl.pdf. Accessed on 15 Apr 2020.

[15] USP, 2007.United States Pharmacopeia and National Formulary (USP 30-NF 25). Rockville, MD: United States Pharmacopeial Convention; 2007.

[16] Yilmaz, B., Bayrak, B., and Kadioglu, Y.(2022). Development and Validation of HPLC Method for the Determination of Flurbiprofen in

Pharmaceutical Preparations. International Journal of Pharm ATA , 2(3): 72-81.