Available online on 15.06.2020 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

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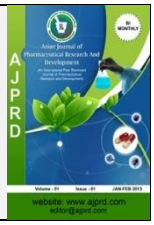

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Research Article

Detection of Impurities in Bulk Drug and Capsule of Fluconazole

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ABSTRACT

Objectives: The purpose of this study was to identify the impurities and their amounts in the fluconazole bulk drug and capsule FLUNAC™ (150 mg).
Method: HPLC with diode array detector was used to carry out the study. The composition of mobile phase was acetonitrile: water (85:15 %) with flow rate of 0.7 mL/min and detected at 260 ± 1 nm.
Results: Two impurities (one is known impurity A and other unknown impurity) were detected in the bulk drug and also in capsule FLUNAC (150mg). The total amount of impurities in fluconazole bulk drug and capsule were 0.368% and 0.392% respectively.
Conclusion: The total amount of impurities was less than 1% which is acceptable.

Keywords: Fluconazole, Impurities, HPLC.

ARTICLE INFO: Received 07 March 2020; Review Completed 03 May 2020; Accepted 08 May 2020; Available online 15 June. 2020

Cite this article as:

Sarker U K[1], Ali[1] M M, Farhana F[1], Islam M A[1*], Kaisar S[2], Misbahuddin M[3], Islam M R[4], Molla M E[4], Detection of Impurities in Bulk Drug and Capsule of Fluconazole, Asian Journal of Pharmaceutical Research and Development. 2020; 8(3):14-17
DOI: http://dx.doi.org/10.22270/ajprd.v8i3.717

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INTRODUCTION

Fluconazole is an anti-fungal drug. The triazole antifungal fluconazole is used against superficial and systemic candidiasis and in the treatment of cryptococcal infection for patients with the accuired immuno deficiency syndrome[1-6]. It is also used for the treatment of fungal infection by inhibiting the action of fungal cytochrome enzyme[7-9]. Fluconazole decreases ergosterol synthesis by interfering with cytochrome P450 activity, thus inhibiting cell membrane formation of susceptible fungi including B dermatitidis, Epidermophyton spp, Candida spp, C immitis, C neoformans, thus leading to cell death. Fluconazole is formulated in different dosage forms. In USP, three potential impurities of Fluconazole are listed (Fluconazole related compounds A, B, and C). Impurity is defined as any substance co-existing with the original drug, such as starting material or intermediates or that is formed, due to any side reactions. At present the control of pharmaceutical impurities is a demanding issue to the pharmaceutical industry.

The International Conference on Harmonization (ICH) has formulated a workable guideline regarding the control of impurities[10]. Drug impurity has become essential as per various regulatory requirements. In the pharmaceutical world, an impurity is considered as any other organic material, besides the drug substance, or ingredients, arise out of synthesis or unwanted chemicals that remains with API's. The presence of these unwanted chemicals, even in small amount, may influence the efficacy and safety of the pharmaceutical products[11]. Impurities can be classified as Organic impurities (process and drug related), Inorganic impurities and Residual solvents. Impurities in new drug substances can be addressed from two perspectives, the chemical aspect which includes classification and identification of impurities, report generation, listing of impurities in specifications, and a brief discussion of

analytical procedures, the safety aspect which includes the specific guidance for quantifying impurities, substantially at lower levels in a drug substance used in clinical studies[12].

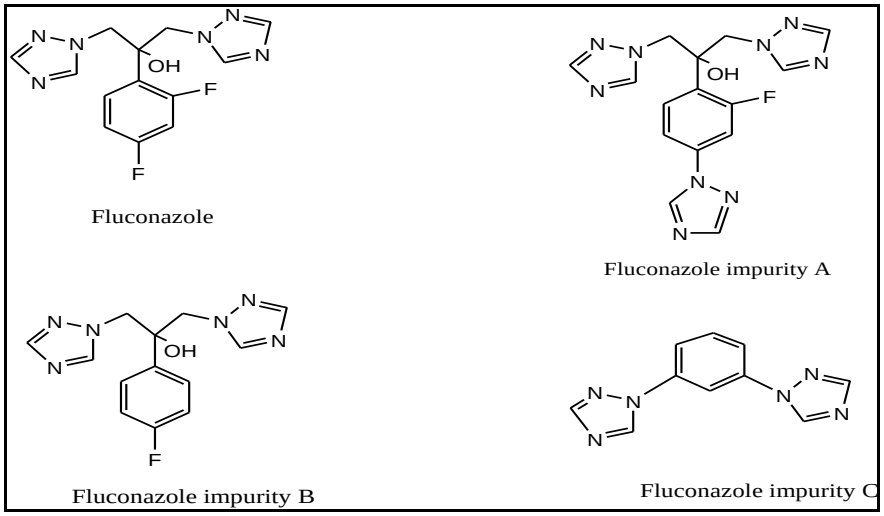

Figure: 1. Fluconazole and its impurity A, B and C

MATERIALS AND METHODS

Chemicals

Fluconazole and its three impurities, impurity A,B and C were supplied by Cadila healthcare Ltd. Ahmedabad, India. HPLC grade accetonitril was purchased from E. Merk (Germany). Capsule FLUNAC™ (150mg); fluconazole, batch number: 0909) was supplied by Drug International Ltd, Bangladesh.

Chromatographic condition

The HPLC-UV diode-array system consisted of Agilent model 1200 series solvent reservoir, binary pump, degasser, auto sampler, column oven and photo diode array detector. Chromatographic data were collected and analyzed using Chemstation software.

A reverse-phase high performance liquid chromatography (HPLC) was used for the determination of fluconazole both in fluconazole bulk drug and capsule FLUNAC™ (150 mg)[13]. The chromatographic analyses were performed on an Agilent 5 µm C18 column (150 × 4.6 mm). The mobile phase used for analysis consisted of 85% acetonitril (HPLC grade; E. Merck, Germany) and 15% water was delivered at a rate of 0.7 mL/min. Separations were carried out at 50°C. The wavelength was set at 260 nm with bandwidth 1 nm. Injection of sample (50 µL) was done using an autosampler. The peak with retention time and area were defined using software.

Identification of impurities

One milligram of each impurity (impurity A, impurity B, impurity C) was dissolve into the mobile phase separately and then diluted into different concentrations. One microgram of each sample was injected into the HPLC system separately to identify the peak using retention time. Then all the above mentioned impurities with fluconazole (1 µg each) were mixed in mobile phase of which 50 µL was injected.

One tablet FLUNAC™ (150 mg) was powdered and dissolved in 150 mL of mobile phase (1 mg/mL). It was then diluted to100 µg/mL using mobile phase and finally filtered using syringe filter 0.22 µm. Fifty microliter of the sample was injected into the HPLC system.

RESULTS AND DISCUSSION

The peak of the impurity A appeared first with a retention time of 6.98 min was shown in **Figure 2**. Other peaks were: impurity B (11.66 min), impurity C (13.02 min). Impurity A=150 µL (10 µg/ml), Impurity B=500 µL (100 µg/ml), Impurity C=8 µL (100 µg/ml), Fluconazole =100 µL (100 µg/ml), mobile phase 242 µL. The volume of drug injected was 50 µL.

In case of FLUNAC™ (150mg) all peaks were similar to fluconazole bulk drug which were shown in **Figure 3 and 4**. An unknown peak was detected in both fluconazole bulk drug and FLUNAC™ (150 mg). Impurity B and Impurity C were not detected in both fluconazole bulk drug and FLUNAC™ (150 mg). Unknown impurity showed the highest amount (0.271%) among all the impurities. The total amount of these impurities in fluconazole bulk drug was 0.368% and in FLUNAC™ (150 mg) capsule was 0.392% shown in T**able 1**. Only 0.024% impurity was increased during the production of capsule.

Table 1: Percentage of impurities of fluconazole present in bulk drug and capsule FLUNAC™

Peak No.	Impurities	Retention time (min)	Present (%)	
			Bulk drug*	Cap. FLUNAC™ (150 mg)*
1	Impurity A	7.3	0.106	0.121
2	Unknown impurity	8.5	0.262	0.271
Total impurities			0.368	0.392

*Data are mean of three samples

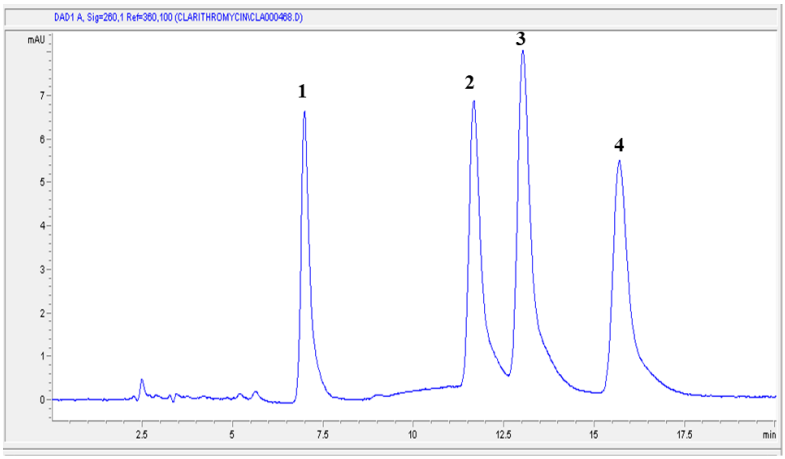

Figure: 2 Chromatogram shows peaks of fluconazole standard and its impurities. [RT = Retention time, Peak 1 (RT, 6.98 min) was impurity A, peak 2 (RT, 11.66 min) was impurity B, peak 3 (RT, 13.02 min) was impurity C, Peak 4 (RT 15.7 min)was the fluconazole]

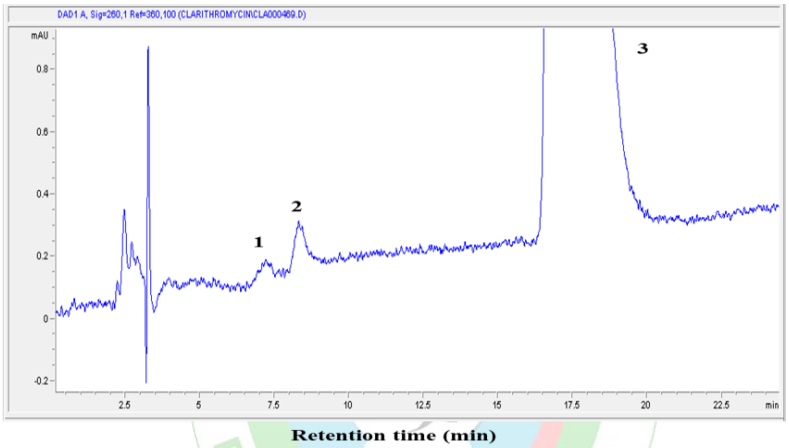

Figure: 3 Chromatogram shows peaks of fluconazole and its impurities in fluconazole bulk drug. [RT = Retention time, peak 1 (RT, 7.20 min) was impurity A, peak 2 (RT, 8.31 min) was unknown impurity, peak 3 (RT, 17.05 min) was the fluconazole]

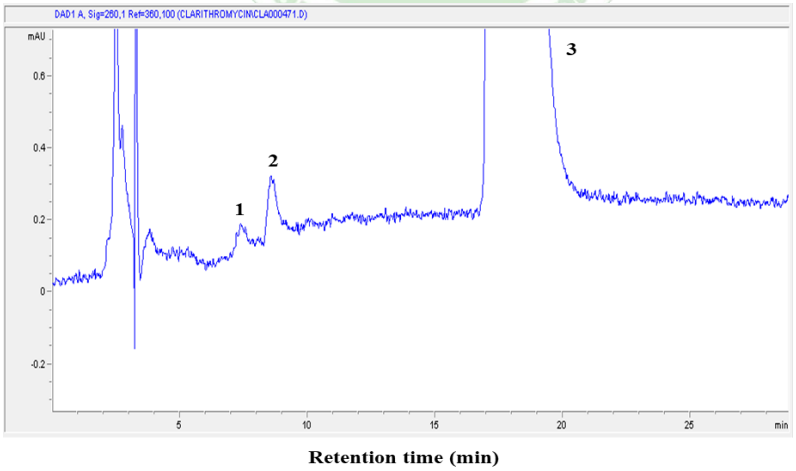

Figure: 4 Chromatogram shows peaks of fluconazole and its impurities in tablet FLUNAC^{TM} (150 mg). [RT = Retention time, peak 1 (RT, 7.36 min) was impurity A, peak 2 (RT, 8.56 min) was unknown impurity, peak 3 (RT, 17.59 min) was the fluconazole]

The International Conference on Harmonization (ICH) has published guidelines on impurities in new drug substances, products and residual solvents. According to ICH guidelines on impurities in drug products, identification of impurities below the 0.1% level is not considered to be necessary unless the potential impurities are expected to be unusually potent or toxic[14]. The maximum daily dose qualification threshold is considered to be less than 1 mg/day.

CONCLUSION

The amount of impurities in finished product (Cap. FLUNAC^{TM}) was less than 1% which is acceptable.

ACKNOWLEDGEMENT

The authors are thanks full to Mr. Mohammed Yusuf, Director (Technical), Khwaja Yunus Ali Medical College Hospital, Enayetpur, Sirajgonj, Bangladesh.

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