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A Greener Spectroscopic Approach for the Quantification of Ketoconazole in Pharmaceutical Formulations

Abubakar S. Muhammad[1*], Akpu M. Francis[1], Augustine U. Abel[1], Baba M. Nasirudeen[1], Labulo H. Ayomide[1], Ibrahim Hassan[1], Safiyanu T. Musa[1], Noopur Srivastava[2], Usman M. Mubarak[3]

[1]Department of Chemistry Federal University Lafia, P.M.B. 146, Lafia, Nasarawa, Nigeria
[2]Department of Chemistry and Biochemistry Sharda School of Engineering and Sciences, Sharda University, Greater Noida, UP, India, 201306
[3]Department of Chemistry Nigeria Police Academy, P.M.B. 3437, Wudil, Kano, Nigeria

***Corresponding Author**: muhammad.said@science.fulafia.edu.ng; +2348039624276*
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Abstract
Ketoconazole as a synthetic imidazole derivative, has emerged as a significant therapeutic agent in the field of antifungal pharmacology. Its unique mechanism of action, pharmacokinetic properties and clinical efficacy have positioned it as an essential component in the treatment of various fungal diseases, leading it to gain significant attention in medical mycology. Unfortunately, incorrect dosage or wrong pharmaceutical formulations of ketoconazole drug, can cause adverse health effects, which may include the reduced efficacy, hepatotoxicity and other unintended side effects. However, most of the conventional methods followed for the quantification of ketoconazole active ingredient in pharmaceutical samples pose significant threat to environment, and are rendered undesirable and damaging to habitable environment. Hence, there is a need for coming up with safer analytical alternatives that minimize the discharge of hazardous chemicals. This research endeavours to address the multifaceted challenges by validating a developed spectrophotometric technique, offering reliable solution in promoting a green methodology for the quantitative determination of ketoconazole active ingredient. Sample solutions of 100 ppm, 50 ppm, 25 ppm, 12.5 ppm and 6.25 ppm were prepared in water for each of the four selected brands (Axo-1, Derm-2, Ketora-3 & Keta-4) and the absorbance of each solution was taken separately at a wavelength of 240 nm, using 1 cm cell. Calibration curve for each of the tested brands indicates a linear correlation between concentrations and measured absorbance, which simply validates the accuracy of this procedure. The research finally recommends engagement of more brands on this approach for further validation and accommodation.

Keywords: Ketoconazole, hepatotoxicity, green methodology.

Introduction

Ketoconazole, as a synthetic imidazole derivative associated with a chemical name of cis-1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]pi perazine, has emerged as a significant therapeutic agent in the field of antifungal pharmacology. Ketoconazole possesses broad-spectrum activity against a diverse range of fungal pathogens, making it a valuable tool in the treatment of various fungal infections (Naveed & Jaweed 2014; Olga et al., 2013). Ketoconazole formulations come in several pharmaceutical forms indicating various routes of administration such as: tablets,

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topical creams, ointments, gels and antidandruff shampoo (Skiba et. al., 2000; Olga et al., 2013). Ketoconazole's unique mechanism of action, pharmacokinetic properties, and clinical efficacy have positioned it as an essential component in the armamentarium against fungal diseases. The discovery of ketoconazole stemmed from the quest for more effective antifungal agents capable of combating the growing incidence of fungal infections which were becoming increasingly prevalent and challenging to manage (Harshal et al., 2024; Belazarian et al., 2014). The development of ketoconazole was based on the success of earlier imidazole antifungal agents, such as miconazole and clotrimazole (Belazarian et al., 2014). These agents were primarily used topically to treat superficial fungal infections. However, due to the limited systemic absorption of these earlier imidazole agents, researchers were prompted to explore the synthesis of a potent oral antifungal agent with broader activity, an effort that led to the emergence of a chemical ingredient with broader spectrum of action, in the name of ketoconazole. The synthesis of ketoconazole involved modifications to the imidazole ring structure, resulting in improved oral bioavailability and enhanced antifungal activity. It was first synthesized in the late 1970s and subsequently introduced into clinical practice in the early 1980s. Since then, it has played a pivotal role in the management of superficial and systemic fungal infections, addressing the need for efficacious and well-tolerated antifungal therapies. The mechanism of action of ketoconazole centers on its ability to inhibit the synthesis of ergosterol, a key component of fungal cell membranes (Belazarian et al., 2014; Beggs 1984). Simply, by targeting the enzyme lanosterol 14α-demethylase, ketoconazole disrupts the conversion of lanosterol to ergosterol, resulting in the accumulation of toxic sterol intermediates and this compromises the fate of fungal cell membrane as it interferes with ergosterol biosynthesis to impair fungal growth and replication, ultimately leading to fungal cell death (Staub & Bergold, 2004; Mungula & Daniel, 2008; Okorie et al., 2011). In addition to its mechanism of action, ketoconazole possesses. The drug undergoes extensive metabolism in the liver, primarily via cytochrome P450 enzymes, leading to potential drug-drug interactions (Arvanitis et al., 2015). favourable pharmacokinetic properties. It is well-absorbed orally, with bioavailability reaching approximately 90%

Ketoconazole was initially approved for the treatment of superficial fungal infections, such as dermatophytosis (ringworm) and candidiasis (yeast infections). However, its amazing level of efficacy as well as its broad-spectrum activity quickly broadened its clinical applications to encompass more challenging fungal infections, such as systemic mycoses caused by organisms like Aspergillus, Histoplasma, and Coccidioides. Over the years, ketoconazole has played a significant role in the management of fungal infections, particularly in immunocompromised individuals, where the risk of opportunistic fungal pathogens is elevated, and it is still serving as one of the leading drugs in that field of therapy (Olga et al., 2013). However, despites its excellent level of efficacy, Ketoconazole is not completely free from limitations as the prolonged use or high doses have been associated with potential adverse effects, including hepatotoxicity and adrenal suppression (Mahler et al.,1993). Ketoconazole, in high doses, can simply bind to androgen receptors, such as that of testosterone and dihydrotestosterone, which can reduce the activity of testosterone and dihydrotestosterone in prostate cancer (Bhasi et al., 1986; 2023). Moreover, since ketoconazole has already established itself as an indispensable antifungal agent due to its broad-spectrum activity, unique mechanism of action, and clinical efficacy therefore, monitoring of liver functioning and adrenal hormone levels is recommended during the treatment period, to ensure patient safety and early detection of any abnormalities. The fact that, incorrect doses of ketoconazole within pharmaceuticals can result in adverse health effects for patients ranging from reduced efficacy, hepatotoxicity to other unintended side effects, accurate determination of the concentration of ketoconazole active ingredient utilized in pharmaceutical formulations is necessary. The recommended concentration according to World Health Organization (WHO) remains 200 - 400 mg per day for an adult

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(Naveed & Jaweed, 2014). Whereas, for the cosmetic dandruff shampoo 2 % concentrations is recommended (Sanfilippo & English, 2006).

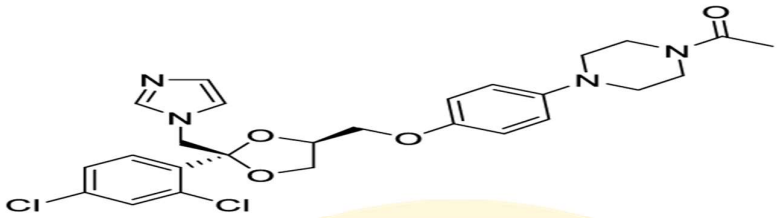

Figure 1: Chemical structure of Ketoconazole

However, the conventional approaches followed for the measurement of these concentration happen to pose significant threat to the habitable environment as these processes involves the use of hazardous chemicals like cn-hexane and methanol which are regarded undesirable and damaging to the living environment (Olga et al., 2013). As a result, the need for a robust and precise analytical method of Ketoconazole quantification in pharmaceutical samples, remains crucial. This research endeavors to address the multifaceted challenges by validating a spectrophotometry-based process using four different brands of ketoconazole. An aqueous medium was used as the solvent in order to avoid using hazardous solvents, offering a reliable solution for the green route to quality control of ketoconazole-based pharmaceutical drugs (Olga et al., 2013; Naveed & Jaweed, 20144).

Materials and Method

These include UV-spectrophotometer, 1 cm cell, 100 mL volumetric flask, pipette, analytical balance, deionized water, and ketoconazole samples coded as Axo-1, Derma-2, Ketora-3, and Keta-4.

Sample Preparation

Four different brands of ketoconazole tablets tagged to contain 200 mg of ketoconazole active ingredient per capsule were obtained from Top Ten Pharmaceutical Co LTD Lafia, Nasarawa State. Each brand was given with a code for identification, average weight of capsule was taken, and the powder containing 10 mg of each sample was taken and separately transferred to 10 mL of water and agitated to dissolve. After dissolving the drug sample, the volume was raised to 100 mL mark of the volumetric flask with deionized water (Fraihat & Bahgat 2014; Naveed & Jaweed 2014).

Procedure

Sample solutions having strength of 100 ppm, 50 ppm, 25 ppm, 12.5 ppm and 6.25 ppm were prepared for each of the four brands and absorbance of each solution was taken by using 1 cm cell at wavelength of 240 nm. Calibration curves were designed for each of the four samples and the nature of the curves were identified and conclusion was depicted (Fraihat & Bahgat, 2014; Naveed & Jaweed, 2014).

Wavelength Selection

Wavelength selection was done based on literature, and a wavelength of 240 nm was chosen and applied for the entire measurements (Naveed & Jaweed 2014).

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Result and Discussion

Table 1: NAFDAC number, manufacture date, expiry date, batch number, and license number of the drugs sample.

Brand Code	NAFDAC Reg. No.	MFG. Date	Exp. Date	Batch No.	MFG. LIC. No
Axo-1	B4-5194	04/20/23	03/2026	A308	G/308
Derm-2	B4-2451	12/2022	11/2025	M2202	G/639
Ketora-3	O4-9538	04/2022	03/2025	KZRT-008	MNB/04/85
Keta-4	A4-6971	10/2022	09/2025	GT22492	G/25/1749

Table 2: Average weight of tablet, and absorbance of 100 ppm of each sample at 240 nm.

Brand Code (given)	Average Weight of tablet (mg)	Concentration (ppm)	Absorbance at 240 nm
Axo-1	299	100	0.0230
Derm-2	309	100	0.0201
Ketora-3	290	100	0.0220
Keta- 4	300	100	0.0190

Table 3: Absorbance at different concentration of each brand at 240 nm wavelength.

Concentration (ppm)	Absorbance			
	Axo-1	**Derm-2**	**Ketora-3**	**Keta-4**
100	0.0230	0.0201	0.0220	0.0190
50	0.0120	0.0195	0.0110	0.0096
25	0.0053	0.0047	0.0052	0.0047
12.5	0.0028	0.0023	0.0024	0.0023
6.25	0.0014	0.0012	0.0012	0.0011

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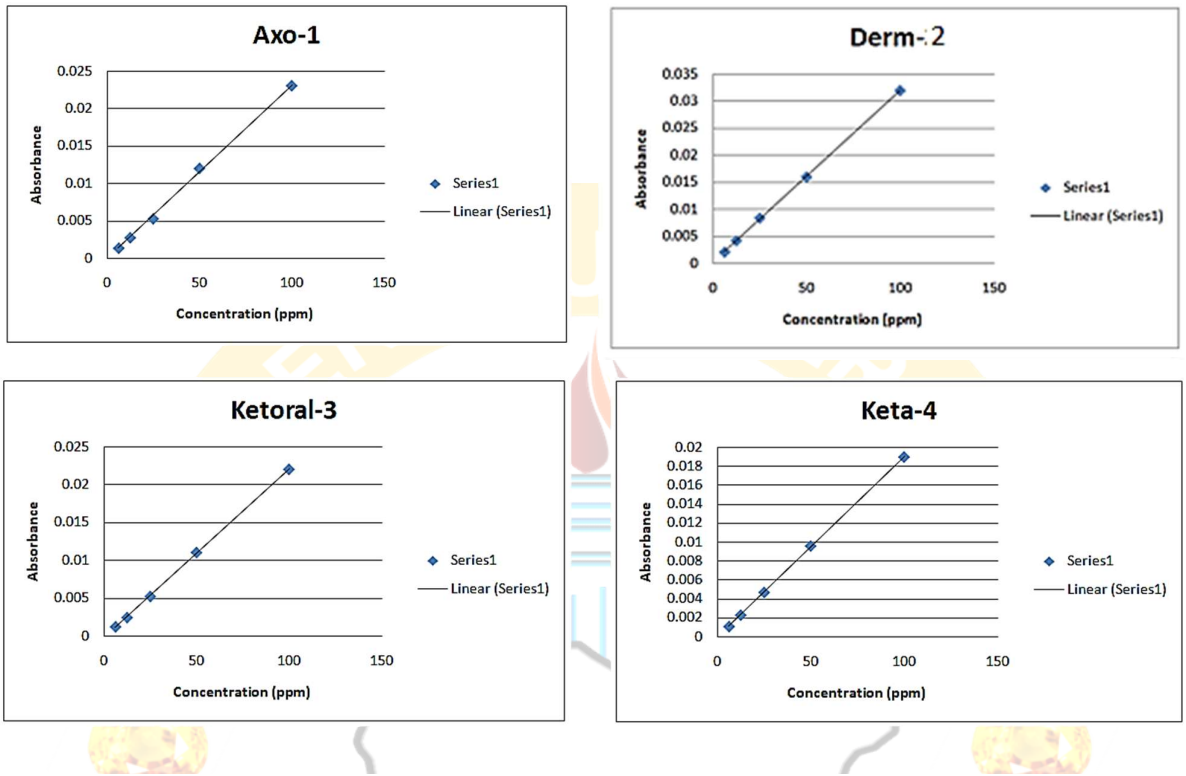

Figure 2: Graphs of Absorbance versus Concentration of all the four tested brands

The result from each of the four graphs (Fig. 2– 5) indicates that for each set of the measurements done on a separate brand, there is a linear relationship between absorbance and concentration. However, it is obvious that, one of the five coordinates in Derm-2 (Fig, 3), correspondence to an absorbance of 0.0195 and 50 ppm concentration; has shown deviation from the linear regression curve. This may have come from certain measurement errors. Moreover, Axo-1 (Fig. 2) shows highest level of UV light absorption at 240 nm wavelength for the varying concentrations of the drug that fell within the range of 100 - 6.25 ppm. This ranking, on the basis of the degree of UV absorptivity in the decreasing order was followed by Ketora-3, then Derma-2 and lastly the Keta-4 which shows the lowest level of UV absorption capability (Table 3). This result which shows a linear pattern of decrease in absorption with decrease in concentration, indicates compliance with the result reported by Naveed and Jaweed (2014), who worked on certain brands of ketoconazole drugs. Since, according to the Beer Lambert Law, concentration of a substance is directly associated with the absorbance of that material, then the brand with highest concertation of ketoconazole ingredient is the Axo-1, followed by Ketora-3, then Derma-2 and finally Keta-4 with the lowest concentration of the active ingredient present. However, these higher and lower concentrations may not indicate the degree of efficacy these brands can produce, since the recommended concentration shouldn't be significantly higher or lower than the usually recommended dose of 200 mg per tablet (Naveed & Jaweed 2014). However, when there is need to further identify the best brand with an amount of this active ingredient that best suits the dose recommendation of ketoconazole drugs, it is necessary to compare from the resulting absorbance; the degree of concentration of ketoconazole active ingredient among the four tested brands, and further analyses concerning

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the quantitative determination of this active ingredient are needed. However, this research is limited to process validation, which was conducted in a similar manner to the work of Naveed & Jaweed (2014), but this time, using new set of brands of the same drug..

Conclusion

According to Beer-Lambert law concentration, is always linearly related to the absorbance. Since, each of the four available brands has yielded a linear calibration curve, signifying a linear relationship between concentration of drugs in each sample and absorbance, the accuracy of the procedure is said to be validated.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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