dx.doi.org/10.14227/DT320125PGC23

Comparative Study of Metronidazole Tablets Marketed in Saudi Arabia

Hassan M. Ghonaim[1*], Munirah A. Alsaleh[1], and Saira Zahoor[1]

[1]*Department of Pharmaceutics, College of Pharmacy, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia.*

**Corresponding author*

email: hmghonaim@iau.edu.sa

ABSTRACT

Introduction: Metronidazole is usually used as an oral antiprotozoal and antibacterial agent to treat skin and mouth infections, bacterial vaginosis, and pelvic inflammatory diseases as well as some sexually transmitted infections (trichomoniasis and giardiasis. **Methods**: This study compared three generic brands of 250-mg metronidazole tablets (Amrizole, Riazole, Anazol) available in Saudi Arabia with the reference brand (Flagyl) according to United States Pharmacopeial (USP) specifications, including in vitro dissolution and physicochemical characteristics (i.e., uniformity of drug content, weight, hardness, thickness, and diameter, friability, and disintegration). In addition, anti-blastocyte activity was studied to evaluate the biological activity of the formulated nanocomposites by using cultured *Blastocystis sp*. suspension from symptomatic patients. *Blastocystis sp.* was subcultured with fresh Jones medium enriched with 10% horse serum to remove stool debris. **Results**: All products met USP specifications for all physical and physicochemical parameters, including the drug content assay (i.e., all products contained 94–99% of the label claim). The dissolution profiles showed no differences between the products. The biological activity showed the metronidazole started to destroy *Blastocystis sp*. within 1 hour, and all cells were dead by the end of 24 hours. **Conclusion**: All tested brands were bioequivalent under biowaiver conditions, with no significant differences in physical and physicochemical parameters as well as biologic activity against blastocystosis. The generic metronidazole tablets can be used interchangeably with the brand name product.

Keywords: Metronidazole, bioequivalence, physicochemical parameters, dissolution, blastocystosis

INTRODUCTION

According to the United States Food and Drug Administration biowaiver procedure for pharmaceutical products containing Biopharmaceutics Classification System (BCS) class I active pharmaceutical ingredients (APIs), the formulation should be freely dissolving, should not contain inactive ingredients that might influence the absorption, should not have a narrow therapeutic index, and should not be designed to be absorbed in the oral cavity. Metronidazole is a cost-effective treatment for both bacterial and parasitic organisms as well as anaerobic infections, with only minor adverse effects (*1*).

Generic medicines have been widely established in the international healthcare market to reduce medication expenditures (*2*). The World Health Organization has encouraged the use of generic products to decrease the cost of drug spending in the public healthcare system, but this requires proof that the generic replacement is interchangeable with the brand name product, and this is accomplished with bioequivalence studies (*3*, *4*). In vitro dissolution studies may be used instead of long and costly in vitro studies to establish bioequivalence for certain drugs under certain biowaiver conditions (*5*).

The aim of this study is to compare generic metronidazole tablets available in Saudi Arabia with the brand name product under biowaiver conditions to establish their bioequivalence and interchangeability (*6*, *7*).

METHODS

Four metronidazole tablet (250 mg) products were purchased from pharmacies in Saudi Arabia. The brand name product was Flagyl (Sanofi), and the generic products were amrizole (Amriya Pharmaceuticals), riazole (Riyadh Pharmaceuticals), and anazol (Jazeera Pharmaceuticals Industries), and a pure drug powder of metronidazole was used to prepare the stock solution (*8*). All chemicals and reagents were of pharmaceutical grade (Sigma Aldrich).

All products were of the same strength (250 mg), pack size, and dosage form, with some price differences (Table 1).

Table 1. Package Details for Metronidazole Tablets (250 mg)

Product	Manufacturer	Batch No.	Expiration date	Cost for 20 Tablets (SAR)	Relative price to the brand
Flagyl	Sanofi	3RX02	09/2026	10.05	Reference
Amrizole	Amriya Pharmaceuticals	9011006	08/2025	7.10	71%
Riazole	Riyadh Pharmaceuticals	23DE93	09/2026	5.80	58%
Anazol	Jazeera Pharmaceuticals Industries	6554	08/2026	6.45	65%

SAR: Saudi Riyals

Weight Variation

Twenty tablets from each product were weighed in milligrams on an electronic balance (A&D Company Ltd., Tokyo, Japan). The mean and percentage of variation were calculated for each batch (*2*, *9*).

Diameter

Six tablets from each brand were randomly selected, and the diameter of each tablet was measured with a digital Vernier caliper.

Hardness

Hardness was measured with a hardness tester (EBT-2PRL, Electrolab) by placing six tablets horizontally between the two jaws of the machine and moving the jaws towards each other until the tablet fractured; the required force to break the tablet was measured and recorded (*3*, *10*).

Thickness

Six tablets from each brand were randomly selected and evaluated by measuring the thickness of each tablet in the hardness tester (*3*, *9*).

Friability

Twenty tablets from each brand were dusted and weighed then subjected to a friability tester (Erweka, Germany), which was operated for 4 minutes at 25 rpm for 100 revolutions. All tablets were re-weighed after removing from the friability tester to calculate friability as a percentage of weight loss per USP standards (*2*, *10*).

Disintegration

A disintegration tester (PharmaTest, Germany) was used for to evaluate six randomly selected tablets from each brand (three with discs and three without discs). The disintegration medium was 900 mL of 0.1 N hydrochloric acid (HCL) with distilled water at 37 ± 2 °C (*8*, *11*, *12*).

Standard Curve

An accurately weighed 250 mg of pure metronidazole was dissolved in 100 mL of 0.1 N HCl solution and sonicated for 20 minutes to get a clear stock solution. A series of dilutions with concentrations of 0.005, 0.01, 0.015, 0.02, and 0.25 mg/mL of metronidazole were prepared from the stock solution. Absorbances were scanned at 278 nm using diluted stock solution as a blank on a UV spectrophotometer (Shimadzu, UV-1800, Japan). The measured absorbances were plotted against the concentrations of the standard drug solutions.

Uniformity of Content and Assay

One metronidazole tablet equivalent to 250 mg of active ingredient was dissolved in 100 mL of 0.1 N HCL using a magnetic stirrer then filtered. A 10-mL sample of filtrate was diluted to 100 mL with 0.1 N HCL, and further dilutions were prepared (0.25 and 0.025 mg/mL) (*3*). Metronidazole content was analyzed at 278 nm with a UV-visible spectrophotometer (Shimadzu, UV-1800, Japan) using the diluted solution as a blank at the lowest concentration of 0.025 (*13*).

Dissolution

Dissolution testing was conducted by qualifying all standards according to USP 32 (*14*). The test was measured for six randomly selected tablets by using a USP apparatus 2 (paddle) (Electrolab, India) at 100 rpm with 900 mL of 0.1 N HCl as dissolution medium at 37 °C. Samples (10 mL) were withdrawn after 5, 15, 30, 45, and 60 minutes and immediately replaced with pre-heated fresh medium to maintain the equilibrium. The samples were filtered using a syringe filter (0.45 µm, Millipore) (*15*). The filtrates were further diluted with 0.1 N HCl to a suitable dilution, and absorbance was measured at 278 nm (0.1 N HCL was used as a blank) on a UV-visible spectrophotometer (*3*). The percentage of cumulative drug release was recorded (*16*).

Antimicrobial Activity

Blastocystis sp. (protozoa) is a parasite that causes many infections including gastrointestinal infections. The first-line therapy is metronidazole; however, it was reported to show frequent inefficacy. Previously, *Blastocystis sp*. isolated from different populations showed metronidazole variable resistance, so the effect of metronidazole treatment on pathogenic *Blastocystis sp*. is unclear (*17*).

Cultured *Blastocystis sp.* suspension from symptomatic patients was used for evaluating the metronidazole biological activity. Following ethical clearance from the institutional review board, *Blastocystsis sp.* were collected from the El-Badry Lab and subcultured using fresh

Jones medium enriched with 10 mL of 10% horse serum to remove stool debris. Cultures with vacuolar forms of *Blastocystis sp.* greater than 106/mL were used to evaluate the biological activity of metronidazole. The number of *Blastocystis sp.* were counted under a microscope in a hemocytometer counting chamber.

The experiments were analyzed in five groups as follows: group 1 served as the negative control group containing cultured *Blastocystis sp.*, and groups 2–5 served as positive treatment groups, containing parasites exposed to Flagyl (group 2), amrizole (Group 3), riazole (group 4), and anazol (group 5). Parasites were exposed in each group with increasing concentration. All culture tubes were incubated at 37 °C in humidified CO_2 for 48 hrs. All tested nanocomposites for each concentration were prepared by dilution with stock suspension in the appropriate amount of phosphate buffered saline (PBS). Cultured *Blastocystis sp.* were tested for their viability using Trypan blue solution (0.4%). All cultured tubes were examined for a percentage of reduction in the growth of *Blastocystis sp.* each hour for 5 hours then at 24 hours (*18*).

RESULTS AND DISCUSSION

The metronidazole standard curve showed an equal concentration of known and standard metronidazole ($R^2 = 0.9994$).

All products complied with the USP specifications regarding all physical and physiochemical parameters for tablets, i.e., uniformity of drug content, weight, hardness, thickness, diameter, friability, and disintegration (Table 2). All brands contained 94–99% (w/w) of label claim.

Table 2. Mean ± SD Values for Physical and Physiochemical Tests of Metronidazole Tablets (250 mg)

Product	Drug Content (% of Label Claim)	Diameter (mm)	Weight (mg)	Hardness (kg/cm²)	Thickness (mm)	Friability (%)	Disintegration (min)
Flagyl	99 ± 0.9	10.1 ± 0.24	352.4 ± 3.8	9.1 ± 0.5	4.1 ± 0.1	0.072 ± 0.15	9.7 ± 1.0
Amrizole	96 ± 0.3	11.1 ± 0.33	500.2 ± 3.3	17.7 ± 0.9	5.2 ± 0.1	0.055 ± 0.002	11.5 ± 0.1
Riazole	94 ± 0.6	10.1 ± 0.15	360.2 ± 4.3	5.5 ± 0.9	4.0 ± 0.1	0.018 ± 0.001	3.2 ± 0.1
Anazol	97 ± 0.6	10.6 ± 0.40	379.1 ± 7.2	18.6 ± 2.8	3.7 ± 0.2	0.163 ± 0.011	9.9 ± 1.1

The dissolution profiles showed no variation (Fig. 1). Within 10 minutes, 40% of metronidazole was released from the tablet, and within 20 minutes almost 100% was released except for amrizole, and 100% was complete released was achieved within 1 hour for all products.

Based on data collection, the rate of apoptosis was noticed high in response to all treatments, when treated with metronidazole (*19*). *Blastocystis sp.* isolates died with metronidazole treatment. A lower cell count was observed with treatment of all metronidazole products, and the rate of apoptosis was high (*19*). When metronidazole was compared to the control, metronidazole treatment increased the destruction of *Blastocystis sp.* (*20*). There were no significant differences between the brand name and generic products, as all *Blastocystis sp.* started to die within 1 hour, and they were all dead in 24 hours (Figs. 2 and 3 and Table 3).

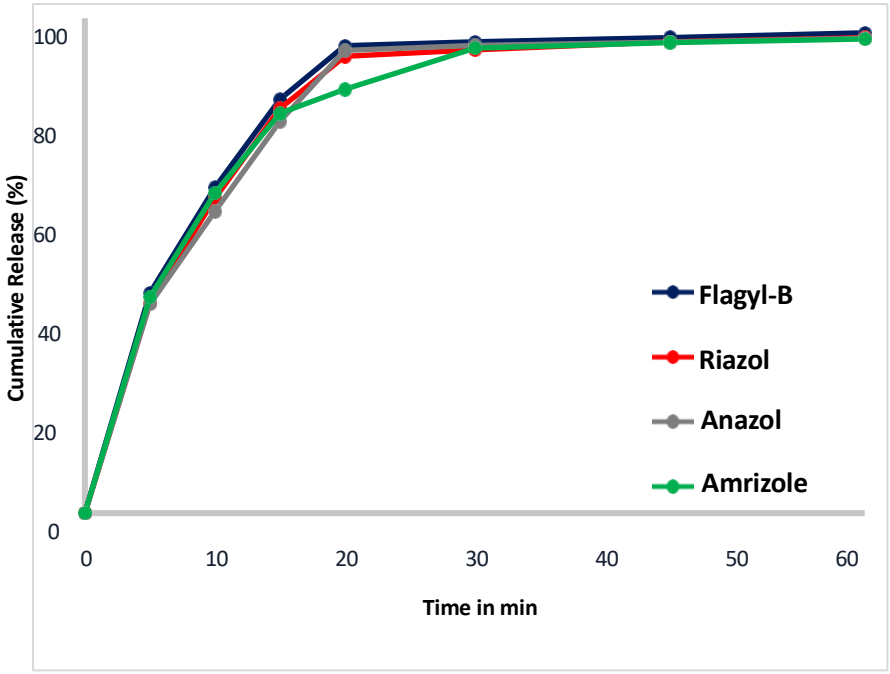

Figure 1. Results of in-vitro drug release dissolution studies of 250-mg metronidazole tablets.

Table 3. Anti-Blastocystis Sp. Effect of Metronidazole Tablets (250 mg)

Group	Product	1 hr	2 hr	3 hr	4 hr	24 hr
1	Standard (Control)	91.05 (1.22)	94.33 (0.58)	96.67 (0.58)	98.67 (0.58)	100 (0.00)
2	Flagyl	91.33 (1.53)	94.67 (0.58)	97.06 (1.08)	98.67 (0.58)	100 (0.00)
3	Amrizole	92.27 (1.06)	95.33 (0.58)	97.67 (0.58)	99.05 (0.63)	100 (0.00)
4	Riazole	91.67 (1.15)	95.07 (1.24)	97.33 (1.15)	98.83 (0.29)	100 (0.00)
5	Anazol	91.33 (1.53)	94.67 (0.58)	97.15 (1.33)	98.67 (0.58)	100 (0.00)

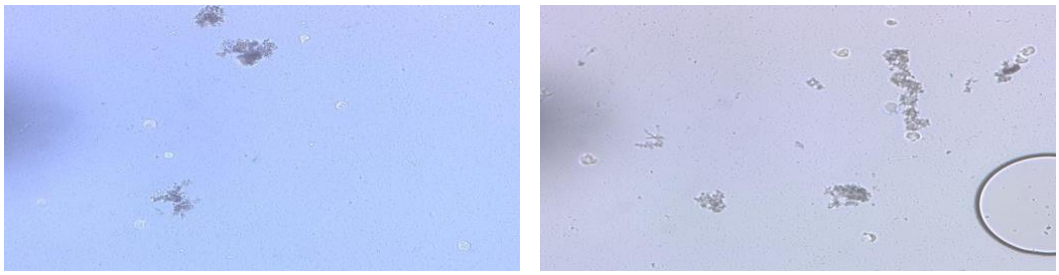

Figure 2. Microscopic staining of Blastocystis sp. with Trypan blue solution (0.4%). Light cells are considered alive (left) and dark cells are considered dead after addition of metronidazole (right).

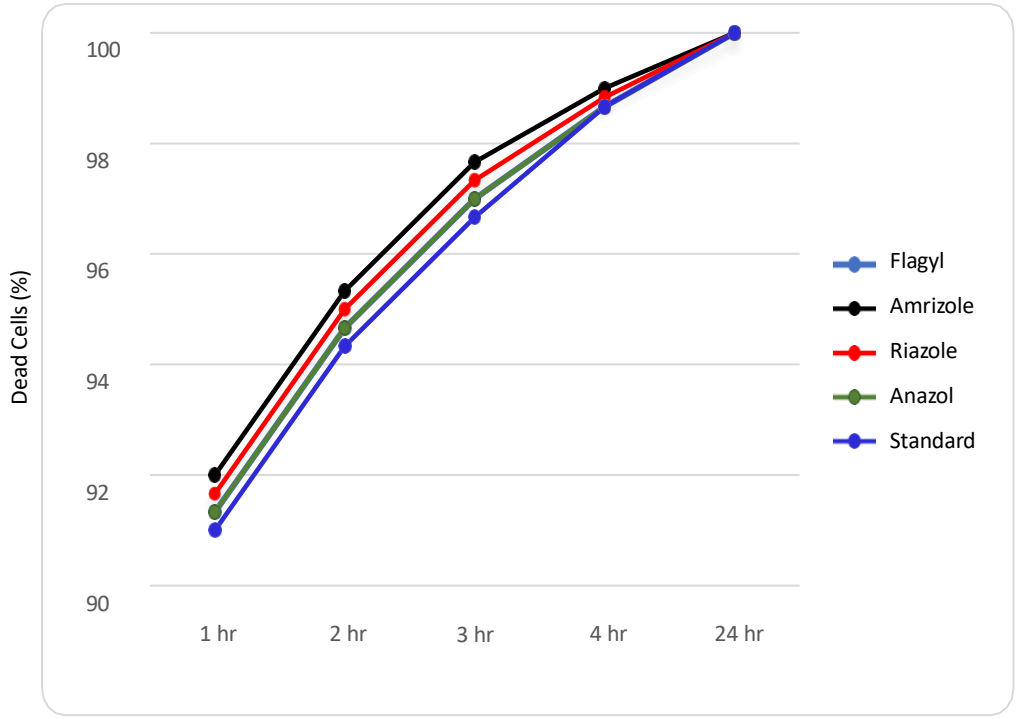

Figure 3. Anti-Blastocystis sp. effect of 250-mg metronidazole tablets.

CONCLUSION

The tested generic (amrizole, riazole, anazol) and brand name (Flagyl) metronidazole tablets available in Saudi Arabia are bioequivalent under biowaiver conditions, with no significant difference in biologic activity against blastocystosis. Therefore, these products can be used interchangeably.

ACKNOWLEDGMENT

The authors thank the College of Clinical Pharmacy at Imam Abdulrahman Bin Faisal University for providing facilities and support.

DISCLOSURES

The authors received no financial support for this study and have no conflicting interests.

REFERENCES

1. Noor, S.; Salam, F. B. A.; Hima, H. N.; Bhuiyan, S.; Chowdhury, S. Comparative in-vitro quality evaluation of some brands of metronidazole tablet available in Bangladesh. *Int. J. Appl. Res.* **2017**, *3* (7), 753–758.
2. Ahmed, E. M.; Ibrahim, M. E.; Magbool, F. F. In vitro-in vivo bio-equivalence correlation study of metronidazole, and its brands of immediate release tablet under bio-waiver conditions. *Universal Journal of Pharmaceutical Research* **2020**, *5* (1), 32–37. DOI:10.22270/ujpr.v5i1.360.
3. Aleanizy, F. S.; Al-Eid, H.; Tahir, E. E.; Alqahtani, F.; Al-Gohary, O. Stability and in vitro dissolution studies of metronidazole tablets and infusions. *Dissolut. Technol.* **2017**, *24* (2), 22–27. DOI: 10.14227/DT240217P22.
4. Mishra, V.; Gupta, U.; Jain, N. K. Biowaiver: an alternative to in vivo pharmacokinetic bioequivalence studies. *Pharmazie* **2010**, *65* (3), 155–161.
5. Kaushal, N.; Singh, S. K.; Gulati, M.; Vaidya, Y.; Kaushik, M. Study of regulatory requirements for the conduct of bioequivalence studies in the US, Europe, Canada, India, ASEAN and SADC countries: impact on generic drug substitution. *J. Appl. Pharm. Sci.* **2016**, *6*, 206–222. DOI: 10.7324/JAPS.2016.60430.
6. Dunne, S.; Shannon, B.; Dunne, C.; Cullen, W. A review of the differences and similarities between

generic drugs and their originator counterparts, including economic benefits associated with usage of generic medicines, using Ireland as a case study. *BMC Pharmacol. Toxicol.* **2013**, *14* (1), 1–19. DOI: 10.1186/2050-6511-14-1.
7. Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System; Guidance for Industry. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, 2017.
8. Silva, D. A.; Curo Melo, K. J.; Davies, N. M.; Bou-Chacra, N.; Ferraz, H. G.; Löbenberg, R. A BCS-based biowaiver approach using biphasic dissolution test. *Dissolut. Technol.* **2021**, *28* (4), 40–48. DOI: 10.14227/DT280421P40.
9. Odunayo, A. B.; Kayode, F. I.; Benjamin, A. A.; Adekola, A. I.; Ruth, O. O. Evaluation of the binding property of some binders in metronidazole tablet formulation. *Int. J. Pharm. Chem.* **2021**, *7* (2), 22–30.DOI: 10.11648/j.ijpc.20210702.11.
10. Nisar, H.; Khan, S. A.; Shah, Y.; Alossaimi, M. A.; Abbas, M. Effect of shape on physico- chemical properties of metronidazole tablet. *Braz. Arch. Biol. Technol*. **2022**; *65*, e22210611. DOI: 10.1590/1678-4324-2022210611
11. Ilomuanya, M. O.; Mbaneme, N. A.; Okubanjo, O. O.; Ofokansi, K. In vitro equivalence studies/comparative assessment of generic metronidazole tablets commercially available in Lagos, Nigeria. *Br. J. Pharm. Res.* **2015**, *7* (3), 196–205. DOI: 10.9734/BJPR/2015/18572.
12. *British Pharmacopoeia*. Medicines & Healthcare Products Regulatory Agency; 2024.
13. Abdullahi, A. K.; Olowosulu, A. K.; Allagh, T. S. Crystallo-co-agglomeration technique for improving physicochemical properties, compressibility and solubility characteristics of metronidazole. *British Journal of Pharmacy* **2023**, *8* (1). DOI: 10.5920/bjpharm.1039
14. *USP–NF.* United States Pharmacopeia, 2023.
15. Lazo, R. E. L.; Teleginski, L. K.; Maciel, A. B.; Silva, M. A. S.; Mendes, C.; Bernardi, L. S.; Murakami, F. S.; Sonvico, F.; Oliveira, P. R. Comparator product issues for biowaiver implementation: the case of fluconazole. *Braz. Arch. Biol. Technol*. **2022**, *58*, e19710. DOI: 10.1590/s2175-97902022e19710.
16. Stuart, A. V.; Zuo, J.; Löbenberg, R. Investigating the dissolution profiles of amoxicillin, metronidazole, and zidovudine formulations used in Trinidad and Tobago, West Indies. *AAPS PharmSciTech* **2014**, *15* (5), 1060–1069. DOI: 10.1208/s12249-014-0132-9.
17. Rajamanikam, A.; Hooi, H. S.; Kudva, M.; Samudi, C.; Kumar, S. Resistance towards metronidazole in Blastocystis sp.: A pathogenic consequence. *PLoS One* **2019**, *14* (2), e0212542. DOI: 10.1371/ journal.pone.0212542.
18. Jermy, B. R.; Al-Jindan, R. Y.; Ravinayagam, V.; El-Badry, A. A. Anti-blastocystosis activity of antioxidant coated ZIF-8 combined with mesoporous silicas MCM-41 and KIT-6. *Sci. Rep.* **2022**, *12* (1), 6403. DOI: 10.1038/s41598-022-10397-4.
19. Girish, S.; Kumar, S.; Aminudin, N.; Hashim, N. M. Comparison of apoptotic responses in *Blastocystis sp*. upon treatment with Tongkat Ali and Metronidazole. *Sci. Rep.* **2021**, *11* (1), 7833. DOI: 10.1038/s41598-021-81418-x.
20. Franklin, F.; Rajamanikam, A.; Raju, C. S.; Gill, J. S.; Francis, B.; Sy-Cherng, L. W.; Kumar, S. Higher amoebic and metronidazole resistant forms of Blastocystis sp. seen in schizophrenic patients. *Parasit. Vectors* **2022**, *15* (1), 313. DOI: 10.1186/s13071-022-05418-0.