

Research: In Vitro Dissolution Study of Metronidazole Tablet in Different Fluids

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ABSTRACT

Objective: The plot of this study is to obtain a grasp regarding the effect of different fluids (Gastric medium, simulated gastric fluid without enzyme, ORS solution, rice water, lentil soup, watermelon juice, apple juice, mango juice, pomegranate juice, black and green tea) on dissolution of metronidazole tablet by alteration of different medium.

Method: Our study had focused on the *in vitro* dissolution study of metronidazole in the presence of different fluids (Gastric medium, simulated gastric fluid without enzyme, ORS solution, rice water, lentil soup, watermelon juice, apple juice, mango juice, pomegranate juice, black and green tea) which has been commonly used in diarrheal condition. Different dissolution medium was prepared from 200 ml of fluids and 700 ml of 0.1 N HCl. pH was adjusted to pH 1.2. One commonly available marketed brand is used for the study. Dissolution study was performed as per mentioned in Indian pharmacopoeia. For the analysis of study, we used Graph Pad prism version 8 and Microsoft excel. ANOVA followed by Tukey's test was performed for the comparison of dissolution in different fluids

Result: The mean dissolution of metronidazole tablet in Rice water, Lentil soup, Watermelon juice, Mango juice, Pomegranate juice, Black tea and Green tea are significantly different from the dissolution on 0.1 N HCl medium ($p < 0.05$, 95% CI). The dissolution in simulated gastric fluid without enzyme, ORS solution and apple juice are not significantly different from 0.1 N HCl medium ($p > 0.05$, 95% CI). The highest percentage drug release of Metronidazole was found in apple juice (102.78 ± 2.10). In lentil soup dissolution of Metronidazole doesn't comply with the IP specification (78.69 ± 1.89).

Conclusion: Metronidazole tablet plays a vital role in treatment of diarrhea but before it shows its therapeutic effect on body, it must have to dissolve first. So, dissolution is considered as an important parameter to determine the bioavailability of drug as well as its efficacy. Our study concludes that administration of apple juice can be beneficial. On other hand lentil soup can decrease the therapeutic effect of metronidazole tablet.

Keywords: Metronidazole; Dissolution; Different fluids; Lentil soup

INTRODUCTION

Metronidazole tablet (400 mg) is included in essential medicine list of Nepal. It is an antiprotozoal anti-parasitic agent used in the treatment of amoebiasis, trichomoniasis, giardiasis, and other parasitic diseases. Since 1960 it has been extensively used as systemic trichomonicide and also shown activity against anaerobic microorganisms including bacteria and protozoa [1]. Metronidazole is used as first line treatment for *Clostridium difficile* infection which is the leading cause of hospital acquired diarrhoea in developed countries [2]. It is the drug of choice for the treatment of various parasitic infections, Anaerobic infections, Pseudomembranous colitis, Helicobacter pylori and Crohn's disease [3]. Metronidazole is a prodrug. Unionized metronidazole is selective for anaerobic bacteria due to their ability to intracellularly reduce metronidazole to its active form. This reduced metronidazole then covalently binds to DNA, disrupt its helical structure, inhibiting bacterial nucleic acid synthesis and resulting in bacterial cell death. It is metabolized in liver through oxidation and conjugation process with glucuronic acid. It has 7-8 hours of half-life and eliminates through urine [4]. Metronidazole

if used during pregnancy may results in high risk of congenital malformations [5]. It shows disulfiram like reactions when administered with alcohol [3].

Metronidazole occurs as pale yellow crystals that is slightly soluble in water and alcohol [6]. According to Indian pharmacopoeia, assay of metronidazole is done by non-aqueous titration method using

Preparation of gastric medium: Preparation of gastric medium (0.1 N HCl, pH 1.2), 50.84 ml of 36.5% w/v HCl was diluted with sufficient water to produce 6000 ml.

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Perchloric acid as titrant and malachite green as indicator. Methods such as UV spectroscopy and polarography have been reported for the determination of metronidazole tablet [7]. There is need to develop dissolution study that better predict *in vivo* absorption of drug products. This could be achieved if the gastrointestinal tract were successfully reconstructed *in vitro* [8]. The drug food interaction may affect health status due to altered pharmacokinetics and pharmacodynamics of the drug by increasing drugs bioavailability with the risks of adverse effects and toxicity or decreasing its bioavailability, leading to therapeutic failure [9].

Tablet Dissolution is a standardized method for measuring the rate of drug release from a dosage form. Actually *in vitro* dissolution of the drug from the tablet matrix relies on many factors, which includes physicochemical properties, nature of formulation and process manufacturing of drugs. Several studies shows that the percentage drug release varies when *in vitro* studies has been conducted in presence of different juices [3,10,11]. Diarrhea is one of the most common disease in Nepal. During diarrheal condition, many fluids has been recommended for replenish of fluid loss and electrolytes and restoring the energy in body. This study deals with the determination of *in vitro* dissolution release of most commonly available metronidazole tablet considering with different fluids during administration.

MATERIALS AND METHODS

Reference standard of Metronidazole has been supplied from Lomus Pharmaceuticals Pvt. Ltd. Nepal. All reagents were analytical grade for dissolution studies.

Collection of samples

One commercially available metronidazole film coated tablet was purposively selected from pharmacy located in Kathmandu, Nepal. The sample was within the expiry date. The labeled active ingredients contents of metronidazole were 400 mg and packed in blister packing. The metronidazole tablet was claimed Indian Pharmacopoeia standard. Those samples were properly stored within room temperature before the experiment.

Preparation of standard: 50 mg of metronidazole was weighed and dissolved with 100 ml of 0.1 N HCl. Then, 1 ml of solution was taken and further diluted to 50 ml of 0.1 N HCl.

Preparation of dissolution medium: The pH of all medium were adjusted to 1.2.

Preparation of simulated gastric fluid without enzyme: 2 gm of NaCl was weighed and dissolved in water then 7 ml concentrated HCl was added and the volume was made 1000 ml with distilled water.

Preparation of oral rehydration salt solution: ORS was randomly purchased from pharmacy and ORS solution was prepared in 1000 ml of water. 200 ml of above solution was taken and the volume was made 900 ml with 0.1 N HCl.

Preparation of rice water: 125 ml (1/2 cup) of rice was taken and rinsed then 2 liter of water was added and the rice was brought to boil on medium heat for 5 to 10 minutes. The rice wasn't fully cooked. The rice from the water was separated and cooled. Then 200 ml of previously prepared rice water was diluted to 900 ml with 0.1 N HCl.

Preparation of lentil soup: The *Vigna radiate* (Mung bean) soup was purchased from hospital canteen. 200 ml of lentil soup and 700 ml of 0.1 N HCl was mixed well before placing in dissolution basket.

Preparation of watermelon juice: The watermelon was sliced and placed in plate with a fork to avoid mess. 500 g of watermelon fresh fruit was taken to which 200 ml of water was added. Then, it was placed in the juicer. The difference between seedless watermelons and watermelons with seeds lies not in their tastes but only in that seedless watermelons contain white edible seeds. After preparation of watermelon juice, 200 ml of watermelon solution was taken and 700 ml of 0.1 N HCl solution was mixed well before placing it in dissolution basket [10].

Preparation of juices (apple, mango and pomegranate): The marketed juices were purchased. Each of 200 ml of juices were taken separately and 700 ml of 0.1 N HCl was added in each vessel. The solution was then mixed well and apparatus were assembled.

Preparation of tea (black and green): 300 ml water was brought to boil and 2 tea bags were put separately in a beaker. 200 ml of boiled water was poured into the beaker and infusion was allowed for 2-3 min, after 2-3 min, tea bags were dipped in the beaker for 8-10 times. After preparation of tea, 200 ml of each tea solution was taken and 700 ml of 0.1 N HCl solution was mixed well before placing in dissolution basket.

Procedure of dissolution method (I.P): Dissolution medium: 900 ml

Apparatus I: 100 rpm

Procedure: Determined the amount of metronidazole dissolved from UV absorbance at wavelength of maximum absorbance at 277 nm of filter portions of solution under test, suitability diluted with 0.1 N HCl, in comparison with a standard solution having a known concentration of IP Metronidazole RS in the same medium.

Tolerance: Not less than 85% of the labeled amount of metronidazole.

Standard curve preparation: 50 mg of reference metronidazole is dissolved in 0.1 N HCl to make volume 100 ml. From above solution 1, 2, 3, 4, 5 ml is taken respectively and each of them was made 100 ml with 0.1N HCl. All gradual concentrations of solution were conducted for plotting standard curve at 277 nm. The corresponding regression data, indicated reasonable linear relationship $R^2 = 0.9981$. The calibration curve was shown in Figure 1.

Statistical analysis

For the analysis of this study we used graphpad prism version 8 and Microsoft excel. ANOVA followed by Tukey's multiple comparison test was performed for comparison of % drug release in different dissolution medium with standard dissolution medium.

RESULTS AND DISCUSSION

In process quality control parameters

Weight variation test: The objective of weight variation test is to ensure appropriate size of the tablet and the content uniformity of the formulation. The Indian pharmacopoeia provides criteria for tablet weight variation test of intact dosage forms which states that the % weight variation should be within 5% for 400 mg tablet. This brand of tablets compiled within the specifications. The average mean weight of tablet was shown in Table 1.

Length and thickness: The tablets of various length and thickness can be prepared without any change of their weights. The length and thickness of tablets depend on the die and punches of the tablet compression machine. As shown as in Table 1, the average length and thickness of tablet was found to be 19.28 mm and 6.37 mm respectively.

Hardness: Hardness influences many tablet properties including disintegration, dissolution, and friability. The average value of hardness was found to be 5.75 (Kg/mm²) which compiled the IP specification which was shown in Table 1.

Friability: Friability test is used to determine physical strength of compressed and uncoated tablets upon exposure to mechanical shock and attrition. The friability of tablet was found to be 0.021% which is within the IP specifications and shown in Table 1.

Disintegration test: Disintegration test is very important for all coated and uncoated tablet because the dissolution rate of drug depends on the disintegration time, which ultimately affect the rate of absorption and subsequent bioavailability of drug. According to IP, film coated tablet should disintegrate within 30 mins. Tablets were disintegrate in 7 min and 45 sec which was shown in Table 1. The Metronidazole tablet meets the requirement of IP.

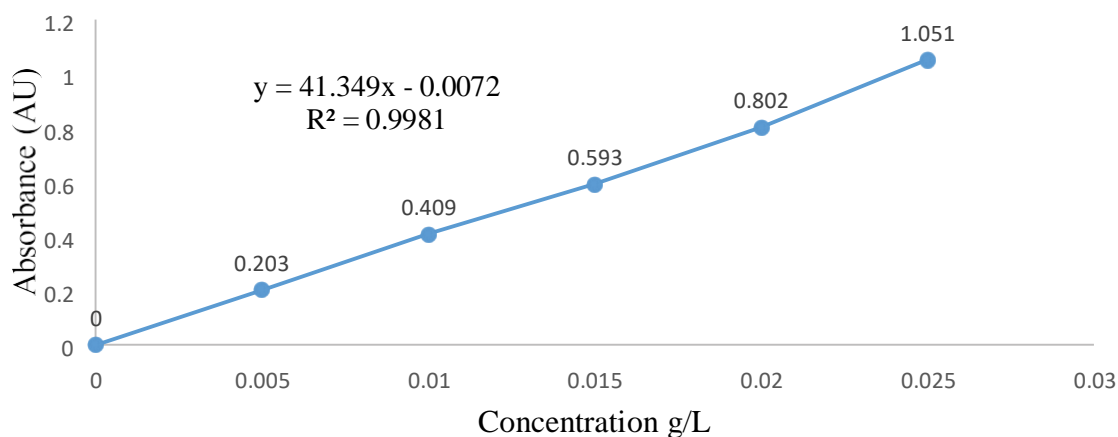
Assay%: Assay is a measure of drug activity expressed in terms of the amount of API (in %) require to produce and effect of given intensity. The assay % of the metronidazole tablet within the range of % of labeled amount of drug and compiled according to IP which was shown in Table 1 average assay of tablet was 101.37%.

Dissolution test

The dissolution of metronidazole tablet was performed in different fluids. The mean % drug release was determined from 6 tablets. According to IP, the limit of % drug release should not be less than 85% of labeled amount in 60 min at 100 rpm. Dissolution percentage of all 6 samples of metronidazole tablets in different fluids were shown in Table 2. The mean % drug release of metronidazole tablet in 0.1 N HCl was found to be 101 ± 2.22 which is within the IP specifications. In simulated gastric fluid without enzyme, the % drug release was found to be 101.28 ± 2.51 which is within the limit range. When dissolution study was performed in ORS solution the % drug release is 100.38 ± 2.52 which passed the test. Dissolution of metronidazole tablet was done in rice water which also pass the specification i.e. 95.16 ± 2.00 . Lentil soup was also used as dissolution medium, in this medium the % drug release of metronidazole tablet is decrease to 78.69 ± 1.89 . In lentil soup, the dissolution of metronidazole tablet doesn't compiled with IP specification. The dissolution of Metronidazole was also performed in watermelon juice and % drug release was found to be 92.42 ± 4.72 which passed the limit. The result of the study was also shown in Figure 2. In the study of Amber Nawab et al. the average % drug dissolve in presence of watermelon juice was found to be 97.51 [10] which is slightly higher value than our study.

Statistical significance was determined between the dissolution on different mediums to investigate the difference between the mean dissolution in all mediums. The ANOVA results indicate that there is significant mean difference between all mediums ($p < 0.05$). The result of ANOVA test is presented in Table 3. Tukey's multiple comparison test is also conducted to compare the differences between the mean dissolution in different medium. The Tukey's test showed that dissolution in Rice water, Lentil soup, Watermelon juice, Mango juice, Pomegranate juice, Black tea and Green tea are significantly different from 0.1 N HCl medium ($p < 0.05$, 95% CI). The dissolution in simulated gastric fluid without enzyme, ORS solution and apple juice are not significantly different from 0.1 N

Calibration Curve Of Metronidazole



In Different Concentration

Figure 1: Calibration curve of metronidazole working standard.

Table 1: Physical parameters of tablets.

Mean (mg)	Weight		Length (mm)	Thickness (mm)	Hardness (Kg/mm ²)	Friability %	DT (min)	Assay%
	%Maximum deviation	%Minimum deviation						
920.17	3.03	2.61	19.28 ± 0.1	6.37 ± 0.3	5.75 ± 1.3	0.021	7.45	101.37

Table 2: %Drug release of metronidazole samples.

S.N.	Dissolution Medium	% Drug release					
		S ₁	S ₂	S ₃	S ₄	S ₅	S ₆
1.	0.1 N HCl (Gastric medium)	98.97	103.98	97.90	101.12	101.83	102.19
2.	Simulated gastric fluid without enzyme	98.25	104.63	103.04	99.53	99.53	102.72
3.	ORS Solution	97.97	101.29	97.97	100.39	104.62	100.39
4.	Rice Water	95.79	95.50	96.37	95.50	96.66	91.18
5.	Lentil Soup	77.56	76.68	76.90	79.75	81.29	79.97
6.	Watermelon Juice	85.51	91.56	88.39	95.88	96.74	96.45
7.	Apple Juice	102.94	104.70	104.00	103.51	98.74	102.83
8.	Mango Juice	96.20	95.95	92.24	89.52	93.97	95.95
9.	Pomegranate Juice	86.29	92.70	88.67	95.26	91.78	91.23
10.	Black Tea	86.54	87.51	86.54	86.22	86.70	87.84
11.	Green Tea	91.56	91.73	92.24	93.43	90.53	90.70

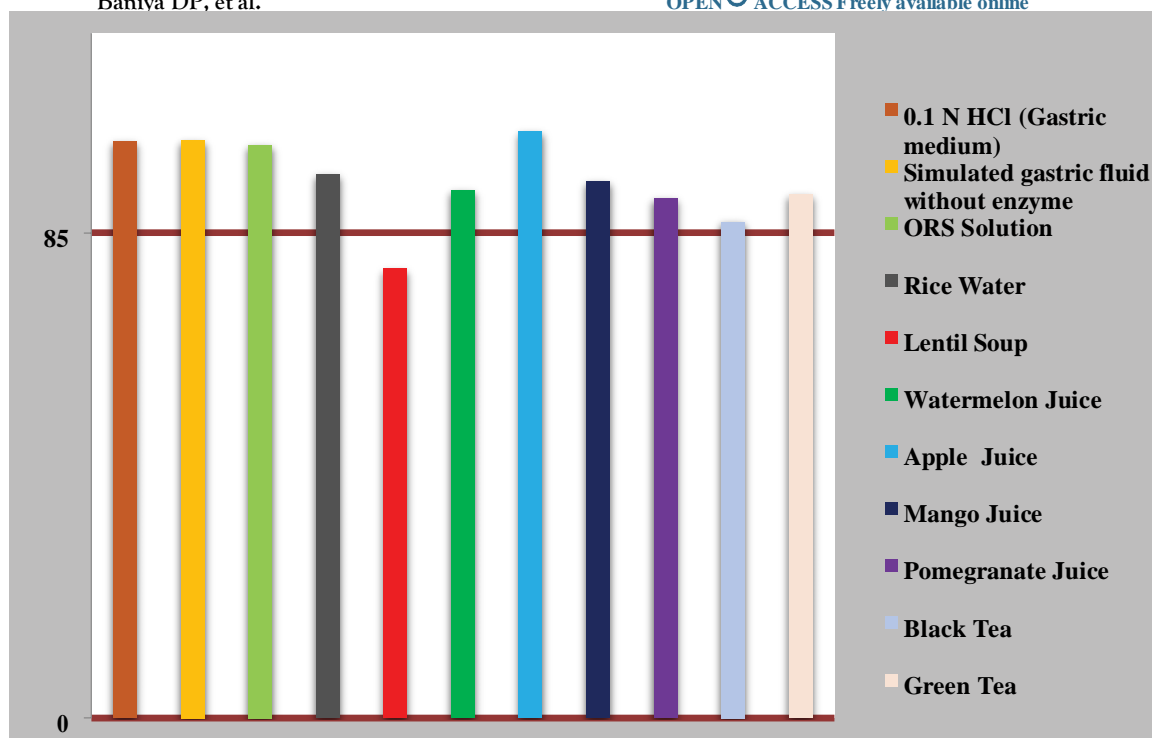


Figure 2: %Drug release in different dissolution medium.

Table 3: Drug release in different mediums (ANOVA Table).

ANOVA Table	SS	Df	MS
Treatment (between columns)	3142	10	314.2
Residual (within columns)	351.3	55	6.388
Total	3493	65	

HCl medium ($p > 0.05$, 95% CI). The result of test is presented in Table 4.

Percentage drug release when evaluated in apple juice, the dissolution of metronidazole tablet was within the limit i.e. 102.78 ± 2.10 which is higher % drug release among all other used mediums. *In vitro* dissolution study of metronidazole tablet was also performed in pomegranate juice and % drug release was found to be 90.98 ± 3.14 which shows significantly decrease the dissolution

Medium	Mean Diff.	q	Significant?	95% CI of diff
Simulated gastric fluid without enzyme	-0.2850	0.2762	No	-5.191 to 4.621
ORS Solution	0.5600	0.5427	No	-4.346 to 5.466
Rice Water	5.832	5.652	Yes	0.9254 to 10.74
Lentil Soup	22.31	21.62	Yes	17.40 to 27.21
Watermelon Juice	8.577	8.312	Yes	3.670 to 13.48
Apple Juice	-1.788	1.733	No	-6.695 to 3.118
Mango Juice	7.027	6.810	Yes	2.120 to 11.93
Pomegranate Juice	10.01	9.701	Yes	5.104 to 14.92
Black Tea	14.11	13.67	Yes	9.200 to 19.01
Green Tea	9.300	9.013	Yes	4.394 to 14.21

Table 4: Result of Tukey's multiple comparison test. Result is presented only comparison between 0.1 N HCl and other mediums.

Profile when compared with 0.1 N HCl dissolution medium. Both dissolution medium passed the dissolution test.

For evaluation of metronidazole dissolution profile we also used black and green tea medium. Both medium shows dissolution profile within the limit. The average % drug release was found to be 86.89 ± 0.63 and 91.69 ± 1.06 in black tea and green tea respectively. In black tea, average % drug release was minimum than other used mediums. In both tea medium dissolution profile significantly decrease when compared with 0.1 N HCl solution. Amber Nawab et al. study shows that 114.66% and 104.12% average drug release in black and green tea respectively [11]. Her study shows higher % drug release in both medium than gastric medium (0.1 N HCl) but our study shows decrease in dissolution profile. The reason behind this may be because of using different brand of tea and different excipients used in different brands.

CONCLUSION

The present study was performed with a purpose to evaluate dissolution pattern of metronidazole tablet in the presence of different fluids. The drug food interaction can decrease the absorption of drug. Thus, undertaken dissolution testing in dissolution apparatus need to be able to deliver precise and consistent results. The metronidazole tablet which was marketed in Nepal showed higher % drug release in apple juice. In the presence of lentil soup, there was significant decrease in % drug release of metronidazole which doesn't comply with Indian Pharmacopoeia specification but the other remaining fluids complied with the IP specification. Conclusively, our study suggests that metronidazole tablet should not be administered with lentil soup.

DATA AVAILABILITY

The data described in this article are new data obtained from this research. The data used to support the findings of this study are included within the article.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest to disclose.

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