

Statistical Evaluation of *In-vitro* Analysis of Different Brands of Metronidazole Tablets Available in Bangladesh

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Abstract:

Introduction: In Bangladesh, there are different brands of metronidazole tablets available. Each brand has its own formulation which affects the release and delivery of drug and produce variable clinical responses. So, there is a need for *in-vitro* analysis of different brands of the same generic.

Aim: The prime objective of this research work was to check and evaluate the quality of different brands of metronidazole tablet 400mg from local market of Dhaka in Bangladesh.

Methods: Five different brands of this drug were tested for uniformity of weight, length, diameter, hardness, friability and *in-vitro* dissolution. Statistical analysis tools like standard deviation for compendia tests and repeated measure ANOVA were also applied to find out the variation in dissolution parameters between brands.

Results: All the brands had passed the compendia tests (variation, length, thickness, hardness, friability) and had showed a significant difference of dissolution patterns between the brands.

Conclusions: Although significant difference was observed in dissolution patterns between the groups, all the brands of metronidazole tablets met the compendial criteria for film coated tablet.

Keywords: Metronidazole tablets; Generic products; *In-Vitro* analysis; Compendial tests; ANOVA.

1. INTRODUCTION

Among the different routes of drug administration, oral route is the most common due to patient compliance. After oral administration of drug, it may have ineffective drug absorption due to improper dissolution rather than absorbed rapidly and completely into the bloodstream [1]. Tablet formulation contains various excipients like diluents, binders, disintegrants, glidants, lubricants and sometimes coloring and flavoring agents that must not affect the stability, dissolution rate and release [2]. The increasing number of generic products demand health care professionals to select one from several products that are assumed to be equivalent. According to a study in US, in 1975 about 9% of all prescription drugs dispensed were generic versions [3]. So, there is a need for *in-vitro* analysis of different brands of the same generic.

Metronidazole is an anti-protozoal, anti-bacterial, anti-parasitic agent that is very effective in the treatment of amoebiasis, trichomoniasis, giardiasis and many other parasitic diseases. It is a prodrug that requires reproductive activation of the nitro group by susceptible organisms. Therefore, it can be inferred that slight change in the dissolution pattern may affect its biopharmaceutics and thus its quality and efficacy. [4, 5]

In Bangladesh, there are many different brands of metronidazole tablets available from different national and multinational companies. Each brand has its own formulation which affects the release and delivery of drug and produce variable clinical responses. It is suggested that their pharmaceutical and therapeutic equivalence should be determined in order to ensure interchangeability [6]. Evaluation of *in-vitro* release and the physicochemical properties of these brands is very important as it can be used to evaluate the bioavailability and pharmaceutical equivalence [7].

The prime objective of the study was to evaluate and compare the physicochemical equivalence of tablets of different brands that were available in local market of Dhaka (Bangladesh). The proposed study had performed to provide a guideline for the health care professionalists that can be used to select metronidazole for patients. The physical parameters i.e. weight variation, length and diameter, thickness, hardness, friability, disintegration as well as dissolution were considered during the present study.

Different physicochemical parameters like variation in weight, length, thickness, hardness, friability and percent release was calculated. Statistical analysis of the dissolution results were performed using repeated measure ANOVA [8].

2. MATERIALS AND METHODS

2.1 Instrumentation

For the analysis of metronidazole content in their dosage form, a Shimadzu UV-1800 spectrophotometer was utilized. Spectrophotometer system was integrated via Shimadzu model to dual core Windows 7 computer loaded with Shimadzu 1800 software for data acquisition. Analytical balance, dissolution test apparatus, friabilator, slide calipers, hardness tester (Monsanto Type) and micropipette were also used. Statistical Analysis like average, standard deviation and repeated measure ANOVA was applied using Microsoft Excel 2007.

2.2 Materials and reagents

Metronidazole API was given as a gift by Incepta Pharmaceuticals Ltd, Dhaka, Bangladesh. Different brands of metronidazole tablets (400mg) were purchased from local market. The details of the tablets like the product code, batch numbers, manufacturing date and expiry date are given (Table 1) along with their codes.

2.3 Uniformity of weight

20 tablets were taken from each product code. Each tablet was weighed individually. Then the average weight was calculated for every brand. The tablets were carefully examined for their uniformity of weight and the percentage deviation allowed by USP (generally $\pm 10\%$ for tablets weighing 130 mg or less, $\pm 7.5\%$ for tablet weighing more than 130 mg to 324 mg and $\pm 5\%$ for tablet weighing more than 324 mg [9]).

2.4 Uniformity of thickness

Thickness of tablet was measured by using slide calipers for 20 individual tablets of different product code. The thickness variation limits allowed were $\pm 5\%$ of the size of the tablet [10].

2.5 Length and diameter

According to British Pharmacopoeia (2002) [11] the percentage deviation allowed by + 5% up to 12.5 mm and by + 3% above 15mm.

2.6 Hardness

The resistance of the tablet to chipping, abrasion, or breakage under condition of storage, transportation and handling before usage depends on its hardness [12]. Hardness testing is very much necessary because it may affect disintegration and dissolution time. The tablets were placed between the jaws of the Monsanto hardness tester on its edge and then force was applied. Six tablets of each product code were used for this test.

2.7 Friability

Approximate 5 grams of tablets were weighed and placed in a friabilator. It was then operated for 100 revolutions. Tablets were dusted, reweighed and percentage of loss was calculated.

2.8 Preparation of the standard curve

A stock solution of pure metronidazole was prepared by dissolving 50 mg of pure metronidazole in 50 ml of 0.1N HCl to produce a 1mg/ml solution. 10 ml of stock solution was then transferred to a measuring flask and the volume was made to 100 ml with 0.1 N HCl. 5 different concentrations of pure metronidazole were prepared from stock solution. The concentrations prepared were 5, 10, 15, 20

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and 25 µg/ml. The absorbance of 6 concentrations including blank was taken at 278 nm using UV-spectrophotometer and calibration curve was plotted (**Figure 1**)

2.9 Dissolution Rate Determination

Dissolution test was carried out by paddle method [13], at 50 rpm. Hydrochloric acid 0.1N (900ml) prepared as dissolution medium, was poured into the vessel and equilibrated to 37±0.5°C. Six tablets from each brand were tested. 5ml of aliquot was withdrawn at the intervals of 10, 15, 30, 45 and 60 minutes. The volumes withdrawn were replaced with fresh dissolution medium. The sample was filtered, using Whatman filter paper and 5ml of filtrate was further diluted as working solution. The absorbance was measured at 278 nm against dissolution medium. Then percentage of average drug release for each brand was plotted against time (**Figure 2**).

2.10 Statistical Analysis

Statistically significant differences among the brands were analyzed using the F-test with repeated measure ANOVA at the 0.05 significance level using Microsoft Excel 2007.

3. RESULTS AND DISCUSSION

The uniformity of weight determination for the five brands of metronidazole tablets gave values that comply with the USP specification with a deviation less than 5% from the mean value (maximum deviation value 0.015). All the brands also passed the friability test; none had a weight loss of up to 1% (w/w), with the maximum percentage being 0.17 (Table 1). Dissolution profiles for five Brands of metronidazole film coated tablets had showed more than 80% release within 10 minutes (except 38.17% for Product code B). Statistical results of repeated measure ANOVA showed a significant difference of dissolution pattern between the groups. From the calculation F values, the corresponding *p*- values were less than 0.001 and rejected the null hypothesis of indifferent release pattern for different brands of metronidazole tablet (Table 3).

Table 1. Tablet Information and Summary of Parameters

Product Code	Batch No.	Mfg. Date	Exp. Date	Avg. Weight (mg)	Avg. Length (mm)	Avg. Thickness (mm)	Avg. Hardness (Kg/cm ²)	Friability %
A	409007	Sep'14	Aug'17	0.66±0.006	17.16±0.015	5.68±0.028	3.21±0.25	0.00
B	8959	Sep'14	Sep'17	0.59±0.009	12.02±0.03	5.15±0.04	2.85±0.09	0.17
C	X81	Aug'14	Jul'16	0.51±0.006	14.87±0.03	5.37±0.033	2.72±0.38	0.00
D	06	Nov'14	Jan'17	0.83±0.014	18.20±0.05	5.87±0.12	2.61±0.32	0.00
E	80914	Sep'14	Sep'16	0.74±0.015	18.20±0.033	5.31±0.06	2.65±0.28	0.00

Table 2. Absorbance at various concentration

Concentration (µg/ml)	Absorbance
Blank	0.00
5	0.190
10	0.375
15	0.562
20	0.719
25	0.924

Table 3. Statistical Analysis of Dissolution for Five Different Brands of Metronidazole

Source	SS	df	MS	F	P
Between time	3944.63	4	986.16	4.34	<i>p</i> <0.001
Between Group	11094.82	4	2773.71	12.20	<i>p</i> <0.001
Error	32065.35	141	227.41		
Total	47104.80	149			

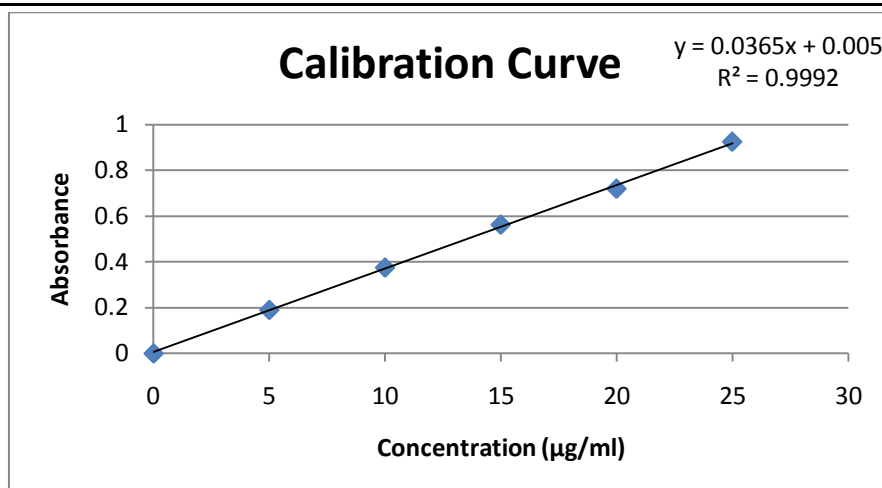


Figure 1. Calibration Curve

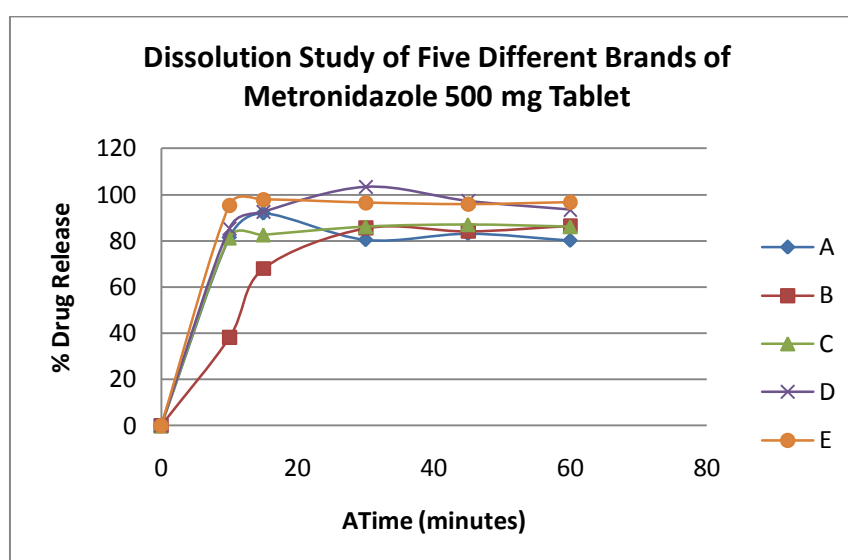


Figure 2. Dissolution Study of Different Brands of Metronidazole Tablets

4. CONCLUSION

From the above results, it is observed that although there was wide variation in the results of the quality control test (Hardness, weight variation, disintegration, and friability), metronidazole tablets from different manufacturers met all compendial tests. Assuming the null hypothesis of having similar dissolution pattern, a significant difference was observed between the brands.

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