Comparative *in-vitro* Pharmaceutical Evaluation of Four Brands of Metronidazole Tablets Marketed in Gulf Region

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ABSTRACT

Metronidazole is an antiprotozoal drug which is also effective against anaerobic bacteria. It exhibits pronounced *in-vitro* and *in-vivo* activity against *Trichomonas vaginalis* and *E. histolytica*. Many different brands and dosage forms of Metronidazole are available in the gulf countries that place physicians and pharmacists in a dilemma of drug substitution in case of non availability of a particular brand. The present study was aimed to evaluate the pharmaceutical equivalence of four brands of 250 mg Metronidazole tablets marketed in Middle East countries. Four brands of Metronidazole tablets were purchased locally from the retail pharmacy outlets in Muscat, Oman and their solubility, partition coefficient (log *P*) and pharmaceutical quality were assessed by using *in-vitro* tests as per the United States Pharmacopoeia (USP) and unofficial standards as recommended by the manufacturers. Selected brands of Metronidazole were found to be highly soluble, highly permeable and also passed all the official and unofficial *in- vitro* quality control tests prescribed for the tablets except hardness test. All brands of Metronidazole tablets released more than 70% of their drug content within 45 minutes. Thus, based on the above results, it can be concluded that tested brands of Metronidazole tablets being eligible for biowaiver, are pharmaceutically equivalent and therefore can be considered for drug substitution for each other.

Keywords: Metronidazole Tablets, Antiprotozoal, Pharmaceutical Equivalence, Dissolution.

INTRODUCTION

Metronidazole is an antiprotozoal and antiparasitic agent commonly used to treat amoebiasis, giardiasis, trichomoniasis and other microbial diseases caused by anaerobic bacteria.[1] It is also listed in the important WHO essential medicine list.[2] Metronidazole in combination with other drugs is quite effective and beneficial therapy in the management of H. pylori infection.[3] Chemically it is 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol (Fig. 1) and occurs as white to pale yellow microcrystalline powder, slightly soluble in water and alcohol.[4] However, it dissolves readily in dilute hydrochloric acid solution. It is a prodrug that requires reductive activation of the nitro group by susceptible organisms. It is usually completely and promptly absorbed after oral intake, reaching concentrations in plasma of 8 - 13 mg within 0.25 - 4 h after a single 500 mg dose.[5] Therefore, it can be inferred that slight change in the physicochemical characteristics and dissolution will affect its biopharmaceutics and thus its quality and efficacy.

There are many multinational brands and dosage forms of Metronidazole available in the market of gulf countries. Some of these brands are manufactured in Middle East countries such as Saudi Arabia, Jordan, UAE, Oman and some are imported from other parts of the world. Various brands available in the market are considered pharmaceutically equivalent if they contain the same amount of active ingredient in the identical dosage form and meet the same compendial or other

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applicable standards (i.e., strength, quality, purity, and identity), but may differ in characteristics such as shape, packaging, excipients (including colors, flavors, preservatives), expiration time, and, within certain limits, labeling requirements etc.[6] The biopharmaceutical equivalent drug products can help the health care providers in substitution of one brand for the other in case of non availability; however this substitution is quite controversial and is often met with suspicion among patients and physicians.[7]

It is the joint responsibility of the manufacturers and the drug law enforcing agencies to ensure that various marketed pharmaceutical products containing the same active ingredient in the identical dosage forms are uniform, safe and effective. The safety and efficacy of drug products can be guaranteed when their quality is reliable and is reproducible from batch to batch. To ensure the requisite quality, drug manufacturers are required to test their products during and after manufacturing and at various intervals during the shelf life of the product.[8]

Metronidazole is one of the commonly used amoebicidal drug in clinical practice for the treatment of amoebiasis and giardiasis, therefore, it is necessary to monitor and ascertain the quality of the various brands available in the market. The quality i.e. safety and efficacy of immediate release oral solid dosage form such as tablets can readily and satisfactorily be assessed by carrying out dissolution studies and *in- vitro* pharmaceutical tests. The present study was carried out to investigate and assess the pharmaceutical quality of four different brands of Metronidazole 250 mg tablets marketed in gulf countries using *in vitro* methods as per the USP and unofficial standards as recommended by the manufacturers to ascertain that all brands are pharmaceutically equivalent. The assessment of tablets included the evaluation of weight uniformity, friability, crushing strength/hardness, disintegration, dissolution rate and chemical assay by UV spectrophotometric method to determine the content of active pharmaceutical ingredient (API).

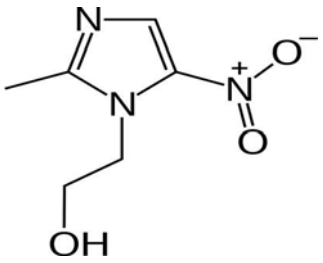

Figure 1: Chemical structure of Metronidazole

MATERIALS AND METHODS

Sample material

Four different brands of Metronidazole 250 mg tablets (Nidazole, Negazole, Amrizole and Metrolag) were purchased from the retail pharmacy and were coded as A, B, C and D respectively. The labeled shelf life was three years from the date of manufacturing and the tablets were evaluated two year before the labeled expiry date.

Pure Metronidazole powder was a kind gift from Hikma Pharmaceuticals, Jordan.

Methods

Identity, uniformity of weight, friability, crushing strength, disintegration, dissolution rate and assay for the content of active ingredient by UV spectrophotometry were done as described in the United States Pharmacopoeia.[9] All the tests were carried out in triplicate. In addition to the compendial tests, solubility and partition coefficient values of Metronidazole tablets were also determined to confirm that the tested brands belong to biopharmaceutics classification system (BCS) class I and are eligible for biowaiver studies.

Hardness

Monsanto hardness tester (Bellstone, Hi-Tech International, India) was used to measure the hardness. Ten tablets from each brand were randomly selected and their hardness was determined (n=10).

Friability

For friability testing, ten randomly selected tablets from each brand were initially weighed and placed in a friabilator chamber (Bellstone, Hi-Tech International, India). The friabilator was operated at 25 rpm for 4 minutes (up to 100 revolutions). Thereafter, tablets were removed, dusted and reweighed. The percent (%) friability was calculated by using following formula.[10] The test was repeated three times for each brand of Metronidazole tablets.

$$\% \text{ Friability} = \frac{\text{Weight before test} - \text{Weight after test}}{\text{Weight before test}} \times 100$$

Weight variation

The weights of twenty tablets were determined individually using an electronic digital balance to evaluate weight variation among tablets. The average tablet weight and standard deviation were calculated and compared with the permissible limits.

Disintegration

A Digital tablet disintegration test apparatus (Bellstone Hi–Tech International, India) was used for disintegration test. A 900 mL beaker was filled with distilled water and was maintained at 37 ± 0.5 ^{o}C. Six tablets of each brand were selected and placed in each of the cylindrical tubes of the basket and connected to the disintegration apparatus. To avoid the floating of tablets while tube move upwards and downwards in water, discs were used. The time taken to break each tablet into small particles and pass out through the mesh at the bottom of the tube was recorded. Mean disintegration time was calculated for each of the brands.

Dissolution or *in- vitro* bioavailability test

USP tablet dissolution test apparatus (Bellstone, Hi-Tech International, India), rotating basket type, was used to study the *in- vitro* drug release pattern of Metronidazole tablets using distilled water as dissolution medium (900 mL). The temperature of water was maintained at 37 ^{0}C and Paddle rotation was set at 100 rpm. Aliquots (5mL) were withdrawn at 15, 30 and 45 minutes time intervals for the analysis of drug concentration (Table 1). The samples were diluted appropriately with 0.1M HCl solution and filtered before measuring absorbance at 277 nm using UV visible spectrophotometer (UV Analyst CT 8200, Taiwan). The content of Metronidazole in each sample was determined based on the calibration curve obtained with serial dilutions of the pure drug at 5, 10, 20 and 40µg/mL (Table 2).

Table 1: Dissolution test conditions

Parameter	Specification
Dissolution medium	Distilled water
Volume of medium	900 mL
Temperature of medium	37±0.5 ^{0}C
Basket rotation speed	100 rpm
Sampling time interval	0, 15, 30 and 45minutes
Wavelength measurement	277 nm

rpm: revolutions per minute, nm: nanometer

Table 2: Standard absorbance values of Metronidazole for plotting standard curve by UV spectrophotometer

Stock number	Stock concentration (µg/ml)	Absorbance
1	5	0.1178
2	10	0.2276
3	20	0.7377
4	40	1.2018

Content Uniformity

A series of working solutions with different Metronidazole concentrations were prepared in 0.1 M HCl solution. The absorbance of each solution was measured at 277 nm and a calibration curve was constructed. Using the standard curve, the amount of Metronidazole in each brand was determined.

Standard solution of Metronidazole

A stock standard solution (500µg/ml) was prepared by

dissolving 50 mg of pure Metronidazole powder in 100mL of 0.1 M HCl. Working standards for constructing a calibration curve were prepared by pipetting 1, 2, 4, and 8 ml aliquots of the stock standard solution into separate 100 mL volumetric flasks and diluting to volume with 0.1 M HCl.

Table 3: Results of official and unofficial quality control tests on four brands of Metronidazole tablets

Brand	Hardness (Kg/cm^2) Mean±SD, (n = 6)	Friability (%) (n= 10)	Weight uniformity (mg) Mean±SD (n=20)	Disintegration time (Sec) Mean±SD (n = 6)
A	9.75±1.62	0.0037	547.9±7.6	46.5 ±9.31
B	11.25±1.33	0.012	482.16±7.91	246.33± 29.15
C	9.8±0.59	0.008	491.57±3.24	125.83 ±15.79
D	9.5±1.64	0.02	446.97±3.92	56 ±4.38
p-value (ANOVA)	0.029	<0.05	<0.05	<0.05

SD: Standard deviation, n: numbers of tablets, all experiments were done in triplicate

Table 4: Content uniformity assay of Metronidazole in four brands by Ultra Violet spectroscopic method

Brand	% Metronidazole content Mean±SD	Remarks as per the USP permissible limit (95-105%)	p-value
A	95.03 ±6.2	Passed	
B	95.08 ±3.43	Passed	0.0069
C	96.91 ±5.84	Passed	
D	102.19 ±11.2	Passed	

SD: Standard deviation, P<0.05 by One way analysis of Variance (ANOVA) single factor

Sample preparation

Three Metronidazole tablets from each brand were weighed individually and powdered. Each powdered tablet was quantitatively transferred to 100 mL volumetric flask and 100 mL of 0.1 M HCl was added to it. 0.8 ml aliquots of each sample were pipetted into separate 100 mL volumetric flasks and each flask was diluted to volume with 0.1M HCl solution. Absorbance of standard solutions and unknown was measured at 277 nm by using 0.1 M HCl as blank.

Log *P* value of Metronidazole tablets

The log *P* of all the selected brands of Metronidazole was determined in n-octanol/water system using shake flask method. Tablet powder equivalent to 10 mg drug was dissolved in minimal amount of methanol and was shaken up with a 20 mL mixture of octanol and water (1:1), for 24 hrs on a mechanical shaker. After 24 hrs, the water layer was separated and its absorbance was measured after appropriate dilution at λmax 320 nm by

using methanol as blank. Amount of metronidazole present in aqueous phase was calculated with the help of standard curve of pure drug. Following equation was used to determine the partition coefficient value of metronidazole tablets:

Log *P*= log [concentration in n-Octanol/concentration in water]

Preliminary solubility determination of Metronidazole tablets

Solubility of Metronidazole tablets was determined in water and buffer solution of pH 1.2 at 20^0C. An excess amount of powdered tablet was dispersed in 30 mL of distilled water or aqueous buffer of pH 1.2 and stirred on a magnetic stirrer for 18 hrs. After 24 hrs, the supernatant was filtered and the concentrations of saturated solution after appropriate dilution was determined spectrophotometrically at 320 nm against suitable blank.

Data analysis

Data for hardness, friability, weight uniformity test, disintegration, % drug release by dissolution and content uniformity of the tablets were analyzed by determining the mean± standard deviation. ANOVA single factor was used for determining significance. P values <0.05 were considered as significant.

RESULTS

The results of various quality control tests performed on four brands of Metronidazole tablets are presented in Table no 3 and Table no 4. Tested brands in water and buffer solution of pH 1.2 at 20 ^{0}C showed high solubility and their dose/solubility (D/S) quotient was well below 250 mL. Partition coefficient (log *P*) values in n-octanol/water were found to be above 0.81, suggesting Metronidazole to be highly permeable. In order to determine the Metronidazole content in tablets, four working standard solutions were prepared and their absorbance was measured to construct standard calibration curve. A liner regression of the standard absorbance data of working solutions (Table 2) in statistical software, SPSS gave the following equation which was used to determine the pure drug content of analyzed tablets.

$$y=0.01384x-0.0248 \qquad (R^2 = 0.9698)$$

DISCUSSION

As per British and US Pharmacopoeia, force of about 4-6 Kg/cm^2 is considered ideal to break the tablets satisfactorily. However, hardness of Metronidazole tablets was found to be quite high (9.5 to 11.25 Kg/cm^2) as compared to permissible limit. Thus all the brands failed to meet the manufacturer's requirement for hardness. The hardness could be more because of the amount of binder, compression force and method of granulation. A significant difference was observed in the mean crushing strength of the tablets by ANOVA test.

Weight loss due to friability in all tested preparations was found to be less than 1% indicating that all brands are mechanically stable and will not undergo any wear or tear during transportation. Brand D showed the maximum % weight loss (0.02%) than all other brands (0.0037-0.012%). However, all the brands met the Pharmacopoeial standard.

Weight uniformity test for tablets is required to ensure that the drug content in each tablet is distributed in a narrow range around the label strength because slight variation in weight of tablet reflects variation in the content of active ingredient. According to the USP, drug products whose strength is >324 mg, permissible limit of ±5% of the average is required to pass the test for weight uniformity. All brands of the commercial products possessed acceptable uniformity of weight as per the pharmacopoeial limit i.e. mean±5%. The p- value for weight uniformity was found to be statistically significant (<0.05) by ANOVA single factor test.

Disintegration evaluates availability of a drug for dissolution and absorption from the gastro-intestinal tract. The results presented in table 1 reveals rapid disintegration of all the products. All the products meet

the disintegration limit set by the USP. Statistical analysis showed a significant difference in mean disintegration time of four brands.

The compendial requirement for content uniformity is met if % content of tablets with average weight above 250 mg falls within 95-105%.[9] Mean average content of analyzed Metronidazole tablets by UV method was found to be in the range of 95.03 -102.19% (Table 4). All the tablets meet the pharmacopoeial limit for the content uniformity test. The p -value obtained by ANOVA single factor was found to be significant as it was less than 0.05.

Pharmaceutical availability or *in – vitro* availability by dissolution testing provides useful and reliable information regarding *in-vivo* bioavailability of drug product.[11] It is considered as reliable, sensitive and rationale for predicting drug bioavailability. Figure 2 shows the % drug release of Metronidazole tablets by dissolution test and was found to be satisfactory for all brands. Brand A showed the highest drug release after 15 minutes whereas brand B had the slowest release rate. After 45 minutes brand D showed almost 99% release thus better dissolution profile than other brands. However, no correlation could be drawn between disintegration time and dissolution profile of Metronidazole tablets.

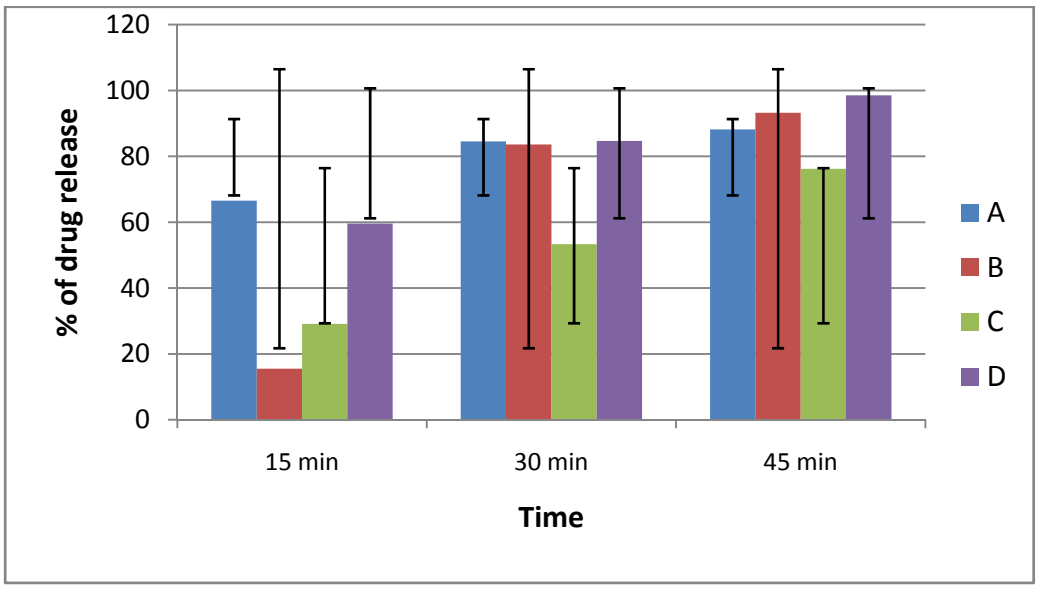

Figure 2: Dissolution rate of various brands of Metronidazole tablets with error bars with standard deviation

CONCLUSION

Metronidazole is a commonly prescribed antiprotozoal drug for amoebiasis and other anaerobic infections. Currently many generic and multinational brands of this drug are available in the pharma market in the gulf region. It has been observed that multi sourcing of a drug product might lead to variability in clinical responses and eventually dissatisfaction among prescribers and consumers. Small differences in the manufacturing process, different formulation factors such as type and amount of excipients, packaging or storage factors and substandard as well as counterfeit products could alter the disintegration, dissolution and other parameters that consequently lead to variation in therapeutic response.[12]

Preliminary physicochemical evaluation of pharmaceutical products is of great importance in

ensuring the quality of drug products. Quality control tests such as *in –vitro* dissolution of drugs belonging to BCS class I provides valuable information about the *in-vitro* bioavailability and bioequivalence of oral solid dosage forms. As per the literature[13], Metronidazole being highly soluble and highly permeable, belongs to BCS class I drugs. Our tested brands also showed high solubility (D/S quotient below 250 mL) and permeability (log *P* values>0.81) and thus is eligible for biowaiver of *in-vivo* bioequivalence (BE) testing. Also many immediate release Metrondiazole formulations have shown very good correlations between *in-vivo* bioavailability and *in-vitro* dissolution results.[14] This study was undertaken to evaluate the physicochemical properties and *in- vitro* bioavailability of four different brands of Metronidazole tablets using *in-vitro* quality control tests (Hardness, Friability, Weight variation, Disintegration time, Dissolution rate and Content uniformity) with an aim to assess whether these four brands are pharmaceutically equivalent or not.

The results indicated that overall quality of all brands was satisfactory as they met the requirements of the official and unofficial quality control tests. All brands failed the hardness test as their mean crushing strength was found to be outside the 4-8Kg/Cm^2, though all the tested tablets were uniform in size, shape, color, thickness and weight. All brands showed good absorption and drug release profile on disintegration and dissolution studies. Metronidazole content of all brands was found to be well within the permissible limit of 95-105% (237.5-262.5mg) of labeled amount.

From the results of this pilot study, it can be inferred that the tested brands of Metronidazole are pharmaceutically or chemically equivalent and most likely will be bioequivalent *in -vivo*. Therefore, these brands can be considered for substitution in case of non availability of other brands.

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مقارنة التقييم الدوائي المختبري لأربع علامات تجارية من حبوب الميترونيدازول المتداولة في منطقة الخليج الشرق الأوسط

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ملخص

الميترونيدازول هو أحد العقاقير المضادة للطفيليات الأولية، ويعرف أيضاً بفعاليته ضد البكتيريا اللاهوائية. لهذا العقار فعالية واضحة مختبريا في حيوانات التجارب ضد ترايكوموناس فاجيناليس وأميبيا هيستوليتيكا. هناك عدة أنواع وأشكال صيدلانية من الميترونيدازول متوافرة في بلدان الخليج، مما يجعل اختيار المنتج الناسب من قبل الصيادلة والأطباء محيرا في حالة عدم توفر نوع معين من الميترونيدازول. الهدف من هذه الدراسة هو تحديد المكافئ الدوائي لأربع علامات تجارية من حبوب الميترونيدازول المتداولة في بلدان الشرق الأوسط. تم شراء أربع علامات تجارية من حبوب الميترونيدازول تم شراؤها من صيدليات محلية بمسقط – عُمان، وتم تحديد ذوبانيتها ومعامل تحللها ونوعيتها الدوائية باستعمال تجارب معملية بمواصفات فارماكوبيا الولايات المتحدة (USP) الرسمية، ومواصفات أخرى غير رسمية. وطبقا لتوصيات المصنعين أظهرت النتائج أن العلاملت التجارية المختارة من حبوب الميترونيدازول لها ذوبانية ونفاذية عالية. كما اجتازت كل اختبارات ضبط الجودة الرسمية وغير الرسمية المناسبة لحبوب الميترونيدازول باستثناء اختبار التحمل (الصلابة). كل العلامات التجارية من حبوب الميترونيدازول المختبرة أطلقت أكثر من 70% من محتويات الدواء خلال 45 دقيقة، واعتمادا على هذه النتائج يمكن الاستنتاج بأن كل العلامات التجارية من حبوب الميترونيدازول المختبرة لا تحتاج للاختبار الحيوي، وأنها متكافئة من الناحية الدوائية، ومن ثم يمكن أن تعوض بعضها بعضا.

الكلمات الدالة: حبوب الميترونيدازول، مضاد الطفيليات الأولية، المكافئ الدوائي، الذوبان.

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