A Comparative In Vitro Evaluation of Different Brands of Metronidazole

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Abstract

Quality of pharmaceutical products is very important because drugs must be marketed as safe and therapeutically active formulations whose performance is consistent and predictable. The evaluation of the physical characteristics of the pharmaceutical products can ensure their quality as well as bioavailability and impart optimum therapeutic activity. Metronidazole was chosen for this comparative study because this drug is widely used worldwide in the treatment of amoebiasis and other microbial diseases. Studies on metronidazole revealed that it is a recommended treatment during pregnancy for infections with bacterial vaginitis and Trichomonas vaginalis. The purpose of this study was to compare the quality of metronidazole tablets of some brands and to evaluate whether all brands obey the USP protocols. There are many different brands and different types of dosage forms of metronidazole under various trade names manufactured by different pharmaceutical companies available in the market. For this current research work Three brands (brand codes A1, A2, A3,) of metronidazole film coated tablets (400mg) commercially available in India were collected and evaluation studies were conducted which includes diameter and thickness measurement, weight variation test, friability test, hardness test, disintegration time, dissolution profile, and potency determination. Tests were performed as per the method described in United States of Pharmacopoeia (USP). All brands were found to comply with the USP specifications. According to this analysis, minimum weight variation was found in brand A3. Brand A3 showed the highest average hardness of 1.79kg/cm^2 and A2 maximum disintegration time (6.58 minutes) and A3 highest friability (0.79%) among other brands. The lowest friability was 0.392% with brand A1. Dissolution pattern and potency of the metronidazole tablets were determined by the UV spectroscopic method at 278nm in the acidic (0.1N HCl) medium. All brands showed satisfactory dissolution profile as they released more than 85% drug in 20 minutes. Among these brands A1 showed highest drug release at required time interval. Dissolution profile of brand A2 and A3 were found similar to that of brand A1 by comparing difference values of all three brands. For better therapeutic effects and safe use of drugs, quality parameters should be maintained strictly.

Keywords: Metronidazole, Comparative study of Metronidazole, Dissolution profile

Introduction:

Metronidazole is often found in film-coated tablets and other medications. Tablets are one of the world's finest pharmaceutical products prepared by molding or compression, with or without appropriate diluents. Almost all drug molecules can be made into tablets, and the tablet-making process is very simple and flexible . It provides comfort to patients by easily covering the taste of bitter components. Metronidazole was first developed by the French Rhone-Poulenc laboratory and marketed under the trade o in the mid-1950s. Its commercial application began in France in the 1960s. It was first approved by the FDA in 1963[(1)] .The antibacterial properties of metronidazole were discovered in 1962 when it treated a patient with trichomonas vaginitis and gingivitis. In 1966, metronidazole was shown to be effective in the treatment of amoebic dysentery. After Giardia lamblia (also known as Giardia duodenalis) was identified as causing malabsorption

and epigastric pain in the 1970s, this luminal infection was treated with metronidazole . This is also the first drug with a cure rate close to 100%. However, the use of metronidazole in the treatment of infections caused by Gram-negative anaerobes such as Bacteroidetes or Gram-positive anaerobes such as Clostridium did not become popular until the 1970s . After oral administration, metronidazole is well absorbed, reaching maximum blood levels one to two hours after administration, and its bioavailability is greater than 80%. Plasma concentration of metronidazole is dose proportional .

Metronidazole is widely distributed in human tissues and body fluids. It is the main substance found in plasma together with minor metabolites. Less than 20% of circulating metronidazole is bound to plasma proteins. Metronidazole concentrations in cerebrospinal fluid, saliva, and milk are similar to plasma concentrations. The bactericidal concentration of metronidazole has also been detected in saliva from liver abscesses . The primary route of elimination of metronidazole and its metabolites is urine (approximately 60% to 80% of the dose), with fecal excretion accounting for 6% to 15% of the dose. Metabolites appear in urine mainly through side chain oxidation [1-(β-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole and 2-methyl-5-nitroimidazole-1-ylacetic acid] Early estimates of metronidazole with glucuronic acid account for approximately 20% of the total[(2)] . It creates the . Both the parent compound and the hydroxy metabolite have antimicrobial activity in vitro. The renal clearance of metronidazole in healthy individuals is 8 hours[(3)].

Metronidazole is an antidote. Ionized metronidazole is selected against anaerobic bacteria and sensitive protozoa because these bacteria can degrade metronidazole intracellularly to its active form[(4)]. Reduced metronidazole then covalently binds to DNA, disrupting its helical structure, inhibiting bacterial nucleic acid synthesis and causing bacterial cell death. The presence of oxygen prevents the degradation of metronidazole, thus reducing its cytotoxicity. Currently, metronidazole is cheap, easy to access and has very few side effects. Metronidazole, which is included in many hospital medicines, is used to prevent anaerobic bacteria after gastrointestinal surgery, wound healing and antibiotic treatment. Caused by Clostridium difficile[(4,5,6)] .

Despite some common side effects the most serious adverse reactions reported during treatment with metronidazole have been convulsive seizures, encephalopathy and aseptic meningitis. In addition, patients have reported headache, syncope, and dizziness, vertigo, in coordination, ataxia, confusion, dysarthria, irritability, depression, weakness, and insomnia. Very rare cases such as reversible abnormal liver function tests, cholestatic hepatitis, reversible neutropenia, reversible thrombocytopenia and hypersensitivity reactions have also been reported [(9,8,7)] .

Materials and method

Collection of samples

Metronidazole tablets (400 mg) of 03 different brands (20 tablets from each brand) of were purchased from different local medicine shops located in Washim, India. The samples were properly checked for their visual appearance, manufacturing company, manufacturing date, expiry date, manufacturing license number, batch number and DAR number at the time of purchase.

Identification of sample

The brands were randomly coded as A1, A2, A3… so that the identity of the manufacturers can be blinded. All brands were labeled with a shelf life of two years and claimed to contain 400 mg of metronidazole per tablet. All the tablets were found packaged in strip or in blister with a good condition. The shape, size and colour of different branded tablets were subjected to visual inspection at the very beginning of the research work. The label information of 03 different brands of metronidazole tablet (400 mg) is represented in Table 1.

Table 1: Label information of Three different brands of metronidazole tablets (400 mg)

Brand code	Mfg.date	Exp.date	Price per strip	Price per unit
A1	June 2023	May 2027	25.53	1.70
A2	May 2023	April 2026	25.53	1.70
A3	August 2023	June 2026	23.85	1.59

Weight variation test

Individual weights of selected 20 tablets of each brand were measured in milligram using electronic analytical balance and from these data mean weight with standard deviation (SD) were calculated.

Hardness test

Crushing strength (N) of 03 tablets from each brand was determined with an Monsanto hardness tester. Mean hardness with standard deviation (SD) were calculated.

Friability test

20 tablets from each brand were weighed and subjected to rotation by employing a Roche friabilator (VEEGO, India) which was operated at 25 RPM for 4 minutes and then all tablets were re-weighted after removing from friabilator.

Disintegration test

Three tablets from each brand were employed for the disintegration test in distilled water at 37 °C using a tablet disintegration tester (VDT-2, Veego, India) as per condition described by United State Pharmacopeia, 2013 . The disintegration time (DT) was noted down and it's the time taken for the entire tablet to disintegrate completely[24].

Standard curve preparation

The powder equivalent to 10 mg of standard metronidazole was taken and dissolved in 0.1 N HCl. Then it was diluted to produce a final concentration of 15µg/ml for working solution. Absorbance values were then measured at the maximum wavelength (λmax) of metronidazole of the serially diluted concentrations (0, 1.5, 3, 4.5, µg/ml) using a UV-VIS spectrophotometer . Maximum wavelength was obtained by scanning sample of diluted standard metronidazole from 200 to 400 nm wavelengths and it was found to be 278 nm.

Measurement of potency

Sample was prepared by weighing and crushing 04 tablets, transferring amount of drug powder equivalent to 10 mg in 0.1 N HCl solution and placing it in sonicator (Hwashin Technology, Seoul, Korea). The portion of solution was filtered and the filtrate was suitably diluted. Absorbance was taken at 278 nm by using UV-visible spectrophotometer. Finally the potency of different brands was calculated using the following equation

Potency= Drug present in a single tablet / Strenght (mg) x 100

Dissolution test

The dissolution test was undertaken for 03 randomly selected tablets using USP dissolution apparatus I (Electrolab). The dissolution medium was 900 ml of 0.1 N HCl which was maintained at 37±0.5 °C. Rotations were 100 RPM. Each time 10 ml sample was withdrawn after 5 min, 15 min, 30 min, 45 min & 60 min, and was then filtered. The filtrates were then suitably diluted with 0.1 N HCl. Absorbance was measured at 278 nm. Using the y =mx + c equation derived from the standard curve of API, concentrations of sample at different above mentioned times were calculated. From these data Cumulative amount release and then % Drug release were calculated using the following equation:

%Drug release = Cumulative amount release(mg) / Stength (mg) x 100

Results and discussion

Price fluctuation

There is minor price variation among the brands. Brand A1and A2 had the maximum price of tablet and brand A3 had the minimum price of tablet while there was no major variation in the quality of the tested drugs.(Table 1).

Table 2: A summary of quality control tests undertaken on different brands of metronidazole tablet

Brand Code	Weight (mg)	%Deviation from average weight	Hardness	Friability %	DT* (min)	Potency %
A1	8.790 ±439.5	461.47 417.52	1.75kg/cm^2	0.79%	2.11	99.42%
A2	9950 ±497.5	522.37 472.625	1.27kg/cm^2	0.67%	6.46	98.49%
A3	14030 ±701.5	736.57 666.43	1.79kg/cm^2	0.39%	5.45	98.79%

Test of uniformity of weight

The objective of the weight variation test is to ensure – good manufacturing practices (GMP), appropriate size of the tablets and the content uniformity of the formulation [27]The United States Pharmacopoeia (USP) provides criteria for tablet weight variation test of intact dosage forms which states that the percent weight variation should be within ±5% for tablets having average weight more than 324mg. The tablets met the USP test if there are not more than 2 tablets outside the percentage limit and if no tablets deviate twice of the percentage limit .All the brands complied with the compendia specification for uniformity of weight as the percent deviations from average weight of all the tablets were within the acceptable range of ±5%. Minimum percent deviation from average weight was found in brand A3 (Table 2)[(13,14)].

Hardness test

Tablet hardness testing is a laboratory technique used by the pharmaceutical industry to test the breaking point and structural integrity of a table tunder conditions of storage, transportation ,and handling before usage [14]. The hardness of the tablet depends on the materials used, amount of binder, space between the upper and lower punches at the time of compression and pressure applied during the process of compression [5] . Hardness influences many tablet properties including disintegration, dissolution and friability. High hardness values may result in increased disintegration times and decreased dissolution times. As opposed to this situation, high friability values may be observed in case of low hardness values [18, 11] .Brand A3 had the highest average hardness (1.79kg/cm^2) whereas brand A2 had the lowest average hardness (1.27kg/cm^2) (Table 2). A force of about 40 N is the minimum requirement for a satisfactory tablet [1]. Hence the tablets of all the brands comply with this requirement[(15,16)].

Friability test

Friability (the condition of being friable) testing is a method, which is also employed to determine physical strength of compressed and uncoated tablets upon exposure to mechanical shock and attrition. In simple words, friability test tells how much mechanical stress tablets are able to withstand during their manufacturing, distribution and handling by the customer. Throughout pharmaceutical industry, friability testing has become an accepted technology [22]It is a compendial test and met the USP specification if friability is not more than 1% [16, 25] The friability was found to be between the ranges of (0.02-0.79) %, thus all the brands met the friability specification (Table 2)[(17).]

Disintegration test

Disintegration test is performed to find out that within how much time the tablet disintegrates. Disintegration test is very important for all coated & 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 [13] According to BP/USP specification, film coated tablets should disintegrate within 30 min . Here film coated metronidazole tablets of all the brands met the requirement as the disintegration time (DT) was found to be between the ranges of (2.05- 14.84) minutes (Table 2).

Potency test

Potency is a measure of drug activity expressed in terms of the amount of API (in percentage) required to produce an effect of given intensity. This test is done for determining the toxic and therapeutic effect of the drug. The potency of the tablet should comply with the specification because very highly potent drug may give toxic effect & very less potent drug may give sub-therapeutic effect. All the brands showed potency within the range of (95-105)% of labeled amount of drug and complied according to USP

Table 3: Dissolution profile of Three brands of metronidazole tablets

Time (Min)	Brand A1	Brand A2	Brand A3
0	0	0	0
5	0.58	0.52	0.323
10	0.75	0.76	0.37
15	0.93	1.96	0.397
20	1.09	1.08	0.399

Dissolution test

The transfer of a drug from its solid dosage form into the solution of GI fluid as dissolved form is called dissolution. This is the slowest step in a series of kinetic processes of drug and called the rate-limiting step. Dissolution study measures the rate and extent of drug release from any dosage form. The test usually reports the % of drug released at a specific period of time. Dissolution tests are determining factors affecting drug bioavailability. For film coated metronidazole tablets, drug release should not be less than 85% of labeled amount in 20 minutes [13] Brand A1 had maximum drug release within the 20 minutes (99.42%) of the in vitro dissolution test, while brand A2 had minimum drug release (98.49%) within the same time interval. Intra-brand comparison of the drug release profile of all the brands indicated an increase in drug release with increasing time although this rate varied from brand to brand (Figure 1). Since all the brands met the USP specification, it can be assumed that all the brands possessed satisfactory dissolution profile although the brands were manufactured by different companies using different excipients in different ratio (Table 3).

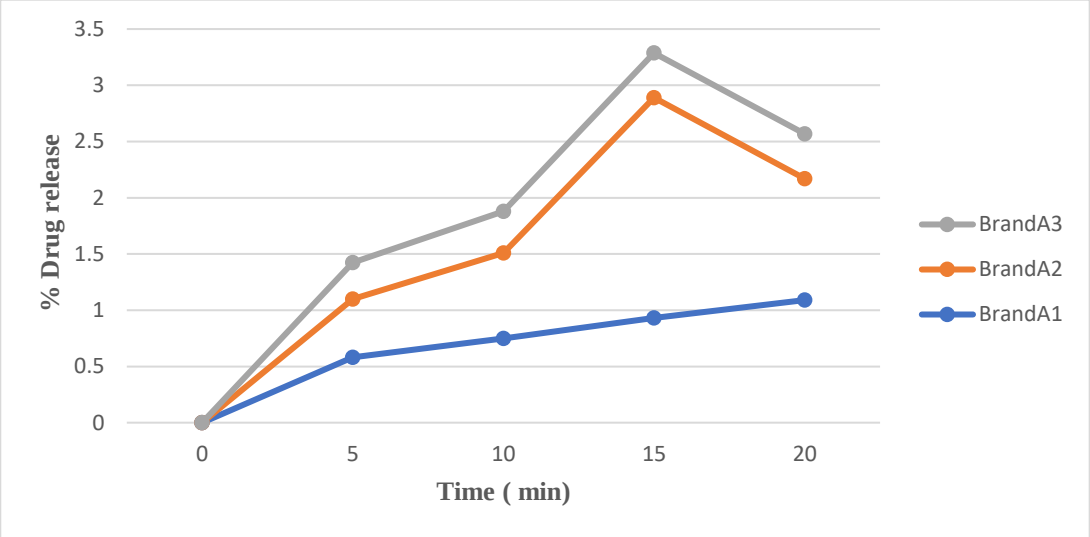

Fig 1: Comparative average % drug release of metronidazole tablet of Three brands

Conclusion

The aim of this study is to evaluate three different formulations of metronidazole available in the Indian market. Metronidazole is a poorly water-soluble drug. Due to its poor solubility, it is difficult to achieve the desired bioavailability. A patient's life depends on the medicine he needs being safe and effective. That's why we do many formal and informal in vitro studies such as weight change, brittleness, hardness, disintegration, dissolution and potency testing. According to our review, almost all types of metronidazole are good and effective and meet the requirements of the pharmacopoeia. Bioavailability and therapeutic efficacy depend on

the quality of the tests. Therefore, careful evaluation of different tablets is necessary before trading. These studies need to be carried out more frequently, both to raise public awareness about the quality of commercial drugs and to be beneficial to the progress of the pharmaceutical industry.

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