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Post-Marketed *In Vitro* Evaluation of Different Tablet brands of Metronidazole in Pakistan

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Abstract

The aim of the present study is to investigate the pharmacopoeia limits of ten brands of tablets containing metronidazole 400mg (oral antiprotozoal and antibacterial) sourced from different retail Pharmacy outlets in Lahore region. The quality and pharmaceutical limits of ten different brands of metronidazole tablets were assessed. The assessment included the physical evaluation, disintegration and dissolution tests. All the ten brands of the tablets passed the British Pharmacopoeia (BP) standards for physical appearance. Two of the brands amongst the ten did not comply with the USP specifications for dissolution test and disintegration for metronidazole tablets amongst them one was local and the other belonged to a multinational pharmaceutical company. Only two brands out of the ten analyzed brands of metronidazole tablets passed all the BP, USP quality specifications and were physicochemical equivalent. This study highlighted the need for constant market monitoring of new and marketed products to ascertain their equivalency to the innovator product and to assure that the medicine received to the patient will meet the standards and provide maximum efficacy to the diseased person.

Keywords: Post-market surveillance; In vitro evaluation; Metronidazole brands; Pakistan; Antibiotic brands

Introduction

The increase in the number of generic drug products from multiple sources includes local and multinational has placed people involved in a position to select one from amongst several apparently seeming equivalent products. Amongst them some drug products can be regarded as supurious, a product may contain no API or the drug content may be completely different to that as stated on the label. This may occur through deliberate falsification, substandard if it has either too much or too little of the API compared with the formulation specifications, counterfeit drugs are medicines that are deliberately and fraudulently mislabelled with respect to identity and/or source, inappropriate packaging can affect formulation content in certain storage conditions. Official National Pharmacopoeias, such as the British Pharmacopoeia (BP) and United States Pharmacopeia (USP), publish the standards to maintain quality for medicinal substances and preparations manufactured or sold in the country. The information given specifies the acceptable limits for the amount of the API (active

pharmaceutical ingredient) that should be present in a given formulation. For instance, in 1975 in United States approximately 9% of all prescription drugs dispensed were of generic versions [1]. This figure increased to 20% in 1984 and 40% in 1991 [2]. Over 80% of the approximately 10,000 prescription drugs available in 1990 were obtained from more than one source and altered clinical responses were documented to those dosage forms supplied by different drug manufacturers [2]. The variability in responses was may be due to formulation ingredients employed, methods of handling, packaging and storage and quality control. Thus, there is need to determine their pharmaceutical and therapeutic equivalence in order to ensure interchangeability of therapeutic equvalents. However, many developing countries do not have an effective means of monitoring the quality of generic drug products in the market. This results in widespread distribution of substandard and/or counterfeit drug products. In view of the facts World Health Organization issued guidelines for global standard and requirements for the registration, assessment, marketing, authorization and quality control of generic pharmaceutical products [3]. This gave technical guidelines to regulatory authority FDA (Food and Drug, Administration and Control), which is responsible for drug administration and control, on the quality of drug dosage forms generally available in the market. Generic drug products must satisfy the standards of quality, efficacy and safety as those applicable to the initiator product. Preliminary physicochemical assessment of the product is very important and in vitro dissolution testing can be a valuable predictor of the in vivo bioavailability and bioequivalence of oral solid dosage forms [4].

Metronidazole is BCS Class I, being highly soluble and highly permeable [5]. Metronidazole drug (400 mg) belongs to a group of drugs called anti-infective agents/anti-biotic agents. Metronidazole belongs to the class of nitrimidazole. It is obtained synthetically and used against bacterial and some protozoal species. It's molecular formula is $C_6H_9O_3N_3$ and a molecular weight of 171.6g/mole [6]. Some chemical or IUPAC names, all used for metronidazole are 2-(2-Methyl-5-nitroimidazol-1-yl)ethanol, 2-methyl-5-nitroimidazole-1-ethanol, 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole and 1-(beta-ethylol)-2-methyl-5-nitro-3-azapyrrole [7].

Metronidazole is a basic compound with p*K*a value of 2.62. The shelf life of metronidazole in different formulations is given as injectables (3 years), tablets (5 years), suspension (2 years), and intravenous pre-mixed infusions (2 years) [8]. BP specifies to store the formulation from direct sunlight to prevent from detorioration. İnfusions should be stored between 15 to 30°C. Orally administered tablets of respective drug provides 93%-95% bioavailability. Metronidazole has been reported against protozoal infections (including Balantidium coli; Blastocystis hominis; Entamoeba histolytica; Giardia intestinalis (Giardia lamblia); and Trichomonas vaginalis), anaerobic bacterial infections (includingBacteroides spp and Clostridium spp.) and amoebiasis [9]. Metronidazole has been used to treat the infections, caused by bacteria in brain, bone, lung, stomach lining, for gum and teeth infections, gastritis or stomach ulcers caused by Helicobacter pylori, infected leg ulcers or pressure sores, post-operative infections prevention, diarrhea or colitis ,Crohn's disease (inflammatory bowel disease)and pelvic area following childbirth or in a wound following an operation. Moreover, the drug may also be used against Protozoal parasites, several Gram-positive and Gram-negative anaerobes. It is also a drug of choice for urinary or genital infections.

Product Dosage Forms

Metronidazole is available as an extended Release tablet, capsule, suspension, topical gel, topical cream, topical Emulsion, topical lotion, vaginal Gel and many more. The action of the metronidazole is to inhibit nucleic acid synthesis by disrupting the DNA of microbial cells. This process includes intracellular electron transport proteins such as ferredoxin, transfer of an electron to the nitro group of the metronidazole, and formation of a short-lived nitroso free radical to interact with DNA leading to inhibition of DNA synthesis and DNA degradation leading to the death of the bacteria.

Thus, in the present study the equivalence of ten brands (including the innovator product) of metronidazole tablets sourced from retail pharmacies in Lahore was determined using in vitro methods. This preliminary study is aimed in obtaining baseline data. Different quality control testing for the final product are performed to confirm the proper release including disintegration and dissolution testing etc of API in vivo which in turn relates to the therapeutic index and bioavailability of the relative API. Dissolution test is mostly used in pharmaceutical industry to check whether the chemical agent will properly dissolve in the required medium and meets the desired target. İnfact number of factors including dissolution rate, solubility, absorption, intestinal permeability and metabolism affects the bioavailability of the drug inside the body [10]. Complete disintegration is the state of the solid pharmaceutical product in which any remains of the unit dosage form

except fragments of the insoluble coating or capsule shell, that remains on the test apparatus screen or adhered to the lower surface of the discs, is a soft mass containing no palpably firm core [11]. The significance of this quality control testing is to find out whether the solid unit dosage form either tablet or capsule disintegrate within the prescribed time in accordance with BP specifications. This break-up of the dosage form is the first step towards the dissolution [11].

Drug dissolution testing normally provides information about in vitro release rate of the drug, which assists in attaining the quality of the batch. This test is important to maintain the batch consistency in accordance with BP specifications [12]. It also explains the in vivo drug release profile. Some dissolution related factors include the choice of excipients, dissolution medium, sorption and humidity [13]. Dissolution rate of a pure active ingredient is found to be alterable with the utilization of several types of adjuncts used in the formulation [14]. These variables include diluents, binders, lubricants, granulating agents, disintegrants, bulking agents, colourants and many more. The difference in the choice of these variables in different formulations is due to the cost effectiveness, the feasibility of the ingredients and more preferably to improve the standard of the product [15]. The change in these excipients results in variation in the release rate of an API. This is one of the considerable reason for an improved ranking of a pharmaceutical product. The test temperature must be adjusted to 37°C according to USP/NF specification [16].

In Pakistan, about 60 pharmaceutical companies are currently manufacturing nearly 80 products (injections, syrups, tablets, mucoadhesive patches) of metronidazole. Out of these, 10 products (all tablets) are analyzed [17]. According to literature, extended release metronidazole tablets are considerably in use as does immediate release tablets. However, metronidazole brands which are being considered here are mostly extended release tablets and some of them are immediate release tablets [18]. The literature survey of the release of metronidazole tablets reveals that the extended release metronidazole tablets dosed at 12 or 24 hour interval attains the same activity against bacterial infection as immediate release metronidazole tablets which are dosed at the time interval of every 8 hour [19]. So this comparative study of metronidazole tablets, taken from local companies of Pakistan is compared with the standard active pharmaceutical ingredient (metronidazole). This type of work has never been performed before. So this study will be a step forward towards quality control testing of under discussion local brands [20].

Materials and Methods

Materials

Reference standard of Metronidazole (99.87%) was gifted from (?).

Drug Samples Employed in this Study

Brands were collected from the retail pharmacies of Lahore. Potency of all film coated brands is 400mg metronidazole. Before dissolution, drug samples were stored at room temperature 25+_5°C.

Assay of metronidazole by UV spectroscopy

Calibration curve was plotted by using the pure metronidazole at a wavelength of 278nm specified in the USP monograph. The regression equation for the calibration curves prepared for pure drug component was y = 0.0037x + 0.0047, r 2 = 0.9994 for metronidazole

Brands Color	Potency mg	Expiry date	Manufacturer	Sample code
Brown	400	10/20	Local	MTZ1
Blue	400	06/19	Multinational	MTZ2
Yellow	400	04/20	Local	MTZ3
Blue	400	09/18	Local	MTZ4
Yellow	400	07/20	Local	MTZ5
Yellow	400	09/21	Multinational	MTZ6
White	400	05/20	Multinational	MTZ7
Light brown	400	08/18	Local	MTZ8
White	400	05/19	Local	MTZ9
Orange	400	04/22	Local	MTZ10

Table 1: Different available brands of MTZ employed in the study.

Disintegration Test

The disintegration time of six tablets per brand were determined in distilled water at 37 ± 0.5°C using the Disintegration Apparatus at a frequency of 29 and 32 cycles per minute through a distance of not less than 53 mm and not more than 57 mm. The fluid volume in the vessel is such that at the highest point of the upward stroke the wire mesh remains at least 15 mm below surface of the fluid and descends to not the less than 25 mm from the bottom of the vessel on the downward stroke. At no time should the top of basket-rack assembly become submerged. The time required for the upward stroke is equal to the time required for the downward stroke, and the change in stroke direction is a smooth transition, rather than an abrupt reversal of motion. The basket-rack assembly moves vertically along its axis. This test is provided to determine whether tablets disintegrate within specified time. Carry out the test for 20 minutes for capsules, 30 minutes for plain tablets, and 60 minutes for coated tablets and pills, for pills containing crude drugs, carry out the test for 60 minutes and for enteric coated 2-3hr [21,22].

Dissolution Test

Dissolution testing is required for all solid oral dosage forms in which absorption of the drug is necessary for the product to exert their desired therapeutic effect. The USP 32 specification for metronidazole tablets is not less than 85% of the labeled amount dissolved in 60 min in 900 mL 0.1 N HCl (0.1N hydrochloric acid 3.636 g of HCI, corresponding to 8.3 ml hydrochloric acid 37% (m/m) per 1000 ml of aqueous solution) using the apparatus II operated at 100 rpm [23].

Dissolution Studies

Ten metronidazole tablets, including the one generic product, two multinational and 7 local brands were subjected to *in vitro* dissolution using the basket at 100 rpm in 900 mL 0.1 N HCl according to USP 23 using the USP Dissolution Apparatus-II fitted with a basket rotated at 100 rpm for 1hour. 0.1N HCl was poured into the vessel maintained at 37±0.5 °C. One tablet of each brand was placed in the basket and lowered into the vessel containing the dissolution medium. Samples (5 ml) were withdrawn at timed intervals (10,20,30,40,60 minutes) and replaced with fresh dissolution medium maintained at 37+-0.5°C [24, 27]. The samples were filtered and diluted appropriately with the 0.1N HCl solution and the absorbance of the solution was measured at 278 nm for metronidazole on a double beam UV spectrophotometer [28,31].

Result and Discussion

According to the specification released drug must lie in the range of 85-115% of the labeled amount and disintegration should be within 15minutes n the specified disintegrating medium. Metronidazole tablets used are film coated. Disintegration test necessitates for all dosage forms as the dissolution rate depends on disintegration time affecting absorption, bioavailability and ultimately the drug efficacy [32,35]. Disintegration test is widely used in the pharmaceutical industry for evaluation of disintegration capability of formulations (ex: tablets) and quality control of different dosage forms. Disintegration test is performed for ten tablets of different brands and their values ranges from 12-15 mints. The disintegration times of tablet per brand were determined in distilled water at 37 ± 0.5°C using the Disintegration Apparatus. Only MTZ 2 is out of range-e a multinational brand. When disintegration test is applied on MTZ 2, it disintegrated up to 55mints [36].

Dissolution rate plays a key role in drug absorption and drug's physiological activity in the bloodstream. So, determination of dissolution rate is vital. The term dissolution is the disintegration of drug into solution. Fast drug dissolution will result in rapid onset of action while delayed drug dissolution results in delayed onset of action. Before absorption dissolution inside the body in gastric medium is important. The USP 32 specification for metronidazole tablets is not less than 85% and not more than 115% of the labeled amount dissolved in 60 min in 900 mL 0.1 N HCl (0.1N hydrochloric acid 3.636 g of HCI, corresponding to 8.3 ml hydrochloric acid 37% (m/m) per 1000 ml of aqueous solution) using the apparatus II (paddle) operated at 100 rpm (USP, 2009). Multiple point dissolution are applied samples (5 ml) were withdrawn at timed intervals (10,20,30,40,60 minutes) and observed reading at double beam UV spectrophotometer.

MTZ1, MTZ3, MTZ6, MTZ7, MTZ9 showed clear solution while MTZ10 showed turbidity in the solution. MTZ2 and MTZ8 showed little dissolution and were improperly dissolved.

MTZ4 andMTZ5 showed colored solution with complete dissolution of the drug.

Sample code	Drug present (mg)	Potency(mg)	DT Test (min)	Compliance	Standard Deviation
MTZ 1	386	400	13.17+1.83	Complied	3.50
MTZ 2	180	400	34+19	not complied	55.0
MTZ 3	381	400	12.67+2.33	Complied	4.75
MTZ 4	388	400	13.7+51.25	Complied	3.0
MTZ 5	391	400	14.83+0.17	Complied	2.25
MTZ 6	396.8	400	14.67+0.33	Complied	0.8
MTZ 7	404	400	14.830+.17	Complied	-1.0
MTZ 8	304.8	400	16.67+1.67	not complied	29.8
MTZ 9	385.9	400	12.75+2.25	Complied	3.5
MTZ 10	397.2	400	14+1	Complied	0.7

Table 2: Disintegration results showed by the brands.

The time attained for disintegration ranges from12-14 minutes, by almost all brands including two multinational and six local brands. MTZ2 and MTZ8 failed the test as they were disintegrated in a longer duration. Only MTZ2 showed slow dissolution at 30 min whereas all brands drug release at half an hour ranged from almost 45-65. Innovator showed very rapid dissolution, that is, more than 65% in 30 min. Release rate of all brands at 60min was in an acceptable range of 87-101%, they all complied the dissolution test. Two products-e MTZ2 and MTZ8 showed slower dissolution rate and released less than 85% in 60min.

Conclusion

Out of ten brands one multinational and one local brand fails to comply with the specifications of disintegration as well as dissolution.MTZ 2 and 8 showed delayed disintegration and so the dissolution. This can be considered due to the defects of formulation, lower API content or the excipients employed were unable to disintegrate the product in the required time. This difference in drug release patterns of the drug shows the altered drug standards which can result in victimization of patient, causing resistance, poor efficacious results to the patient and last but not the least our major concern i-e the patient will suffer by not getting the desired effects.

Future Direction

Bioequivalence of batches should be carried out to scrutinize the substandard and malpractice at industrial level. A biannual analysis of all drugs by authority should be made compulsory.

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