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IN VITRO COMPARATIVE QUALITY EVALUATION OF LEADING BRANDS OF METRONIDAZOLE TABLETS AVAILABLE IN BANGLADESH

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Abstract

Metronidazole is a nitroimidazole derivative used for the treatment of antiprotozoal and bacterial diseases. It is one of the commonly produced and sold drugs of several pharmaceutical firms in Banglad esh.

The physicochemical equivalence of five separate brands of metronidazole 400 mg tablets was calculated through examination of both official and non official USP pharmacopoeia specification, including weight uniformity, friability, strength, disintegration, dissolution and assay measures.

Metronidazole tablets are film-coated tablets; all five Metronidazole brands tested comply with USP specifications for material standardization, weight variability, strength, friability, thickness, diameter, disintegration and dissolution. The sum of active metronidazole ranges between 97.0±0.43 and 101.50±0.25 percent of the goods. The average hardness of the items ranges from 12.5±0.77 kg/cm to 14.3±0.60 kg/cm, respectively. Metro-02 had the lowest mean friability test (0.06%) and Metro-03 had the height mean friability test (0.69%). All products had a disintegration time of 7.25±0.69 to 17.0+±0.63 minutes, with 85.34±0.21 to 98.87±0.38 percent release of active ingredient within 60 minutes of dissolution testing. This can confirm the absorption of the drug from the gastrointestinal tract for optimal therapeutic benefit.

The present study revealed that all of the leading brands of this tablet met the quality control parameters as per pharmacopoeial specifications.

Keywords: *Metronidazole, Quality control, Disintegration, Pharmacopoeial specifications*

Introduction

Metronidazole is a synthetic oral nitroimidazole antibiotic drug used to treat infections caused by anaerobic bacteria and protozoa, In addition to antibiotic; it is both an amebicide and antiprotozoal. Metronidazole is less soluble in alcohol and water and is more soluble in ammonia. chloroform, acetone and methanol [1]. Once metronidazole is administered, it prevents nucleic acid synthesis by destroying microbial cell DNA [2]. Metronidazole is a well-tolerated and effective antibiotic since it does not cause any significant adverse effects [3]. Metronidazole is available in the form of white-to-white, circular biconvex, film-coated tablets, i.e. round or oblong. The name of the international union of pure and applied chemistry for Metronidazole is 2(2-methyl-5-nitro-1-H-imidazole-1-y) ethanol, having molecular formula $\mathbf{C_6H_9N_3O_3}$, molecular weight 171.15g/mol, while the melting point is 159-163°C.

It is one of the most important medications needed in the basic health care system and available on the World Health Organization's (WHO) list of essential medicines [4]. Quality of medicine is an absolute necessity in terms of both therapeutic efficacy and safety of the patients. World Health Organization claimed that the manufacturers must undertake responsibility for the quality of the medicines that they manufacturing [5].

Oral delivery of the drug is the most preferable route of drug delivery due to the ease of administration, patient compliance and flexibility in the formulations. [6]. The clinical effectiveness exerted by tablet formulation depends on at least two factors such as; the drug must be present in the labeled amount and its availability to the body [7]. The main objective of an oral tablet is to deliver the drug to the human body at certain and defined amount through the gastro-intestinal system for producing therapeutic effect [8]. A drug from its dosage form is absorbed from gastrointestinal tract, only when it is dissolved in gastric and intestinal fluids [9].

Studies on bioavailability of drugs from different manufacturers showed that in many situations tablets with same drug and drug content did not give the same therapeutic response. Formulation additives in the tablet, physical form of the drug used in the tablet and manufacturing process variation from manufacturer to manufacturer are responsible for the variations observed in the dissolution profile and therapeutic effect [10]. Poor quality medicines do not meet official standards for strength, quality, purity, packaging and labeling. Use of counterfeit and substandard drugs bears serious health implication; such as treatment failure, adverse reactions, drug resistance, increased morbidity and mortality. In combating such type of problems studies on quality assurance must be carried [11]. The prevalence of substandard and or counterfeit medicines is significantly higher in poor and developing countries. This work will increase awareness among the health practitioners and medicine control authority so that, pharmaceutical manufacturers are forced by them to manufacture quality medicine. This study will also provide a comprehensive knowledge about the weight variation, hardness, disintegration, dissolution, the percentage of the potency of Metronidazole tablets available in the market and compares these values with the official specifications.

Methods

Sample Collection:

Each film-coated tablets contains metronidazole 400 mg. Total of five different brands of metronidazole tablets were collected from retail and wholesale medicine shops located at different locations of Bangladesh including Dhaka, Gazipur, Rangpur, Tangail, etc. Here, we select five companies, from which one is multinational (Flagyl 400mg), one is a locally leading company (Filmet 400 mg), and one is locally medium level company (Metryl 400 mg), another two companies are new rising company of Bangladesh (Antipro 400mg, D-metro 400mg). The samples were properly checked by evaluating their batch number and shelf life, name of manufacturer, manufacturing license number, and DAR no. (Drug Administration Registration). The samples were then coded to conceal the identity for analysis. All the selected brands were encoded by the following way given below: Met-01 (Filmet 400 mg; Sanofi-Aventis Bangladesh Ltd.); Met-02 (Filmet 400 mg

Beximco Pharma Ltd.); Met-03 (Metryl 400 mg; Opsonin Pharma Ltd.); Met-04 (Antipro 400 mg Rangs Pharmaceuticals Ltd.); and Met-05 (D-Metro 400mg; Desh Pharmaceuticals. Ltd.). The label information of 05 different brands of metronidazole tablet (400 mg) is represented in Table 1.

Instruments

Instruments used in this study were mortar, pestle, electronic balance (Model: D455007359, Shimadzu Corp.), Roche friabilator (Model: 902, Intech REV), Monsanto hardness tester (Model: Mht-20, Campbell Elec.), USP disintegration apparatus (Model: LTD-DV, Intech), USP dissolution apparatus (Model: VDA-6DR, Veego Instruments Cor.) and ultra violet (UV) spectrophotometer (Model: UV-1800, Shimadzu Corp.).

IN VITRO QUALITY CONTROL TESTS:

Weight variation test

For this test according to the USP-NF weight variation test was run by weighting 20 tablets for each of the ten brands individually using an electronic balance, then calculating the average weights and comparing the individual tablet weights to the average. The difference in the two weights was used to calculate weight variation by using the following formula [12, 13].

Weight variation = (Iw – Aw)/Aw × 100%

Where, Iw=Individual weight of the tablet and

Aw = Average weight of the tablet.

The tablet complies with the test if not more than 2 of the individual weights deviate from the average weight by more than the 5% [12, 13].

Friability test

For this test Roche friabilator was used. Twenty tablets from each of the ten brands were weighed and placed in the friabilator and then operated at 25 rpm for 4 min. The tablets were then de-dusted and weighed. The difference in the two weights was used to calculate friability by using the following formula [12, 14].

% Friability = [(Initial weight – Final weight)/Initial weight]×100

The tablet complies with the test according to USP-NF if tablets loss less than 1% of their weight [12, 14].

Hardness test

For this test Monsanto hardness tester was used. Ten tablets were randomly selected from each of the ten brands and tested. This test measures the pressure required to break diametrically placed tablets by applying pressure with coiled spring.

Disintegration test

For this test USP disintegration apparatus was used. To test for DT, one tablet was placed in each tube for each brand and the basket rack was positioned in a 1000 ml vessel containing 900 ml of water maintained at 37 ± 2 °C, so that the tablets remained 2.5 cm below the surface of the liquid on their upward movement and descent not closer than 2.5 cm from the bottom of the beaker. A standard motor driven device was used to move the basket assembly containing the tablets up and down through a distance of 5–6 cm at a frequency of 28–32 cycles per minute. Perforated plastic discs were used to prevent the floating of tablets. The apparatus was operated for 30 min [12, 15].

To comply with the USP-NF standards, the tablets must disintegrate and all particles must pass through the 10-mesh screen within 30 min. If any residue remains, it must have a soft mass with no palpably firm core.

Standard curve preparation

The powder equivalent to 100 mg of standard metronidazole was taken and dissolved in 0.1 N HCl. Then it was diluted to produce a final concentration of 100µg/ml for working solution. Absorbance values were then measured at the maximum wavelength (λmax) of metronidazole of the serially diluted concentrations (1, 2, 3, 4, 5, 6, 7. 8 & 9 µg/ml etc) using a UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan). 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 in fig.1.

Measurement of potency

Preparation of standard solution:

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:

$$\text{Potency of sample} = \frac{\text{Absorbance of sample x weight of standard}}{\text{Absorbance of standard x weight of sample}} \text{ X purity of standard}$$

Dissolution test

For this test USP dissolution apparatus was used. To test for dissolution, one tablet was placed in each vessel (6 vessels) for each brand, containing 900 ml of 0.1 M hydrochloric acid (HCl) as a dissolution medium maintained at 37 ± 0.5 °C. The rotational speed of the apparatus was held constant at 50 rpm. A sample of 5 ml was withdrawn at a fixed time intervals (10, 20, 30 and 60 min) and this was immediately replaced with the same volume of fresh test media (13, 16, 17).

The sample was filtered and 1 ml of filtrate was taken and diluted to 50 ml with distilled water. So the solution was 50 times diluted. The absorbance of the diluted filtrate was determined spectrophotometrically at the wavelength of 278 nm, using 0.1 M HCl as blank. The percentage of drug release at each interval was calculated by using standard metronidazole. As per USP-NF tablets meet with this test if not less than 75% dissolves in 45 min. According to BP tablet comply with this test if not less than 80% dissolves in 45 min [12, 13, 17].

Results & discussion:

All the brand of Metronidazole tablets used in this investigation was within their shelf life. The labeled shelf life of all tablets was 2 years from the date of manufacturing. All tablets obtained from local market were subjected to a number of tests in order to assess quality parameters like assay, weight variation, hardness, and disintegration time.

The weight variation test is a satisfactory method of determining the medicine content uniformity of tablets and does serve as a pointer to good manufacturing practices (GMP) maintained by the manufacturers as well as the amount of active pharmaceutical ingredient (API) contained in the formulation. When the weight variation is within the specifications the tablets are thought to contain a uniform active ingredient to give desired therapeutic response. But when the weight variation is out of the specification the tablets are thought to contain less or more active ingredient to give an ineffective therapeutic response or toxic effect respectively. It may vary due to result from, poor granulation flow properties, resulting in uneven die fill. 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. Twenty tablets were selected from each of the brands and weighed individually using electronic balance. Their average weights were calculated. For all tablet brands following mathematical equation was used for weight variation [18]. It was observed that all of the brands meet the USP specification. It can be seen in fig.2.

Hardness test of material is indicative of its strength. Most important physical feature for assessing tablet is hardness [19]. The average hardness of tablets was determined by using Monsanto hardness tester. The tablets of different brands possessed good mechanical strength with sufficient hardness. Figure: 3 shows the hardness test results and clearly indicates that the results of all the samples significantly differ from each other. The maximum hardness 14.3 ± 0.60 Kg/cm was observed for Metro -01 while minimum hardness (12.5 ± 0.77Kg/cm was shown by Metro-05 as in fig. 3.

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 [20]. It is a compendia test and met the USP specification if friability is not more than 1% [21, 22]. The friability was found to be between the ranges of (0.06 - 0.69) %, thus all the brands met the friability specification. It can be seen in fig. 4.

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 [23]. According to BP/USP specification, film coated tablets should disintegrate within 30 min [21, 22]. 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 (7.25 ± 0.69 to 17.0 ± 0.63) min as in fig.5.

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 (97.0 ± 0.43 to 101.50 ± 0.25) % of labeled amount of drug and complied according to USP (20,21) as in fig. 6.

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. 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 60 min [21, 22]. Brand Metro -04 had maximum drug release within the 60 min (98.87%) of the *in vitro* dissolution test, while brand Metro -02 had minimum drug release (85.34%) 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 7). 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 as in fig. 7.

Conclusion

Metronidazole tablets were analyzed to find their correct quality status. For this purpose, the marketed sample of Metronidazole tablets was analyzed by using established methods and apparatus. The result of weight variation, hardness, disintegration time, dissolution and assay potency tests of all marketed products comply with Pharmacopoeial limit. So, on the basis of those results, we can conclude that the products of Metronidazole available in Bangladesh meet the quality parameter to satisfy therapeutic efficacy. All of the brands have proved that they have the quality, which meets the USP specification. The present study, although performed on a limited scale yet on the basis of professional judgment the data reported in this study can help the Drug Control Authority to get an idea about the quality status of the marketed Metronidazole preparations in Bangladesh.

Limitations

For this study little number of company was assessed to assess the quality. Cumulative the number of company will give accurate idea about the whole consequence of Metronidazole tablet in Bangladeshi Pharmaceutical market. Authors also faced problems during collection of chemical

reagents and the reference standard of Metronidazole for the analytical tests as that was limited in the research laboratory.

Abbreviations

USP: United State Pharmacopeia; SD: standard Deviation; BP: British Pharmacopeia; RPM: rotation per minutes; NMT: not more than; NLT: not less than; Kgf: kilogram force.

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

Brand code	Shape of the tablets	Mfg. date	Exp. date	Pack size found	Price of pack found (BDT)	Price per unit (BDT)
Met-01	Oval	June 2018	June 2020	250	380.00	1.52
Met-02	Caplet	April 2018	April 2020	200	254.00	1.27
Met-03	Caplet	March 2018	March 2020	300	474.00	1.58
Met-04	Caplet	July 2018	June 2020	210	266.70	1.27
Met-05	Caplet	May 2018	April 2020	100	115.00	1.15

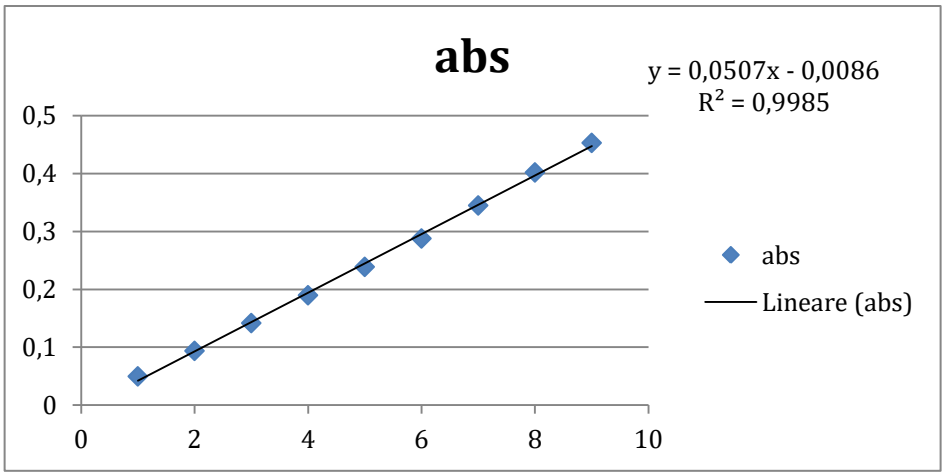

Fig. 1: Standard curve of Metronidazole.

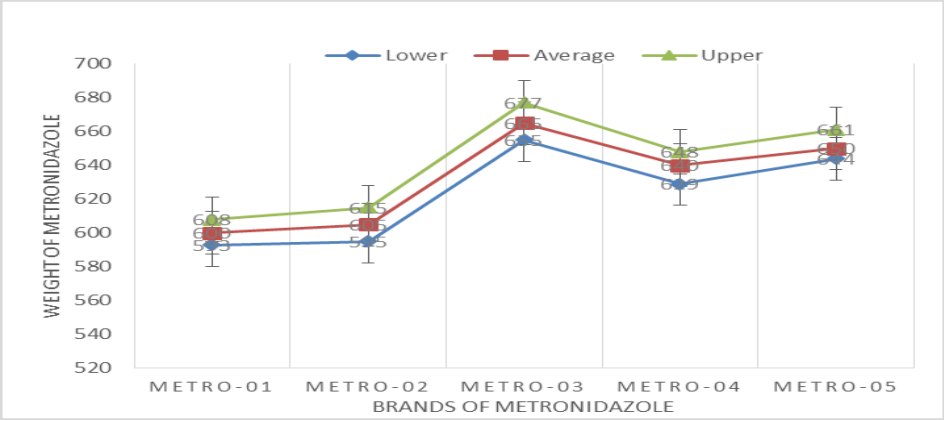

Fig. 2: Weight variation of different brands of metronidazole.

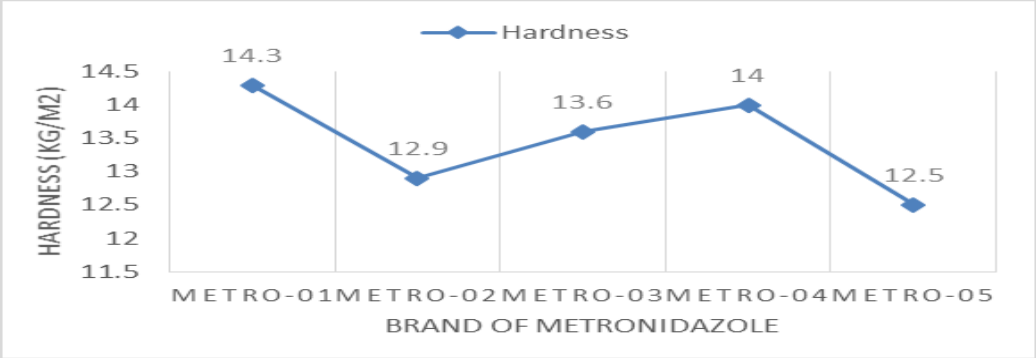

Figure 3: Hardness (Kg/m2) of different brands of metronidazole.

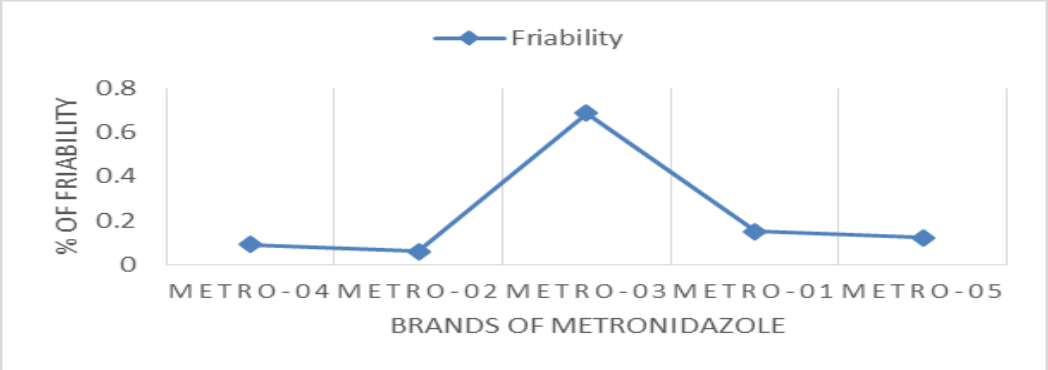

Fig. 4: Friability (%) of different brands of metronidazole

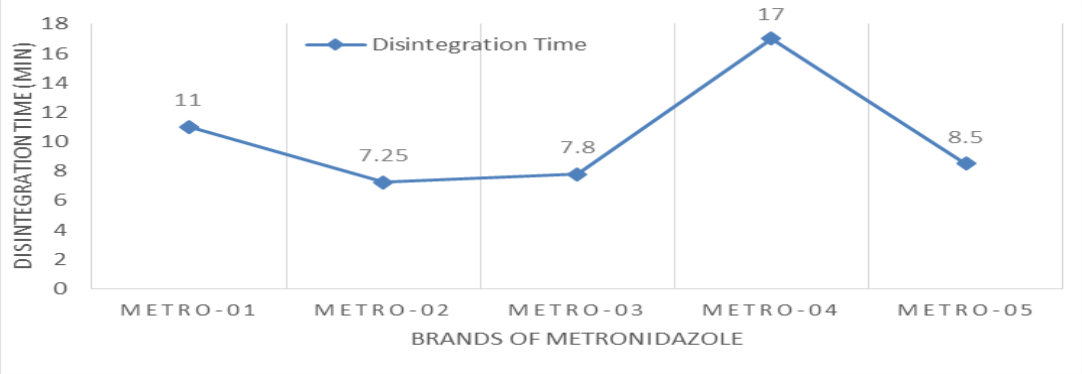

Fig. 5: Disintegration time (min) of different brands of metronidazole

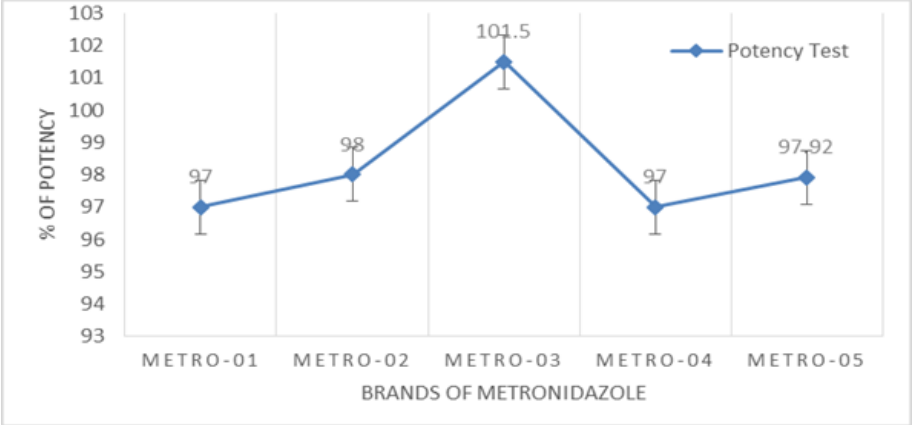

Fig. 6: Potency of different brands of metronidazole.

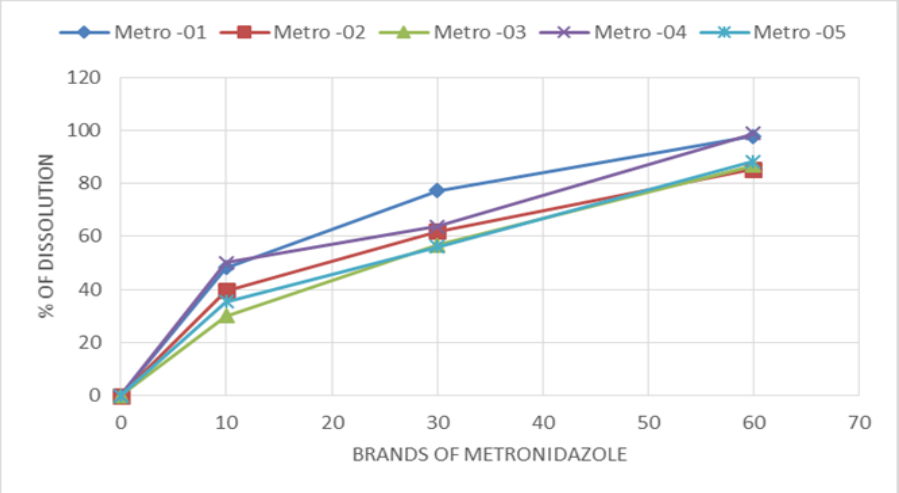

Fig. 7: Dissolution (%) of different brands of metronidazole.