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Quality of Marketed Metronidazole Preparations in Bangladesh- An Analytical Overview

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Abstract: In Bangladesh, about 100 pharmaceutical companies are manufacturing about 160 products of metronidazole. Of them 40 products (30 tablet and 10 suspension) were analyzed. Potency of the selected products were assayed spectrophotometrically and their various physical parameters (color variation, thickness, weight variation, hardness, friability, disintegration time, dissolution rate, pH of suspension) were analyzed according to the official (BP/USP) pharmacopoeial methods to evaluate their quality. The results showed that 26 brands of tablets meet the BP specification of potency and the remaining 4 were less potent. 8 brands of suspensions meet the BP specification of potency and the remaining 2 were less potent. All brands tested, showed a good result for color variation, thickness, hardness, disintegration time and dissolution rate. 2 brands failed to meet the weight variation test specification. It is evident from the study that most of the brands tested showed good results but a few of them failed to meet the specification.

Key words: Quality, metronidazole, Bangladesh

Introduction

Metronidazole was introduced as an antiprotozoal agent but it is also active against anaerobic bacteria such as ***Bacteroides***, ***Clostridium*** and ***Helicobacter*** species and some streptococci (Oldenburg and Speck, 1983). It is effective in the therapy of pseudomembranous colitis and aclostridial infection. It is the most effective drug available for invasive amoebiasis involving the intestine or the liver (Roe, 1977). Metronidazole was found to have particularly high activity ***in vitro*** and ***in vivo*** against ***Trichomonas vaginalis*** and ***Enterobacter histolytica*** (cosar ***et al.***, 1961). Durel and associate (1960) reported that oral doses of the drug imparted trichomonacidal activity to semen and urine and that high cure rates could be obtained in both male and female patients with trichomoniasis. Recent studies have suggested that metronidazole therapy may benefit some patients with peptic ulcer who are infected with ***H. pylori*** (Hopkins and Morris, 1994).

Because of the increasing complexity of modern pharmaceutical manufacture arising from a variety of unique drugs and dosage forms, complex ethical, legal and economic responsibilities have been placed on those concerned with manufacture of modern pharmaceuticals. An awareness of these factors is the responsibility of all those involved in the development, manufacture, control and marketing of quality products. The major causes that lead to

substandard drugs are given below:

Addition of incorrect quantity of active ingredient or date expired sub-potent materials.

Non-uniform distribution of active ingredients and Poor stability of active ingredients in the finished product.

Substandard or spurious drugs could endanger patient's life. After the implementation of the National Drug Policy in 1982 the quality of marketed drug, no doubt, improved, but not as expected. This realization influenced to evaluate the metronidazole preparations available in the market. The major purpose of this study is to investigate the overall quality of the marketed metronidazole (tablets and suspensions) preparations available in Bangladesh. We hope that the findings of this study will help to make awareness both in physicians and consumers to select quality products. The investigation was performed in "Pharmaceutical Chemistry Laboratory" of Pharmacy Discipline, Khulna University, Bangladesh during March, 2002 to August, 2002.

Materials and Methods

Instruments

Name of the instrument	Manufacturer
Analytical balance	Mettler, Toleds, Switzerland
Mortar and Pestle (300 mm)	China.
UV-Spectrophotometer	Thermospectronic type: Helias Gamma,
England	
Many	
Germany England.	
Tablet hardness tester	India.
Dissolution test apparatus	India.
Disintegration test apparatus	India.
Friability test apparatus	India.
pH Meter	Switzerland.
Slide calipers	Shanghai, China.
Distilled water plant	Japan.

Reagents

(a) Concentrated HCl (Merck, Germany), Molecular weight: 36.46, Melting point: 25°C, Boiling point: 85°C, Purity: 32%, Specific gravity: 1.16

(b) Standard buffer solution pH 4 (Merck, Germany)

Composition: Citric acid / NaOH solution / NaCl.

Collection of sample

There are about 160 products of metronidazole (tablets and suspensions) in Bangladesh. These are alphabetically arranged and every forth was selected for analysis. Thus 40 different products were collected from retail medicine shop of different areas of Bangladesh. About 40-50

tablets of each brand were collected for the analysis of tablets and 3 unit files of suspension of each brand were collected for suspension analysis. Tablets of 30 different brands were coded as MT01---------to MT30 and suspension of 10 collected brands were coded as MS01---------to MS10. The samples were properly checked for their physical appearance, name of the manufacturer, batch number, manufacturing date, expiry date, manufacturing license number, D.A.R. number and maximum retail price at the time of purchase. No samples were bought and analyzed whom date of expiry had already been passed.

Weight variation test of tablets

Procedure

Twenty tablets were taken and weighed individually by an analytical balance. The average weight of the tablets was calculated. Then % of weight variation is calculated by using the following formula.

$$\% \text{ of weight variation} = \frac{\text{Individual weight - Average weight}}{\text{Average weight}} \times 100$$

Hardness test of tablets

Tablet hardness is defined as the load required to crush or fracture a tablet placed on its edge. Sometimes it is also termed as tablet crushing strength. In this study Monsanto Hardness Tester was used.

Disintegration time test of tablets

Disintegration time is the length of time required for causing disintegration of tablet. This test is important to evaluate a tablet since it directly influences the onset of action. This test not only evaluates the quality but also the bioavailability and effectiveness of tablets.

Procedure

USP disintegration apparatus contains 6 glass tubes that are 3 inches long, open at the top and held against a 10-mesh screen at the bottom end of the basket rack assembly. To test for disintegration time, one tablet is placed in each tube and the basket rack is positioned in a 1 l beaker containing 0.1 N HCl solution at 37±2°C, such that the tablets remain 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 is used to move the basket assembly containing tablets up and down through a distance of 5 to 6 cm at a frequency of 28 to 32 cycles min^{-1}. The disintegration time of each tablet was determined and the average disintegration time was calculated.

Dissolution rate test of tablets

Dissolution is the property or tendency of a drug to undergo solution, which affects the rate of drug absorption.

Medium: 0.1 N HCl; 900 ml.

Apparatus: USP dissolution apparatus 1; 2. Whatmann filter paper; 3. Pipette; 4. Volumetric flask; 5. UV-visible spectrophotometer USP dissolution apparatus 1: In general, a single tablet is placed in a small wire mesh basket fastened to the bottom of the shaft connected to a variable speed motor. The basket is emerged in the dissolution medium (0.1 N HCl; 900 ml) contained in a 1000 ml flask. The flask is cylindrical with a hemispherical bottom. The flask is maintained at a 37±0.5°C by a constant temperature bath. The motor is adjusted to turn at the specified speed.

Procedure

1) The flask was filled with 900 ml 0.1 N HCl.
2) The dissolution medium was heated up to 37±0.5°c by an autoheater.
3) One tablet was put in to the basket and stirred immediately at 100 r.p.m.
4) 10 ml of sample was withdrawn from the flask after 60 min.

The dissolved metronidazole was determined from UV absorbance at the wavelength of maximum absorbance at about 278 nm of filtered portion of the solution under test, suitably diluted with 0.1 N HCl in comparison with a standard metronidazole solution having known concentration of USP metronidazole RS in the same medium.

pH test of suspension

About 30 ml of the suspension sample was transferred to a clean, dry beaker. Then pH of the sample was measured by a pH meter, which was previously calibrated with two standard buffer solutions (Citrate buffer solution of pH 4.0 and phosphate buffer solution of pH 7.0).

Preparation of standard curve

A series of standard solutions with different concentration of standard metronidazole e.g., 2, 4, 6, 8, 10, 12, 14 and 16 mcg ml^{-1} were prepared by dissolving 100 mg of standard metronidazole in a 100-ml volumetric flask and volume was adjusted by 0.1 N HCl (stock solution). Then 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4 and 1.6 ml of stock solutions were taken in a series of separate 100-ml volumetric flask and the volume was adjusted by 0.1 N HCl. Absorbance was taken at 278 nm against a blank for each solution and the average was calculated (Table 1). The measured absorbances were plotted against the respective concentrations of the standard solutions which give a straight line in the concentration range of 2 to 16 mcg ml^{-1} (Fig. 1).

Potency determination of tablets

(a) Preparation of standard solution: 100 mg of standard metronidazole was weighed accurately in an analytical balance and was taken in a 100 ml volumetric flask. 50 - 60 ml of 1 N HCl was added and was shaken mechanically for 30 min. The volume was made upto the mark with the same solvent. 1 ml of the above solution was diluted to 100 ml with 0.1 N HCl solution.

(b) Preparation of assay solution: 20 tablets were weighed and powdered in a mortar with a pestle. An amount of powder equivalent to 100 mg of metronidazole was transferred in a 100-ml

volumetric flask. 50 - 60 ml of 1 N HCl was added and was shaken for 45 min. The volume was made up to the mark with the same solvent and filtered with whatmann filter paper. 1 ml of the filtered solution was diluted to 100 ml with 0.1 N HCl solution.

© Calculation: The absorbance of both standard and sample were measured in a suitable UV-VIS spectrophotometer at 278 nm using 0.1 N HCl solution as blank. Each sample was run in duplicate and average of the results was taken in to consideration.

$$\text{Potency of sample} = \frac{\text{Absorbance of sample} \times \text{Weight of standard}}{\text{Absorbance of standard} \times \text{Weight of sample}} \times \text{Purity of standard}$$

Potency determination of suspension

(a) Preparation of standard solution: Standard solution was prepared according to the aforementioned procedure.

(b) Preparation of sample solution: 5 ml of suspension was taken in a 200-ml volumetric flask. 100 ml of 1 N HCl was added and was shaken for 45 min. The volume was made upto 200 ml by the same solvent and filtered by whatmann filter paper. 1 ml of the filtrate solution was taken in a 100-ml volumetric flask and the volume was made up to 100 ml with 0.1 N HCl solution.

Table 1: Absorbance of different concentrations of standard metronidazole

Concentration (mcg ml^{-1})	Average of the absorbance
2	0.09
4	0.18
6	0.27
8	0.356
10	0.435
12	0.521
14	0.606
16	0.684

© Calculation: Potency was calculated by using the following formula.

$$\text{Potency of sample} = \frac{\text{Absorbance of sample} \times \text{Weight of standard}}{\text{Absorbance of standard} \times \text{Weight of sample}} \times \text{Purity of standard}$$

Results and Discussion

Weight variation test of tablets

The weight variations of 30 different brands of metronidazole tablets were determined and the observed results are shown in the Table 2. BP/ USP specification of weight variation: ± 5% (w/w).

It is observed from the above result (Table 2) that 2 brands (MT06 and MT16)out of 30 did not comply with the specification where the number of tablets out of USP weight variation range were 3 in both cases. The rest of the 28 brands meet the specification.

Table 2: Weight variation of various brands of metronidazole tablets

Sample code	Number of tablets taken	Average weight	Weight variation number of tablets within BP/USP range	Number of tablets out of BP/USP range
MT01	20	344.48	20	0
MT02	20	401.74	20	0
MT03	20	637.17	20	0
MT04	20	763.16	20	0
MT05	20	666.23	20	0
MT06	20	568.75	17	3
MT07	20	503.39	20	0
MT08	20	487.19	20	0
MT09	20	570.0	20	0
MT10	20	661.08	20	0
MT11	20	570.40	20	0
MT12	20	620.8	18	2
MT13	20	590.2	20	0
MT14	20	710.5	19	1
MT15	20	650.7	20	0
MT16	20	570.7	17	3
MT17	20	670.5	20	0
MT18	20	595.2	20	0
MT19	20	402.78	20	0
MT20	20	637.18	20	0
MT21	20	344.48	20	0
MT22	20	520.4	20	0
MT23	20	480.8	20	0
MT24	20	550.8	20	0
MT25	20	560.8	20	0
MT26	20	610.7	20	0
MT27	20	570.8	20	0
MT28	20	630.8	20	0
MT29	20	596.2	20	0
MT30	20	660.7	20	0

Hardness test of tablets

The hardness of 30 different brands of metronidazole tablets were measured and the observed results are shown in the Table 3. BP / USP specification of hardness: Not more than 7.0 kg-cm^{-1}.

It is seen from the results (Table 3) that none of the samples exceeded the specification for hardness (maximum 7.0 kg cm^{-1}). Therefore, it can be said that all of the studied samples complied with the BP / USP specification for tablet hardness.

Disintegration time test of tablets

The disintegration time were measured according to the aforementioned procedure and the observed results are shown in the Table 4. BP /USP specification of disintegration time: 5-30 min. It is seen from the above results (Table 4) that none of the samples exceeded the specification

Table 3: Hardness of various brands of metronidazole tablets

Sample code	Average hardness (kg cm^{-1})	Sample code	Average hardness (kg cm^{-1})
MT01	3.5	MT16	5.0
MT02	4.0	MT17	4.5
MT03	3.0	MT18	5.0
MT04	2.5	MT19	4.5
MT05	4.25	MT20	3.5
MT06	4.5	MT21	5.0
MT07	3.5	MT22	4.5
MT08	4.0	MT23	5.0
MT09	3.0	MT24	3.5
MT10	3.25	MT25	3.0
MT11	4.0	MT26	5.0
MT12	4.5	MT27	3.5
MT13	3.25	MT28	4.5
MT14	3.0	MT29	5.0
MT15	4.25	MT30	3.5

Table 4: Disintegration time(D.T.) of various brands of metronidazole tablets

Sample code	Average D.T. (min)	Sample code	Average D.T. (min)
MT01	15	MT16	9
MT02	6	MT17	11
MT03	18	MT18	12
MT04	2	MT19	10
MT05	25	MT20	15
MT06	18	MT21	16
MT07	21	MT22	12
MT08	25	MT23	20
MT09	10	MT24	8
MT10	3	MT25	11
MT11	28	MT26	12
MT12	15	MT27	13
MT13	13	MT28	16
MT14	8	MT29	12
MT15	10	MT30	10

for disintegration time. Therefore, it can be said that all the studied samples complied with the BP/USP specification for tablet disintegration time.

Dissolution rate test of tablets

The dissolution rate of tablets were measured and the observed results are shown in the Table 5.

USP specification

Not less than 85% of the labeled amount of metronidazole to be dissolved in 60 min.

It is seen from the above results (Table 5) that every sample fulfills the USP specification for tablet dissolution rate.

Table 5: Dissolution rate of various brands of metronidazole tablets

Sample code	% of drug release after 45 minutes	% of drug release after 60 minutes	% of drug release after 75 minutes
MT01	82.30	91.90	93.73
MT02	82.76	90.99	93.28
MT03	82.30	86.87	88.01
MT04	82.76	86.42	87.56
MT05	80.01	85.75	90.30
MT06	77.73	86.19	88.02
MT07	82.53	88.93	91.45
MT08	80.01	86.87	93.73
MT09	77.73	85.05	87.56
MT10	82.07	86.42	89.62
MT11	82.99	88.70	91.68
MT12	78.08	86.02	90.01
MT13	80.82	88.20	93.09
MT14	75.50	85.03	89.91
MT15	76.75	85.88	90.87
MT16	77.09	86.01	91.10
MT17	80.20	86.77	91.70
MT18	81.32	86.79	90.23
MT19	78.50	89.90	95.95
MT20	80.20	91.20	97.75
MT21	81.30	90.10	99.80
MT22	79.77	90.20	98.70
MT23	78.20	89.99	97.77
MT24	80.20	89.79	92.20
MT25	77.73	88.20	95.60
MT26	81.40	90.30	96.70
MT27	80.50	91.50	97.80
MT28	81.40	92.30	98.15
MT29	79.78	85.10	99.10
MT30	81.50	89.90	97.70

Table 6: pH of various brands of metronidazole suspensions

Sample code	Observed pH
MS01	5.5
MS02	5.3
MS03	5.6
MS04	5.2
MS05	5.0
MS06	5.4
MS07	5.5
MS08	5.3
MS09	5.6
MS10	5.2

Table 7: Potency of various brands of metronidazole tablets

Sample code	Potency (%w/w)	Sample code	Potency (% w/w)
MT01	98.54	MT16	99.45
MT02	98.99	MT17	96.70
MT03	90.99	MT18	97.50
MT04	93.73	MT19	98.98
MT05	96.02	MT20	99.25
MT06	91.68	MT21	97.75
MT07	96.02	MT22	98.25
MT08	97.16	MT23	99.50
MT09	93.73	MT24	98.45
MT10	96.02	MT25	98.75
MT11	98.08	MT26	99.75
MT12	97.20	MT27	97.65
MT13	96.97	MT28	99.65
MT14	98.50	MT29	99.50
MT15	99.01	MT30	98.50

Table 8: Potency of various brands of metronidazole suspension

Sample code	Potency (% w/w)
MS01	95.64
MS02	89.93
MS03	93.10
MS04	102.62
MS05	98.70
MS06	99.02
MS07	98.50
MS08	98.75
MS09	97.80
MS10	99.20

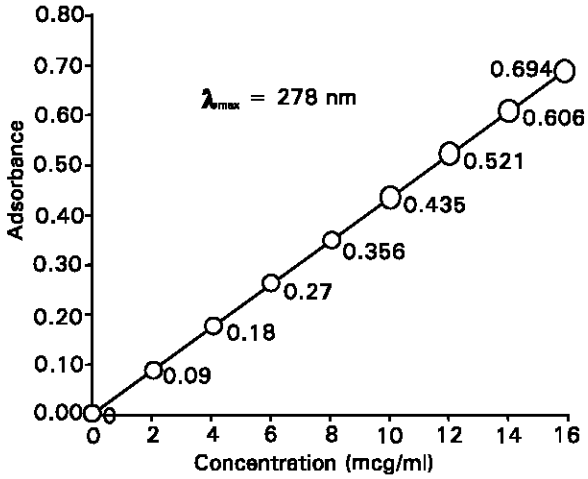

Fig. 1: Standard Curve of Metronidazole

pH of various brands of suspensions

The pH of 10 brands of metronidazole suspensions were measured according to the aforementioned procedure and the observed results are shown in the Table 6.

From the above results (Table 6), it is clear that none of the studied samples exceeded the specification for pH of suspension (maximum pH 7). Therefore, it can be concluded that all of the studied samples complied with BP/USP specification for the suspension.

Potency determination of tablets

The potency of 30 different brands of metronidazole tablets were determined within their shelf-life and the obtained results are shown in the Table 7. BP specification of potency: 95 - 105% (w/w).

From the result (Table 7), it is evident that 26 out of 30 brands of tablets meet the specification of potency. 4 brands (MT03, MT04, MT06 and MT09) are slightly less potent than the BP specification. This may be due to the degradation of active ingredient or due to the addition of less amount of active ingredient at the time of manufacture.

Potency determination of suspension

The potency of 10 brands of suspensions was determined within their shelf-life and the observed results are shown in the table-8. BP specification of potency: 95-105% (w/w).

From the above results (Table 8) it is observed that 8, out of 10 meet the BP specification of potency. 2 brands (MS02, MS03) are found less potent. This may be due to the degradation of active ingredient or the addition of less amount of active ingredient at the time of manufacture.

At present about 95% of the essential drugs are being produced in Bangladesh. In 2000, only 5% drugs are imported which include different types of vaccines and drugs, which require high technology for manufacturing. The overall quality of the drug is satisfactory but some spurious and substandard drugs are also available in the market. Although Bangladesh Drug Control Authority monitors the quality of pharmaceutical products by collecting samples from the market and evaluates the quality, unfortunately some pharmaceutical companies escape the observation of the Drug Control Authority and supply spurious and substandard products. Substandard drugs cause not only wastage of money but also create health hazards. The present study, although performed on a limited scale yet on the basis of professional judgement 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. This study emphasizes the need of constant surveillance and continuous evaluation of marketed drug products by the governments, manufacturers and independent research group to ensure proper supply and availability of quality medicines.

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