QUALITY ASSESSMENT AND COMPARISON OF METRONIDAZOLE ANTIBIOTIC FORMULATION UNDER GOVERNMENT SUPPLY OF DELHI AND BRANDED FORMULATIONS OF INDIA

[1]SURAJ KUMAR PRAJAPATI, [2]RUCHI SINGH

Delhi Institute of Pharmaceutical Sciences and Research, New Delhi, India,
Saroj College of Pharmacy, Lucknow, U. P.

***Abstract*- In this report, an attempt was made to determine the consistency and pharmaceutical equivalence of six Metronidazole samples available in Delhi, India. Three samples were taken from the industry and three from the Government supply of Delhi. Metronidazole was chosen for this analysis because it is widely prescribed as an antiamoebic medication. The research was conducted using in vitro methods as per Indian Pharmacopoeia 2018. All six samples were assessed through different official and non-official tests like Hardness, Friability, Weight Variation, Disintegration Time, Dissolution Profile, Assay, And Impurity testing. All six samples passed the defined limits and were safe and reliable and of high quality. All samples were pharmaceutically equivalent.**

***Keywords*: Metronidazole, Weight Variation, Hardness, Dissolution, Assay, Related Substances.**

INTRODUCTION

The major population of India relies on the Government hospitals for medical care and drugs. The goal of this research was to examine whether patients get good quality medicines. Metronidazole was chosen because it is widely prescribed drug in a government as well as private hospitals.

This study also compared the outcome of following classes of metronidazole.

a) Generic Vs Generic
b) Branded Vs Branded
c) Generic Vs Branded.

Metronidazole is a synthetic antiamoebic drug derived from azomycin [1].It inhibits the nucleic acid synthesis by the formation of nitroso radicals, which lead to disruption of the DNA and then DNA breaks into fragments. Therefore it causes cell death in protozoan [2,3]. It is used in the treatment of giardiasis and epigastric pain. It was effective against dysentery, dracunculiasis, and trichomoniasis [4,5]. The oral absorption is approximately 91% with 1.1 L/kg volume of distribution. Its plasma protein binding is 20% and half-life is 6 hrs [1,6].

MATERIALS AND METHODS

The design of the research involved the collection of three samples each from local pharmacy stores and government hospitals. All samples were assessed for different tests like Hardness, Friability, Weight Variation, Disintegration, Dissolution profiling and Assay. All tests were performed according to Indian Pharmacopoeia 2018.

Sample collection

Metronidazole tablets with 400 mg label statements have been used in this study. Three samples were taken from the Delhi government hospitals and the remaining three samples were purchased from the nearby drug stores in Delhi. Finally, six different samples were taken for quality assessment as shown in Table 1.

Table: 1 Details of samples

Sample code	Company	Sample type	Sample collection site	Manufacturing date	Expiry date	Prices
M1	CMG Biotech Pvt. Ltd.	Generic	NDMC Dispensary New Delhi	October 2019	September 2021	-
M2	Omega biotech ltd.	Generic	Mohalla Clinic Malviya Nagar New Delhi	May 2019	April 2021	-
M3	Daffodils Pharmaceutical Ltd.	Generic	Khanpur Dispensary New Delhi	April 2019	March 2021	-
M4	Abbott Healthcare Pvt. Ltd.	Branded	Local pharmacy store	November 2019	October 2022	Rs 28/strip
M5	JB chemical and pharmaceutical Ltd.	Branded	Local pharmacy store	November 2019	October 2023	Rs 25/strip
M6	PAAM Drugs	Branded	Local pharmacy	June 2019	May 2022	Rs 22/strip

			store			

Chemicals used in the study

Analytical grade chemicals were used in the study, namely Monobasic Potassium Phosphate, Sodium Hydroxide, Ethanol, Acetonitrile and Distilled Water or HPLC Water.

Types of equipment used in the study

Pfizer hardness tester (mLabs), Roche Fribilator (Panomex Inc.), UV Visible Spectrophotometer (Lamba 35 Perkin Elmer), Digital Balance (AL500 Mettler Toledo), Disintegration Test apparatus (ED2SAPO Electrolab), Dissolution Test Apparatus (DS 8000 Lab India), pH meter (seven compact pH/Ion S220 Mettler Toledo), Sonicator (Branson Sonicator Mettler Toledo), Water purification system (integral3Q-POD Millipore, Bedford, USA), HPLC System (Thermo Scientific Dionex Ultimate 3000U) were used.

Weight Variation

The weight variation test was carried out to check whether each tablet contains the labeled amount of Metronidazole. The test was conducted by weighing twenty tablets using a digital balance. The average weight was calculated in milligrams. Percentage deviation was calculated from the average weight and is given in Table 8. Percentage deviation was calculated using the formula [7]

% deviation = Average weight of tablet - Individual weight tablet × 100
Average weight of the tablet

Hardness Test

The hardness test is the structural integrity of the tablet which determines the resistance for breaking of the tablet. For this, the Metronidazole tablet was placed vertically in the Pfizer hardness tester. The load was then applied along the radial axis of the tablet for crushing. Similarly, readings were taken for 10 tablets [8].

Friability

A Friability test was done for the determination of the physical strength of tablets upon exposure to mechanical shock and attrition. It was done by Roche Friabilator. The 10 tablets were weighed and placed in an apparatus and after revolving for 5 min at the speed of 25 RPM, weights were compared with the initial weights. The percentage friability was calculated using the formula [9]

Friability (**% F) = [1 - (W/W$_0$) x 100]**

Where, % F = Friability in percentage, W_0 = Initial weight of tablets, W = Weight of the tablets after the revolution.

Identification

The identification test of Metronidazole was carried out to validate the presence of Metronidazole molecule in the tablet. The identification was done using Retention time (RT) obtained in the chromatogram while performing the related substance test. It is shown in Fig. 8 to Fig. 14

Tablet Disintegration

This test was conducted to determine, whether the Metronidazole tablet disintegrates within the specified time when it is inserted in a liquid medium under laboratory conditions. Disintegration is the first step before Dissolution. The faster the tablet disintegrates, the faster it will dissolve in the gastric or enteric fluid. Six Tablets of each sample were placed in the disintegration apparatus. The volume of the disintegration medium was 900 ml of water and temperature was maintained at 37 ± 0.5℃. The time taken by each tablet to break into small parts that can cross the mesh was observed and recorded. The average time was calculated in minutes.

Tablet Dissolution

For tablets, dissolution is an important step to achieve the bioavailability and therapeutic effect. Dissolution test is important because it measures the rate and extent of solution formation from the dosage form. Dissolution plays major role in drug absorption. If the dissolution of the drug is good then its absorption will be better and it will be more bioavailable for therapeutic effect.

This test was performed using the USP type II apparatus and the samples were analyzed by UV.

Dissolution medium - The dissolution medium was 900 ml of 0.1M Hydrochloric acid. It was transferred to the dissolution basket. The instrument was allowed to rotate at 100 RPM for 60 minutes and the temperature was maintained at 37 ± 0.5^{0}C.

Standard solution - The standard solution was prepared by dissolving 22 mg of the reference standard of (99.8% w/w) Metronidazole in 50ml of dissolution medium. 2 ml of aliquot was diluted to 100 ml by dissolution medium to get a solution of a concentration of approximately 8.88 PPM.

Sample solution – One tablet was placed in 900 ml of dissolution medium using sinker. After 60 minutes the desirable quantity of sample was withdrawn and filtered. 2 ml of aliquot was diluted to 100ml with dissolution medium to get a solution of a concentration of approximately 8.88 parts per million (PPM).

Absorbance of samples was recorded by a UV spectrophotometer at the wavelength of 277 nm. The percentage of content dissolved was calculated using the formula

% Content dissolved = Test area × Std wt × Test dilution × Potency × 100
Std area × Std dilution × Test wt ×100 × Claim

ASSAY

This test was performed to determine the specified label claim or potency of the drug in individual samples and to observe differences among the samples. The assay was done by the non-aqueous titration method. Triturated 20 tablets to powder form. From this 200 mg powder was transferred into a sintered glass crucible where it was extracted by passing 10 ml of hot acetone, the acetone after dissolving the contents filtered out, leaving residue in the crucible. The extraction process was repeated five

more times using 10 ml of hot acetone each time. All extracts were mixed and after cooling, 50 ml of acetic anhydride was added and then the mixture was titrated with 0.1M Perchloric acid. The indicator used was 0.1ml of 1% w/v solution of brilliant green in anhydrous glacial acetic acid. The endpoint is from colorless to yellowish-green.
Standardization of Perchloric acid was done by titrating 20.4mg Potassium hydrogen phthalate in 200 ml Glacial acetic acid with 0.1M $HClO_4$ to calculate the actual strength of perchloric acid, which was calculated as 0.098814 M [10].
Determination of percentage purity was done by formula

$$\%\text{purity} = \frac{17.12\ (\text{IP factor}) \times 0.098814 \times \text{volume consumed} \times \text{average weight} \times 100}{(0.1\text{M})\ \text{Strength of Perchloric acid} \times \text{weight taken} \times \text{label claim}}$$

RELATED SUBSTANCE
Formulations should not contain any impurity but it is practically not possible. Impurities leach in through solvents, raw materials, water, etc. Impurities within the specified limits are permissible but excess impurities can cause adverse effects [11].
Determination of impurities was done by HPLC method [12]. For the determination of impurities in the sample, the standard impurity used was 2-methyl-5-nitroimidazole.
Test solution - It was prepared by dissolving 50 mg of Metronidazole in 50 ml of solvent to get 1000 ppm solution.

Reference solution (a) - It was prepared by dissolving 0.25 mg of 2-methyl-5-nitroimidazole in 50 ml of solvent, then 5 ml aliquot of this mixture was diluted to 50 ml using a fresh solvent to get 0.5 ppm solution.
Reference solution (b) - It was prepared by dissolving 0.25 mg of 2-methyl-5-nitroimidazole in 50 ml then 5 ml aliquot of this mixture was diluted to 50 ml using test solution to get 0.5 ppm solution.
Chromatographic conditions
Column – C18, 25 cm x 4.0 mm, 10 µm
Mobile phase – It is prepared by mixing 30 ml of methanol with 70 ml of 0.68 % w/v solution of monobasic potassium phosphate whose pH was adjusted to 5 using potassium hydroxide.
Flow rate - 1ml/min
Spectrophotometer - set at 315 nm
Injection volume – 20 µl

RESULTS
Weight variation
As per IP the limit for weight variation for the tablet of more than 250 mg is given as not more than two of the individual weight of tablets should deviate from the average weight by the percentage deviation of ± 5% [13]. All samples were within the specified limit and passed the test. The average weight and % deviation of all samples of Metronidazole is shown in Table 2.

Table: 2 Weight variation of Metronidazole samples

Brand Code	Average weight (mg)	Range of % weight variation (n=20)	Limit in mg	Result
M1	500.54	-1.5 to 1.6	475.513 - 525.567	Passed
M2	501.78	-2.6 to 7.30	476.691 - 526.869	Passed
M3	498.90	-1.1 to 1.8	473.955 - 523.845	Passed
M4	468.68	-1.8 to 3.1	445.246 - 492.114	Passed
M5	499.95	-2.0 to 1.8	474.952 - 524.947	Passed
M6	446.25	-4.5 to 2.7	423.937 - 468.562	Passed

Hardness
Being an unofficial test, the official monograph does not prescribe any limit for hardness. The general limits for hardness are given as 4-10 Kg/cm^2. The Hardness of all six samples of Metronidazole was found to be within the prescribed limit and is given in Table 3.

Table: 3 Hardness of Metronidazole samples

Brand Code	Hardness (Kg/cm^2)	Limit	Result
M1	5.66	4.0 - 10 Kg/cm^2	Passed
M2	6.33		Passed
M3	6.44		Passed
M4	6.46		Passed
M5	6.56		Passed
M6	6.86		Passed

Friability

Friability is a non-official test. Friability loss of 10 tablets should not be more than 1% according to Indian Pharmacopoeia 2018. Friability data of Metronidazole samples is given in Table 4.

Table: 4 Friability of Metronidazole samples

Sample codes	Initial weights(W)	Weights After revolution (W_0)	W/W_0	%F	Result
M1	4890.50	4881.25	0.9982	0.18	Passed
M2	5002.75	4992.13	0.9979	0.21	Passed
M3	4995.89	4985.23	0.9979	0.21	Passed
M4	5032.12	5015.15	0.9967	0.33	Passed
M5	4998.87	4985.12	0.9972	0.28	Passed
M6	5000.35	4989.25	0.9978	0.22	Passed

Retention time

Identification was done using the Retention time of Metronidazole reference standard, which was found to be 2.808 minutes in the related substance test as per IP 2018, the limit for retention time is ±10% of Standard RT. Thus all samples correspond with that of standard. All samples were identified as Metronidazole. The retention time of all the samples, taken from Chromatograms, are given in Table 5.

Table: 5 Retention time of the Metronidazole samples

Sample Code	Retention time (in min)	Limit(±10% min)	Result
M1	2.910	2.5272 to 3.0888	Passed
M2	2.910		Passed
M3	2.910		Passed
M4	2.903		Passed
M5	2.908		Passed
M6	2.908		Passed

Disintegration

As per IP, the disintegration Time for the uncoated tablet should not be more than 30 min. The disintegration time of the six samples is given in Table 6. The disintegration Time of all samples was found to be within the prescribed limit.

Table: 6 Disintegration Time of Metronidazole Samples

Brand Code	Disintegration time (min-sec)	Limit	Result
M1	5.02	30min	Passed
M2	5.22		Passed
M3	5.29		Passed
M4	5.48		Passed
M5	5.52		Passed
M6	5.82		Passed

Dissolution test

The % content dissolved for all samples is given in Table 7. Sample M5 shows maximum dissolution while M6 shows the least among all the six samples but all samples show dissolution above the prescribed minimum percentage.

Brand Code	% label claim dissolved	Limit	Result
M1	93.34	Not less than 85%	passed
M2	93.13		passed
M3	92.47		passed
M4	97.51		passed
M5	98.70		passed

Table: 7 profile of samples	M6	92.1		passed

Dissolution Metronidazole

Assay

The % purity for all six samples fall within the prescribed range given in the IP monograph of Metronidazole. Branded sample M5 showed maximum content of 101.67 % while Government Generic sample M1 showed the least content of 96.52%. The percentage purity of all six Metronidazole samples is listed in Table 8.

Table: 8 Assay of Metronidazole samples

Brand code	Amount (mg/tab)			Content %	Limit	Result
	Volume consumed	Label claim	Assay (mg)			
M1	9.2	400mg	386.08	96.52	Not less than 95 % and not more than 105%	Passed
M2	9.1	400mg	390.00	97.50		Passed
M3	9.1	400mg	390.28	97.57		Passed
M4	9.6	400mg	391.44	97.86		Passed
M5	10.2	400mg	406.68	101.67		Passed
M6	10.2	400mg	394.12	96.68		Passed

Related substance

1. **5- Nitro imidazole impurity**

Chromatogram of reference standard of Metronidazole, depicting the retention time and the peak area of related substance, is used for determination of impurities.

As per IP, the sample solution chromatogram of any secondary (unknown) peak area should not be more than the peak area of 2-methyl-5- nitroimidazole in the chromatogram obtained with Reference solution (a). The limit for the sample was 2.808 mAU(milli-Absorbance Unit) per minute. Details of secondary peaks (unknown) in the sample are given in Table 10. HPLC chromatograms depicting the results are given below.

The area of 2-methyl-5-nitroimidazole is 2.808 mAU per min.

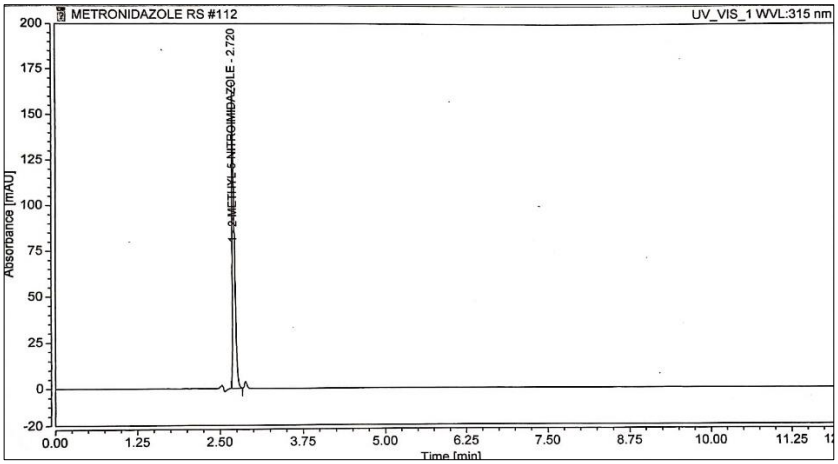

Fig: 1 Chromatogram of 2-methyl-5-nitroimidazole, Reference (a)

Table 9: Related Substance test reference (b)

Sample	**RT(in min) of** 2-methyl-5-nitroimidazole
M1 test Reference (b)	2.703
M2 test Reference (b)	2.708
M3 test Reference (b)	2.705
M4 test Reference (b)	2.711
M5 test Reference (b)	2.705
M6 test Reference (b)	2.705

2**. Other impurities**

The Retention time of test reference (b) was used to detect the impurities present in the sample other than 2-methyl-5-nitroimidazole, so that unknown impurities can be detected easily in the sample.

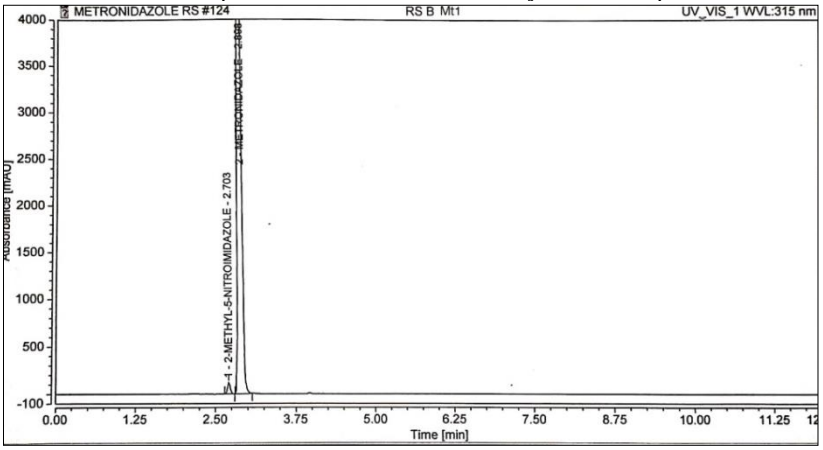

Fig: 2 Chromatogram of M1test Reference (b)

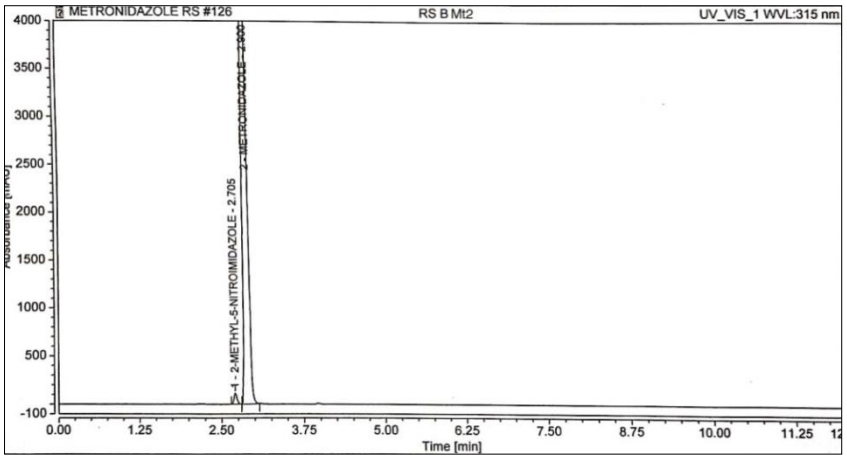

Fig: 3 Chromatogram of M2 test Reference (b)

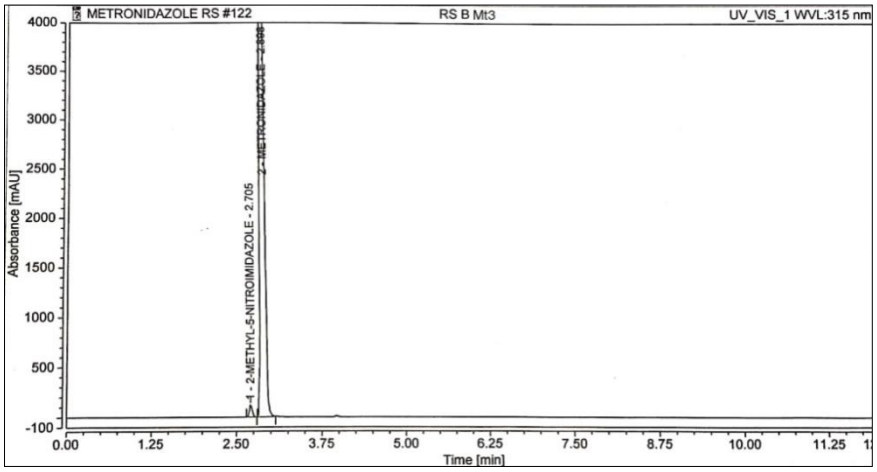

Fig: 4 Chromatogram of M3 test Reference (b)

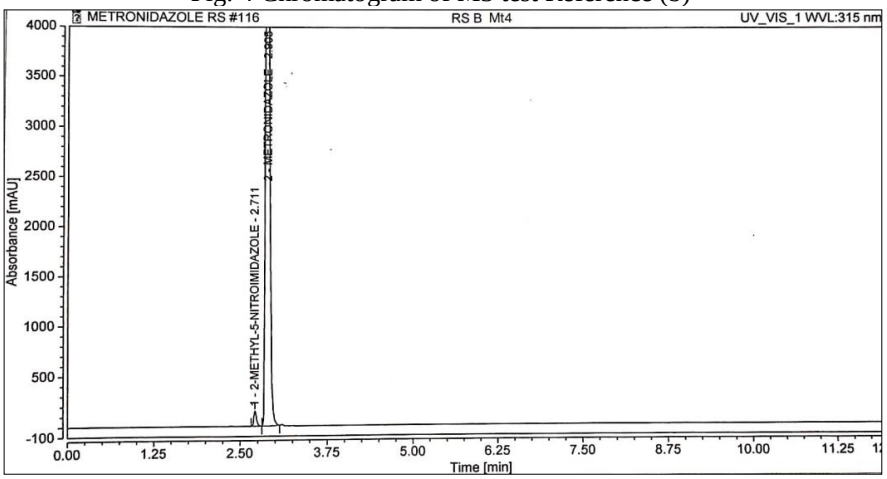

Fig: 5 Chromatogram of M4 test Reference (b)

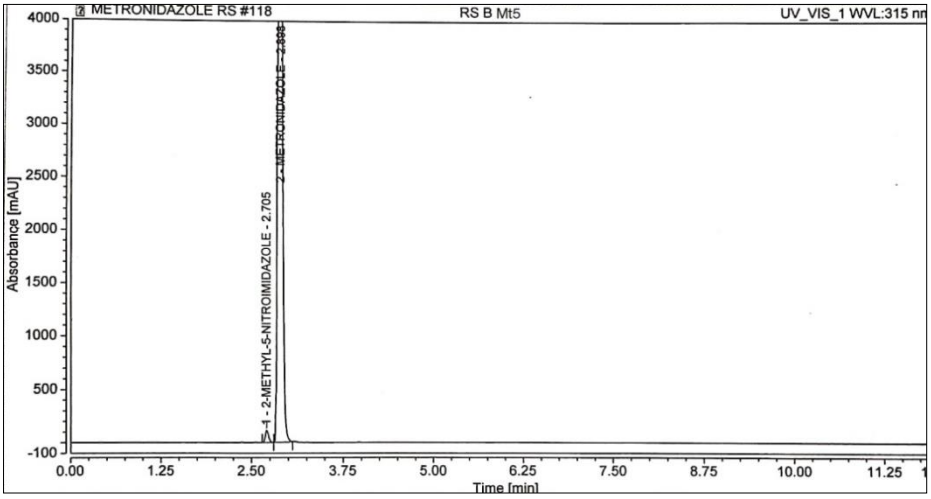

Fig: 6 Chromatogram of M5 test Reference (b)

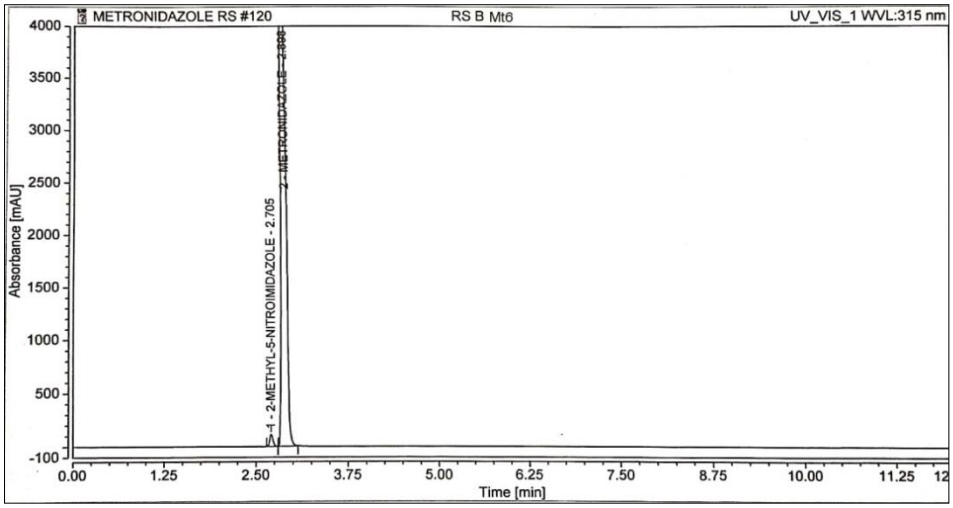

Fig: 7 Chromatogram of M6 test Reference (b)

The chromatogram of Metronidzole sample are shown below:

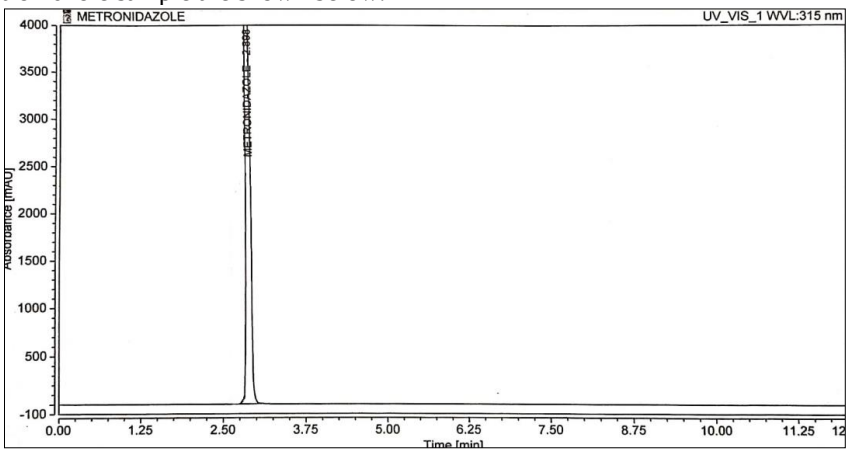

Fig: 8 Chromatogram of Metronidazole Reference Standard and Retention Time is 2.808 min.

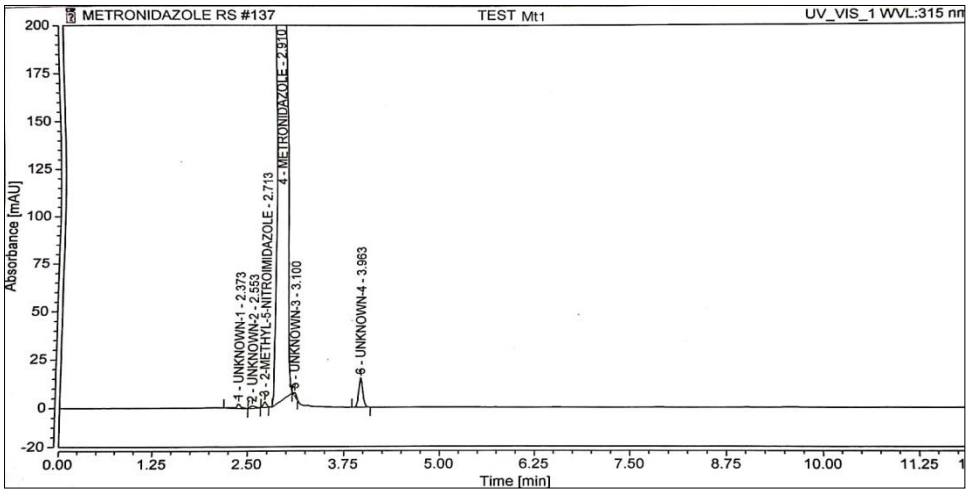

Fig: 9 Chromatogram of sample M1

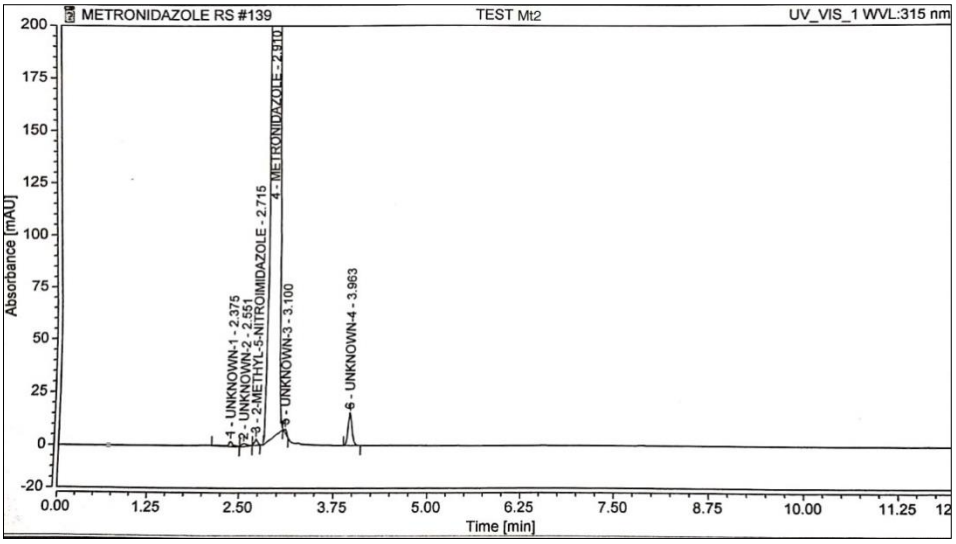

Fig: 10 Chromatogram of sample M2

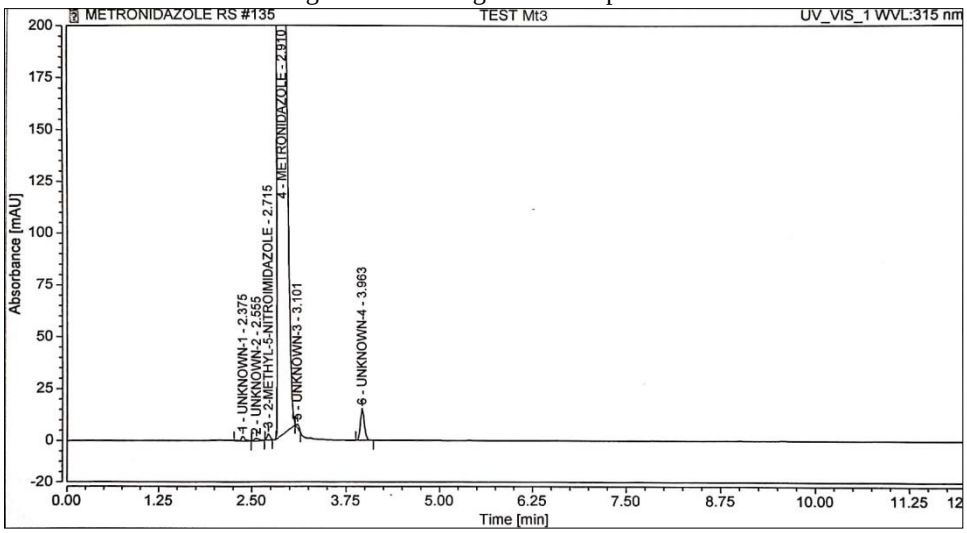

Fig: 11 Chromatogram of sample M3

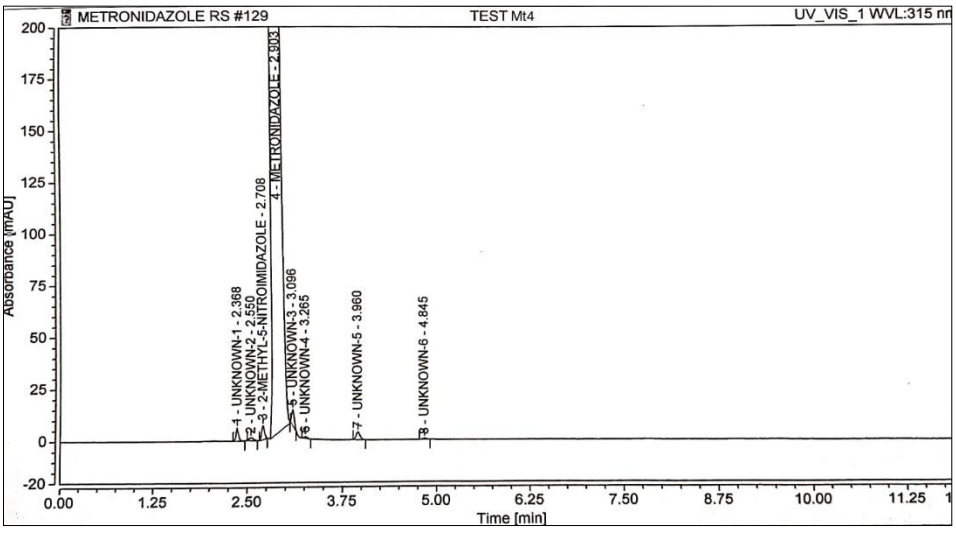

Fig: 12 Chromatogram of sample M4

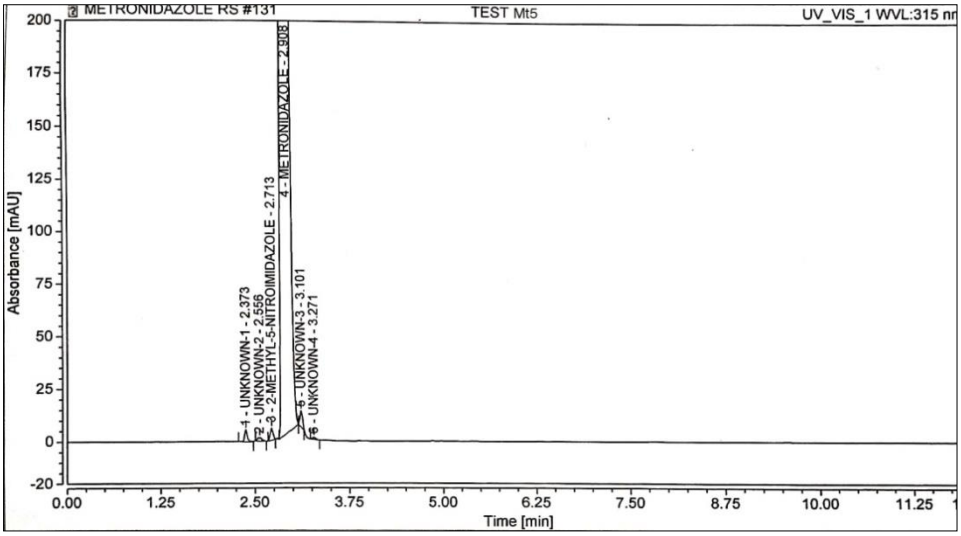

Fig: 13 Chromatogram of sample M5

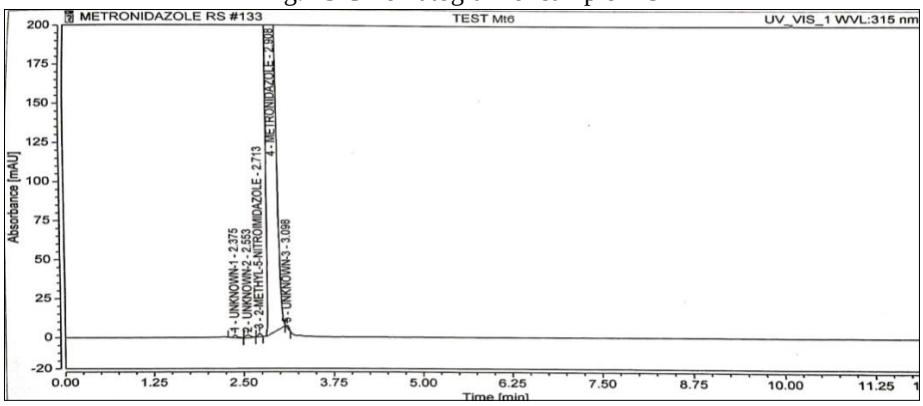

Fig: 14 Chromatogram of sample M6

Table 10: Details of impurities present in Metronidzole samples

Sample code		**Area** (mAU per min)	**Limit** (mAU per min)	**Result**
M1	Metronidazole	393.090	The Peak area of the unknown should not be more than 2.808	Passed
	2-methyl-5-nitroimidazole	0.119		
	Unknown 1	0.155		
	Unknown 2	0.100		
	Unknown 3	0.075		
	Unknown 4	0.900		
M2	Metronidazole	389.372		Passed
	2-methyl-5-nitroimidazole	0.120		
	Unknown 1	0.167		
	Unknown 2	0.101		
	Unknown 3	0.075		
	Unknown 4	0.899		
M3	Metronidazole	387.961		Passed
	2-methyl-5-nitroimidazole	0.121		
	Unknown 1	0.120		
	Unknown 2	0.086		
	Unknown 3	0.076		
	Unknown 4	0.906		
	Metronidazole	393.900		
	2-methyl-5-nitroimidazole	0.279		

M4	Unknown 1	0.239		Passed
	Unknown 2	0.088		
	Unknown 3	0.297		
	Unknown 4	0.042		
	Unknown 5	0.215		
	Unknown 6	0.037		
M5	Metronidazole	386.294		Passed
	2-methyl-5 -nitroimidazole	0.236		
	Unknown 1	0.210		
	Unknown 2	0.114		
	Unknown 3	0.309		
	Unknown 4	0.043		
M6	Metronidazole	391.092		Passed
	2-methyl-5-nitroimidazole	0.099		
	Unknown 1	0.111		
	Unknown 2	0.139		
	Unknown 3	0.078		

The impurities table should come at the end after chromatograms

DISCUSSION

Our study compared Metronidazole generic tablets prescribed in government supply with branded metronidazole tablets sold in the open market. It was found after the literature survey that previously no study is undertaken to compare the Metronidazole tablets of government supply with the branded supply of Metronidazole tablets. This makes our study unique.

Following studies have been undertaken by different researchers to study the metronidazole formulation for its content and quality control. **Musa et al.** in 2011 from Nigeria conducted a comparative study on commercially available fifteen brands of Metronidazole tablets and found that only nine brands passed quality tests as per BP [14]. **Naimi et al.** in 2014 from gulf countries, conducted a comparative evaluation of commercially available four Brands of Metronidazole tablets and found that all the samples passed the quality testing as per USP [15]. **Rahman, et al.** in 2020, from Bangladesh, performed a comparative dissolution study of five commercially available brands of Metronidazole tablets and found that all the five samples passed specified limits of pharmacopoeia as per USP [16].

In our study, all the samples passed quality tests given in IP 2018.Thus, our study is in coungruence with results of Naimi et al and Rahman et al while it is not in a congruence with the results of Musa et al. It is found that M5 sample has released maximum content in dissolution test which is in coherence with the maximum content found in assay as per IP 2018.

CONCLUSION

All Metronidazole samples comply with the specifications given in IP 2018. Thus all six samples are pharmaceutically equivalent. Negligible difference was found among generic vs generic, branded vs branded and branded vs generic. Thus, all samples can be interchanged with each other. For the economic advantage of patients, a medical practitioner may substitute high-cost brand with a lower-cost brand. The study also concludes that the medicines supplied in the government dispensaries and hospitals are of good quality and have efficacy equivalent to branded samples. The study concluded that all samples were safe, reliable, potent and appropriate for use in clinical practice.

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