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Comparative evaluation of Metformin tablets available under government supply and brands available in open market in Delhi, India.

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ABSTRACT
In the present study, an attempt was made to evaluate the quality and pharmaceutical equivalence of six samples of Metformin available in Delhi, India (three from market and three from government supply). Metformin was selected for the present study because it is the most commonly used drug for diabetes. The study was performed using *in-vitro* methods as per Indian Pharmacopoeia 2018. All six samples were assessed through both official and non-official tests like hardness, friability, weight variation, disintegration time, dissolution profile, assay and impurity testing. All six samples met the prescribed limit and found to be of good quality, safe and effective. All samples were pharmaceutically equivalent and interchangeable.

KEYWORDS - Metformin, Weight Variation, Dissolution, Assay, Related Substances, Quality control parameter

I. INTRODUCTION

The present study aimed to compare the quality of branded Metformin tablets available in Indian market with generic Metformin tablets available under government supply of Delhi. The study was also designed to compare (a) Generic Vs Branded (b) Generic Vs Generic (c) Branded Vs Branded (d) Intraday and Interday (e) Intra-instrument and Inter-instrument.

Metformin is a biguanide with anti-hyperglycaemic effect. It is chemically known as 3-(diaminomethylidene)-1,1-dimethylguanidine [2]. It is freely soluble in water, slightly soluble in alcohol and insoluble in acetone. Bioavailability of Metformin 500 mg tablet administered orally in fasting state is 50-60%. It has negligible plasma protein binding, 2.5 hours half life with 63-276 L volume of distribution. It gets excreted unchanged in urine [2].

Metformin does not stimulate insulin secretion and therefore does not produce hypoglycaemia [1]. Metformin decreases hepatic glucose production, decreases intestinal absorption of glucose and improves insulin sensitivity by increasing peripheral glucose uptake and utilization [3]. Resulting in lowering of both basal and post-prandial plasma glucose.

II. MATERIALS AND METHODS

The design of study included the sample collection from the community pharmacy and nearby government hospitals. All samples were checked for their shelf life and assessed for different tests like hardness, friability, weight variation, disintegration, dissolution profiling, potency test (assay), and impurity testing (related substances). All tests were performed according to Indian Pharmacopoeia 2018 [4].

Sample collection

Six samples of Metformin uncoated tablets with label claim of 500 mg were used. Three brands of Metformin tablets were procured from the community pharmacies while the remaining three generic Metformin tablets were obtained from government hospitals of Delhi. Samples were coded before the study to prevent study bias. Table 1 depicts the details of the samples used in this study.

Table 1: Details of samples

Sample code	Company	Sample type	Sample collection site	Cost (in Rs)	Manufacturing date	Expiry date
M1	Cipla Ltd.	Branded	Local pharmacy store	21.72/20 tabs	September, 2019	August, 2022
M2	Franco-Indian Pharmaceuticals Pvt. Ltd.	Branded	Local pharmacy store	32.82/20 tabs	August, 2019	July, 2022
M3	USV Private Ltd.	Branded	Local pharmacy store	15.79/10 tabs	June, 2018	May, 2021
M4	Vivek Pharmachem	Generic	Mohalla Clinic	-	June, 2019	May, 2021
M5	Anglo-French Drugs & Industries Ltd.	Generic	Municipal Corporation of Delhi	-	March, 2019	February, 2021
M6	Omega Biotech Ltd.	Generic	Central Supply	-	August, 2019	July, 2021

Chemicals used in the study

All analytical grade chemicals were used in the study. Metformin (Indian Pharmacopoeia Reference Standard), Potassium dihydrogen phosphate, dicyandiamide, sodium pentanesulphonate, sodium chloride, sodium hydroxide, ethanol, distilled water [4].

Equipments used in the study

UV visible Spectrophotometer (Perkin Elmer- Lamba 35), Infrared Spectroscopy (Perkin Elmer- Spectrum One), Digital Balance (Mettler Toledo), Disintegratation Test apparatus (Electrolab - ED2SAPO), Dissolution Test apparatus (Lab India - DS 8000), HPLC (Agilent - Infinity 1200) pH meter (Mettler Toledo - Seven Compact pH/Ion S220), Friability Apparatus (Roche Friabilator), Hardness tester (Pfizer Hardness tester) were used.

IDENTIFICTION

Identification was done by taking infrared spectrum of each sample. Pellet method for sample preparation was employed. Powder containing 20 mg of Metformin was extracted with 20 ml of ethanol. The content was filtered and evaporated to dryness on water-bath. Residue was dried at 105°C for 1 hour. The residue was triturated in mortar along with potassium chloride and converted into a fine disc. The disc was placed in the sample holder and spectrum of samples were recorded. The sample spectrums were compared with the spectrum of the reference spectrum of Metformin standard graph given in IP [4].

WEIGHT VARIATION

Weight variation test was carried out to check that each of the tablet contains the labeled amount of Metformin. The test was conducted by weighing twenty tablets using a digital balance. The average weight was calculated in milligrams [4]. Percentage deviation was calculated using the formula.

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

HARDNESS

Hardness test was carried out to check the breaking point and strength of tablets. Sufficient hardness in tablet is necessary for damage resistance during packaging and transportation. Hardness also plays role in the disintegration time of a tablet. Higher the hardness, higher will be the disintegration time. Ten tablets of each sample were taken and each tablet was placed vertically in the Pfizer hardness tester to check its crushing strength. Average hardness was calculated in Kg/cm^2.

FRIABILITY

Shock and frictional forces can damaged or break the tablets. The friability value is an indication of the ability of the tablet to withstand stress due to abrasive forces without crumbling, during transportation, packaging, handling and dispensing. Twenty tablets were initially weighed (W_o) and placed in Roche friabilator. The apparatus was rotated at a speed of 25 rpm for 4 minutes. The tablets were again weighed and the final weights (W) were compared with initial weights.

The % friability was calculated using the formula-

$$\% F = [1 - (W/Wo)] \times 100$$

Where, %F = Friability in percentage

Wo = Initial weight of tablets
W = Weight of the tablets after revolution

DISINTEGRATION TEST

This test is useful to know about the disintegration time under experimental conditions as it is correlated with dissolution profile of sample. Six tablets of each sample were placed in disintegration apparatus. The volume of disintegration medium was 900 ml of water maintained at 37 ± 0.5°C. The time taken to break each tablet into small parts and pass through the mesh was recorded and average time was calculated in minutes [4].

DISSOLUTION TEST

Dissolution testing helps to measure the extent and rate of dissolution. It affects the absorption and bioavailability of the drug. Dissolution test was performed using USP type II apparatus.

Dissolution medium - 0.68% w/v solution of potassium dihydrogen phosphate was prepared by dissolving 6.8 gm of potassium dihydrogen phosphate in 1000 ml of water.

One tablet was added in dissolution basket placed in dissolution jar containing 900 ml of dissolution medium. The temperature was maintained at 37 ± 0.5°C and instrument was allowed to rotate at 100 rpm for 45 minutes. 1 ml of aliquot was taken after 45 minutes which was further diluted to 50 ml with water to get the concentration of approximately11.11 PPM.

Absorbance of resulting test solution was recorded at the maximum of 233nm taking 806 as specific absorbance [4]. Calculation of % dissolved content was done using the formula,

$$\% \text{ Content dissolved} = \frac{\text{Test Abs} \times 1 \times \text{Test dil} \times 100 \times 1000}{\text{Specific Abs} \times 100 \times \text{Test wt} \times \text{Claim}}$$

ASSAY

This test helps to find out the content of active pharmaceutical ingredient present in the samples which is responsible for therapeutic action. UV method was used to determine the content of active ingredient in the Metformin tablets.

Test solution - Twenty tablets were weighed and powdered. Powder containing 0.1 g of Metformin was weighed and dissolved in 70 ml of water and volume was made upto 100 ml with water.

Further dilutions were made to get a concentration of approximately 11 PPM. Absorbance of resulting solution was measured. Content of Metformin was calculated bytaking 798 as the specific absorbance at maximum of 232 nm, as given in IP [4]

The % assay was calculated using the formula,

$$\% \text{ Assay} = \frac{\text{Test Abs} \times 1 \times \text{Test dil} \times \text{Avg wt} \times 100 \times 1000}{\text{Specific Abs} \times 100 \times \text{Test wt} \times \text{Claim}}$$

RELATED SUBSTANCE

HPLC method was used to determine the impurities present in the samples. Ideally, formulations should not contain any impurity but in reality, impurities enter through solvents, raw materials, water, etc. Impurities within the specified limits are allowed but exceeding that level of impurities may cause harmful effects.

Chromatographic conditions:

Column used - C18 (25 cm × 4.6 mm, 5 µm)
Column temperature- 25°C
Flow rate- 1 ml per minute
Injection volume- 20 µl
Mobile phase- It was prepared by dissolving 870 mg of sodium pentanesulphonate and 1200 mg of sodium chloride in 1000 ml of distilled water.
Spectrophotometer was set at 218 nm.

Test solution- Quantity of powdered tablets containing 0.5 g of Metformin was dissolved in 100 ml of water and filtered to get the solution of concentration of approximately 5000 PPM.

Reference solution (a) - It was prepared by diluting 0.1 ml of the test solution to 100 ml of water to get the solution of concentration of approximately 5 PPM.

Reference solution (b) - It was prepared by dissolving 1.0183 mg of dicyandiamide in 100 ml of water. 1 ml of aliquot was diluted to 10 ml with water to get the solution of concentration of approximately 1 PPM [4].

III. RESULTS AND DISCUSSION

Identification- IR spectrum of all the samples correspond with the IR spectrum of Metformin standard given in Indian Pharmacopoeia. IR spectrum of samples (Fig - 1 to 6) are given below. Details of wavelength of functional groups are given in Table 2.

Table 2: IR analysis of Metformin samples

Brand code → / Wavelength ↓	Standard	M1	M2	M3	M4	M5	M6
C-N	1170 - 1040	1167.47	1046.93	1060.19	1049.94	1058.41	1048.17
N-H deformation	1650 - 1581	1592.97	1593.47	1590.36	1596.72	1590.24	1598.94
N-H wagging	736	736.19	736	736.29	735.14	735.94	735.47
NH_2	800	800.16	800.41	800.57	800.93	800.18	800.36
CH_3	2816 - 1619	1628.62	1627.62	1623.51	1628.61	1626.94	1627.57
C-N-C	580 - 418	579.14	576.57	575.19	577.19	579.06	577.25

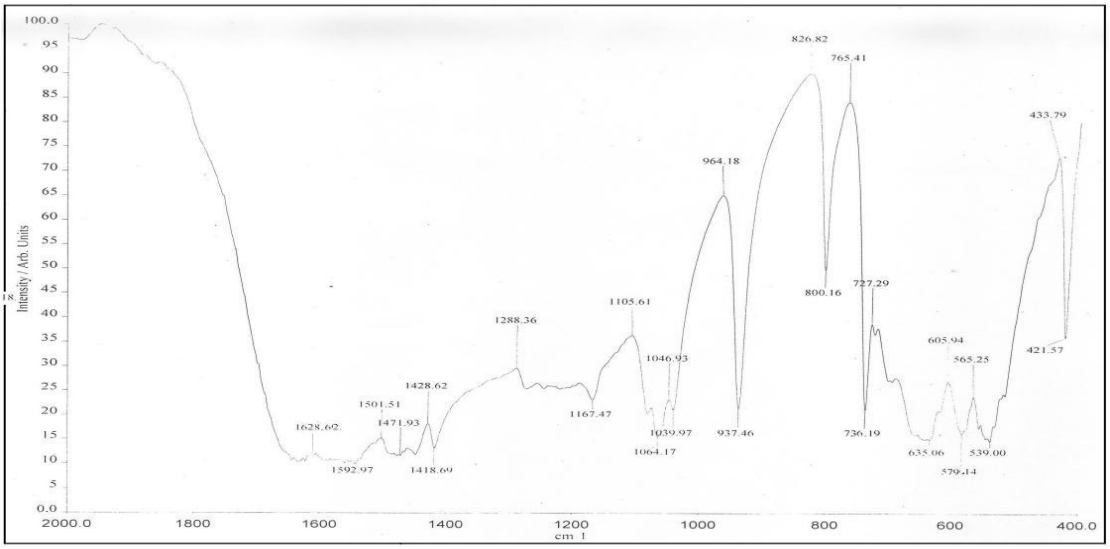

Fig 1: M1 IR spectrum

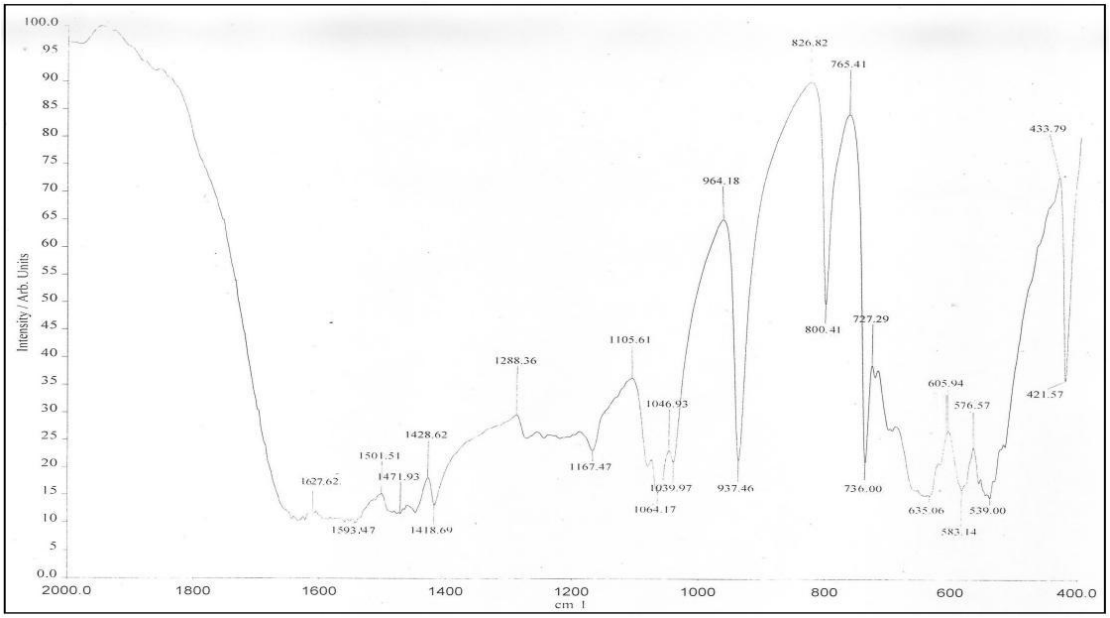

Fig 2: M2 IR spectrum

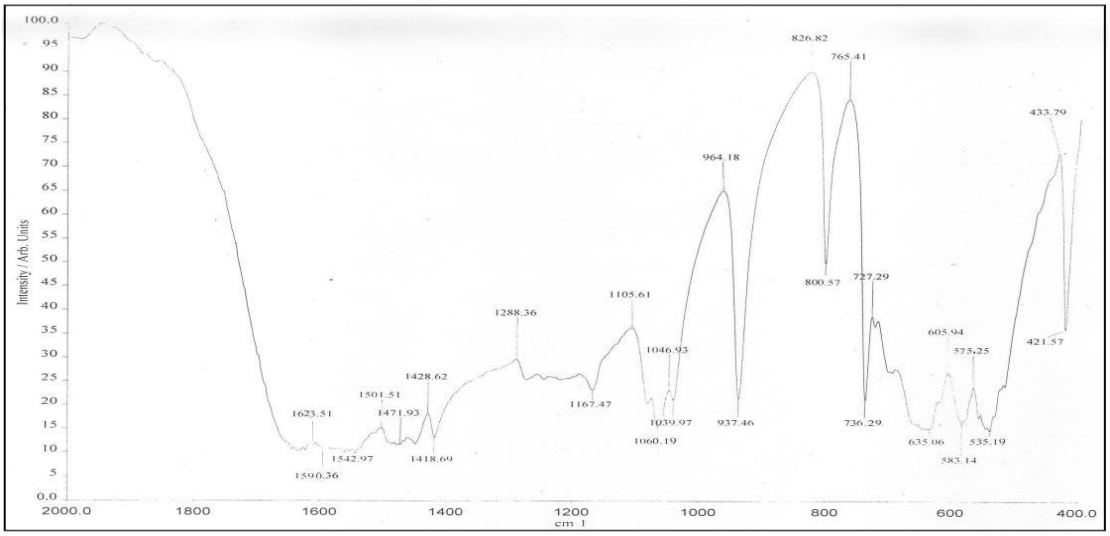

Fig 3: M3 IR spectrum

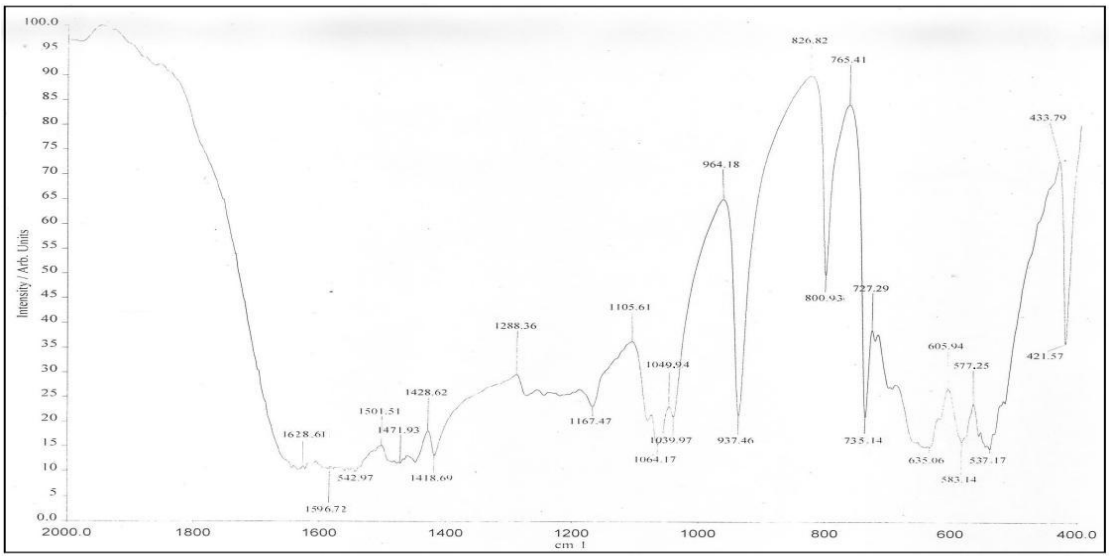

Fig 4: M4 IR spectrum

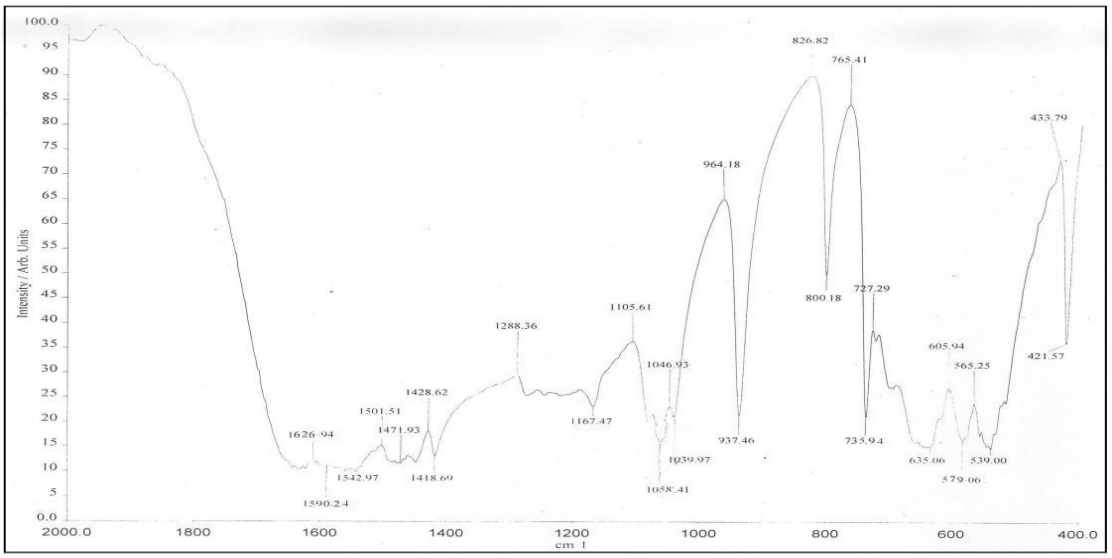

Fig 5: M5 IR spectrum

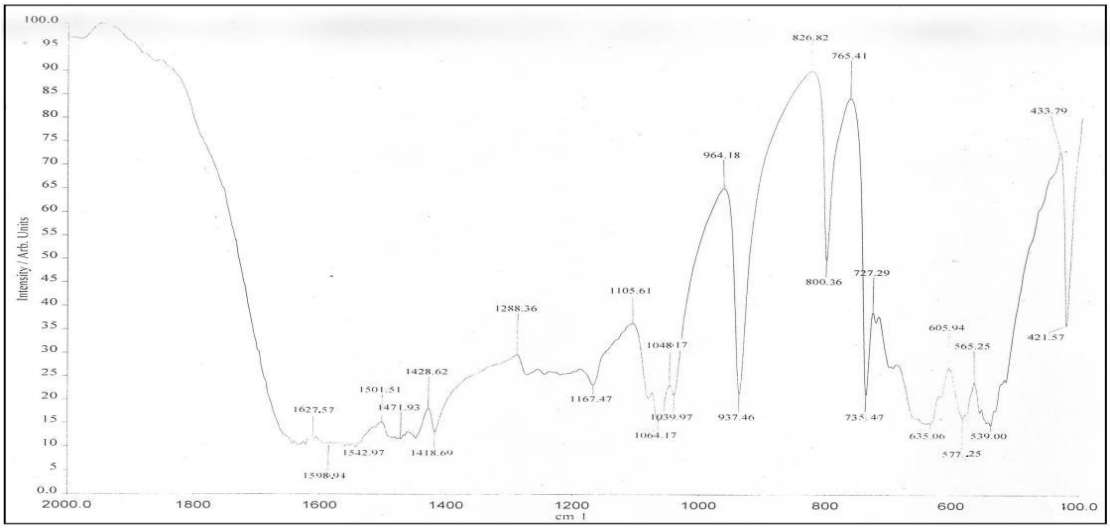

Fig 6: M6 IR spectrum

Weight variation - For 20 tablets of Metformin, the mean and range of % deviation for each sample were calculated. As per IP guidelines, the weight variation was then calculated as Mean ± 5% of the mean. The sample passes if, either no tablet falls outside the limit or if 2 tablets fall outside the limit but are not be outside the double of the limit. All samples were found to be within the prescribed range and hence all samples passed the test. The data of weight variation is given in Table3.

Table 3: Average weight and % deviation

Brand Code	Average weight (mg)	Range of % weight variation (n=20)	Limit (± 5%) (mg)	Result
M1	528.988	-2.56 to 1.95	502.54 - 555.42	Pass
M2	553.343	-2.22 to 2.81	525.68 - 581.00	Pass
M3	590.720	-1.29 to 2.77	561.19 - 620.25	Pass
M4	559.142	-15.12 to 4.73	531.19 - 587.09	Pass
M5	704.342	-1.30 to 1.20	669.13 - 739.55	Pass
M6	695.869	-2.54 to 1.39	661.07 - 730.65	Pass

Hardness- Being an unofficial test, official monograph does not prescribe any limit for hardness. The general limits for hardness is given as 4-10 Kg/cm^2.The hardness of samples are given below in Table 4.

Table 4: Hardness of samples

Brand Code	Hardness (Kg/cm^2)	Limit	Result
M1	5.70	4-10 kg/cm^2	Pass
M2	6.55		Pass
M3	6.49		Pass
M4	6.96		Pass
M5	6.58		Pass
M6	6.73		Pass

Friability-Friability is a non-official test. Friability loss of 20 tablets should not be more than 1% according to the Pharmacopoeia. Friability data of Metformin tablets is given in Table 5.

Table 5: Friability

Weights (in mg)	Brand code					
	M1	M2	M3	M4	M5	M6
Initial weight (W_0)	10579.77	11066.86	11814.40	11182.83	14086.83	13917.38
Weight after revolution (W)	10576.53	11063.34	11807.11	11176.51	14083.21	13910.90

% loss (%F)	0.04	0.04	0.07	0.06	0.03	0.05
Result	Pass	Pass	Pass	Pass	Pass	Pass

Disintegration time - The time taken to break each tablet into small parts and pass through the mesh is the first step towards dissolution. Disintegration time of all six samples was under prescribed limit mentioned in Indian pharmacopoeia (IP). Disintegration time for uncoated tablet should not be more than 15 min. Disintegration time of all six samples is given in Table 6.

Table 6: Disintegration time

Brand Code	Disintegration time (min-sec)	Limit	Result
M1	4.32	Not More Than 15 minutes	Pass
M2	5.07		Pass
M3	5.04		Pass
M4	6.47		Pass
M5	6.00		Pass
M6	6.15		Pass

Dissolution test- Dissolution test is used to determine the extent and rate of drug release. Limit of dissolution test given in pharmacopoeia for Metformin is not less than 75% in 45 minutes. All samples have shown % content dissolved above the prescribed limit. Branded sample M2 showed maximum release of 99.58 % while generic sample M4 showed least release of 84.87%.
It was also observed that though government generic supply of Metformin tablets showed less or equivalent release still it was above the permissible limit given in IP thus proving their good quality. 6 tablets of each sample were used in dissolution test. Average percentage and range observed of content dissolved after 45 min is given in Table 7.

Table 7: Dissolution profile

Brand Code	% label claim dissolved	Range observed (%)	Limit	Result
M1	99.43	98.61 - 100.21	Not Less Than 75 % in 45 minutes	Pass
M2	99.58	98.33 - 101.13		Pass
M3	98.67	98.13 - 99.03		Pass
M4	84.87	84.58 - 85.10		Pass
M5	86.60	85.68 - 87.58		Pass
M6	98.60	98.48 - 98.79		Pass

Assay - This test was an attempt to calculate the label claim specified on the label and quantity difference among the samples. Intraday and Interday (Intra-instrument and Inter-instrument) study was performed and variations were calculated. As per IP, the labeled content should be in the range of 95 - 105 %. All 6 brands are within the range prescribed in the monograph proving their efficacy. Government generic sample M5 showed maximum content 102.61 %. While branded sample M3 showed least content of 98.69%. No notable difference was found in between generic Vs generic, branded Vs branded, generic Vs branded formulations. Intraday as well as interday results comply with the limits set in IP, similarly intra-instrumental and inter-instrumental variation was not found. The percentage of drug content was shown in Table 8.

Table 8: Percentage of drug content

Brand code	Content %				Limit	Result
	Main	Intraday	Interday	Inter-instrument		
M1	102.56	102.55	102.54	102.60	95 - 105 %	Pass
M2	102.11	102.07	102.07	102.13		Pass
M3	98.73	98.69	98.67	98.74		Pass
M4	100.96	100.89	100.89	100.94		Pass
M5	102.58	102.56	102.52	102.61		Pass
M6	100.91	100.99	100.88	100.93		Pass

Related substance - Detection of related substances is necessary to know about the percentage of impurities present in any sample. According to IP, known impurity (dicyandiamide) should not be more than 0.02 % and single highest unknown impurity should not be more than 0.1 %. known impurity (dicyandiamide) was not detected in any sample. Unknown impurities were detected but single highest unknown of all samples were within the prescribed limit. HPLC chromatograms depicting the results are shown below. Percentage of impurities are given in Table 9.

Table 9: Percentage of known and single highest unknown

Brand code	Impurity		Limit	Result
	Dicyandiamide (%)	Single highest unknown (%)		
M1	Not detected	0.008	Dicyandiamide = NMT- 0.02% and Single highest unknown = NMT 0.1%	Pass
3M2	Not detected	0.002		Pass
M3	Not detected	0.009		Pass
M4	Not detected	0.036		Pass
M5	Not detected	0.005		Pass
M6	Not detected	0.044		Pass

*NMT = Not More Than

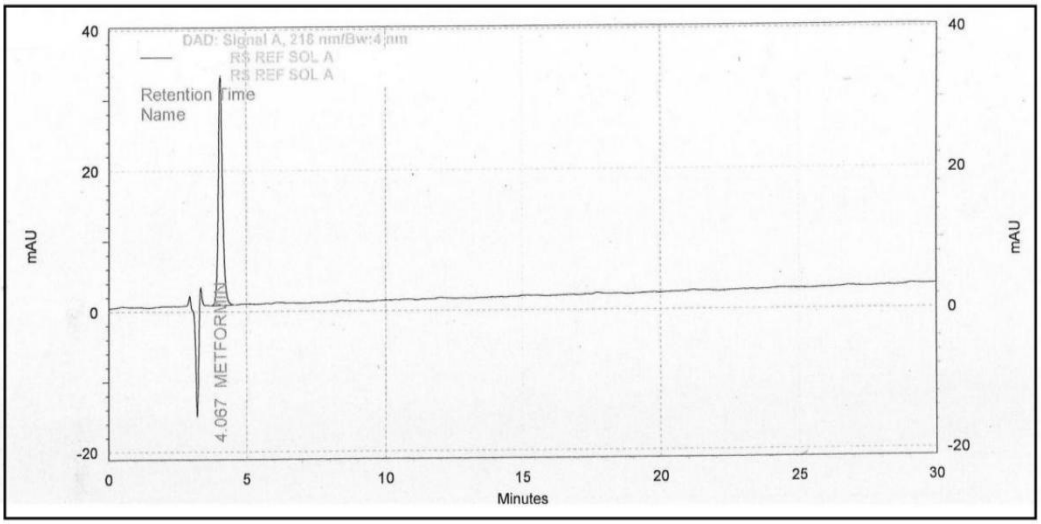

Fig 7: Reference solution A M1

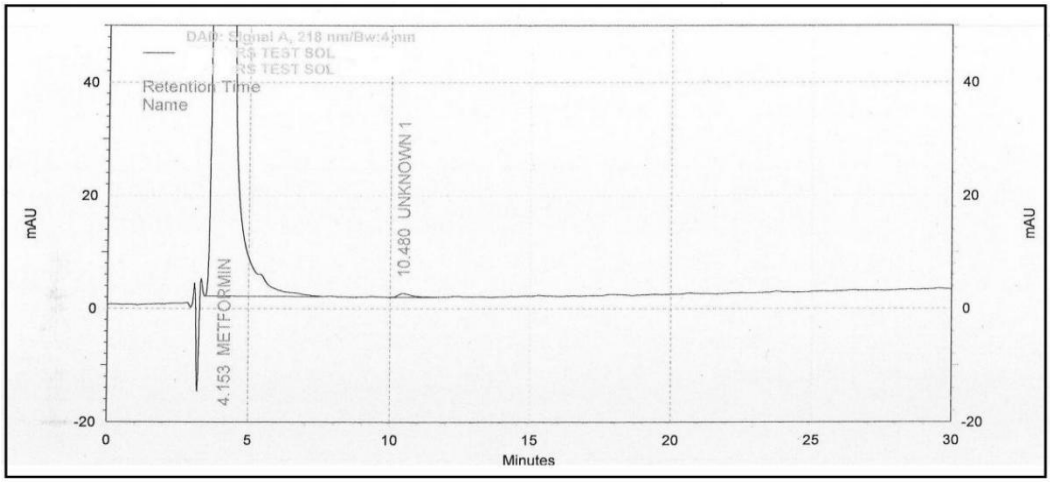

Fig 8: Related substance Test M1

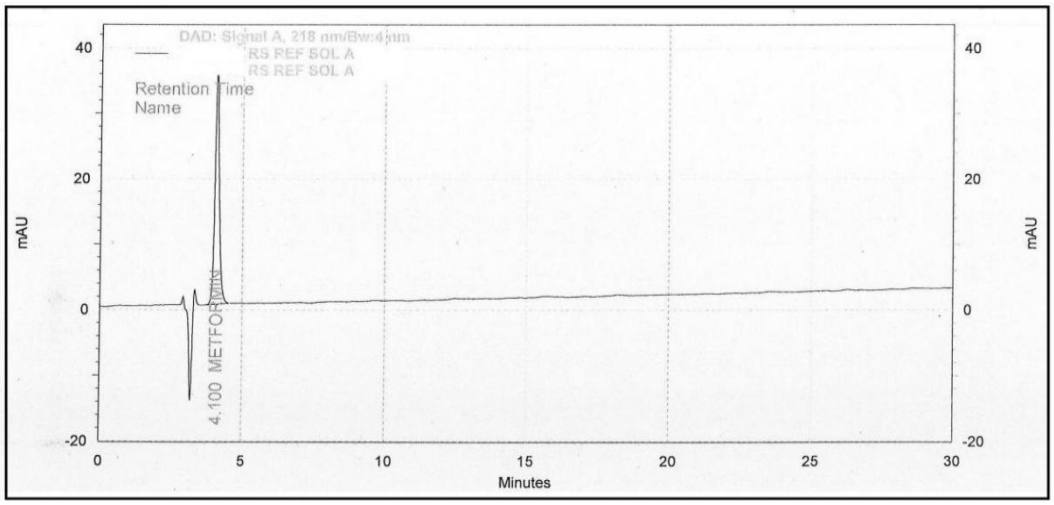

Fig 9: Reference solution A M2

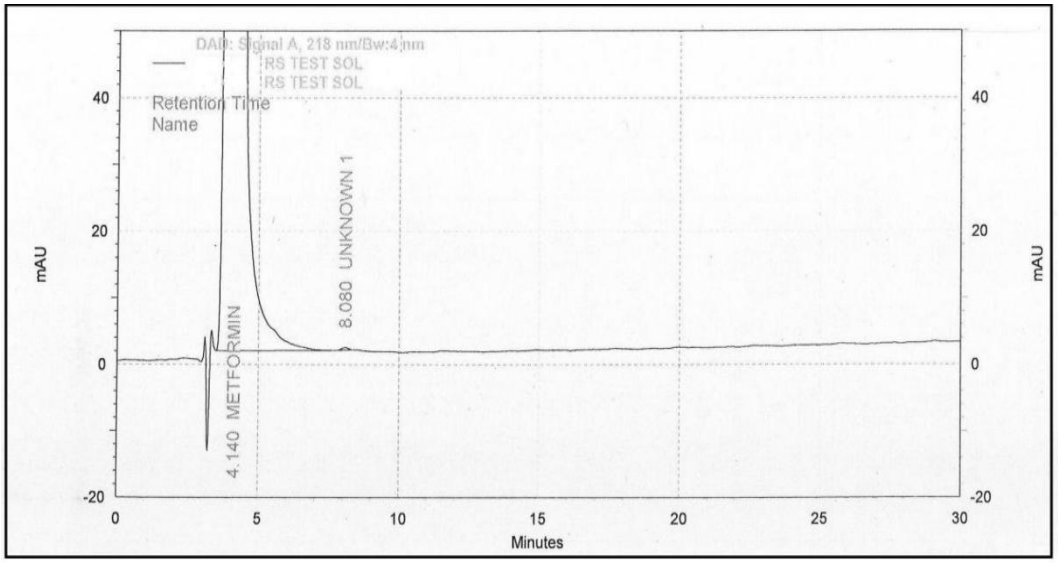

Fig10: Related substance Test M2

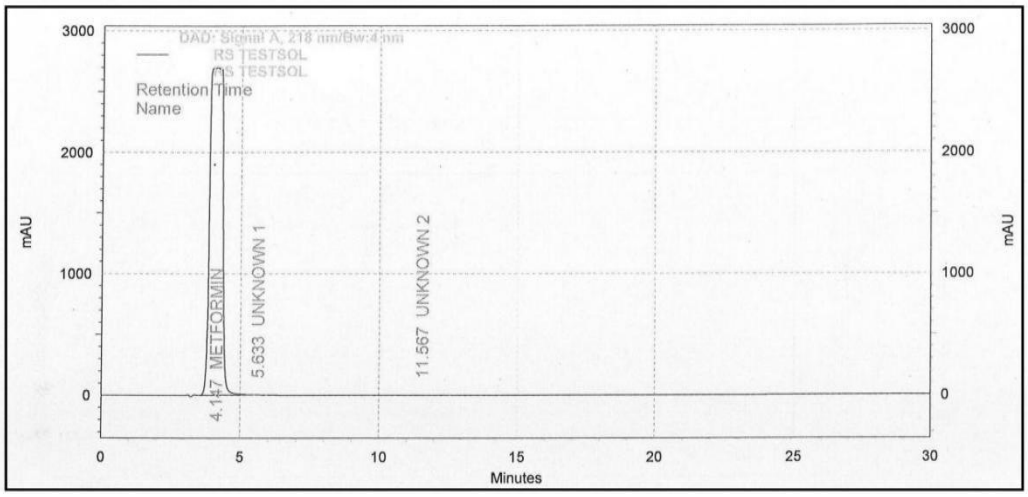

Fig 11: Reference solution A M3

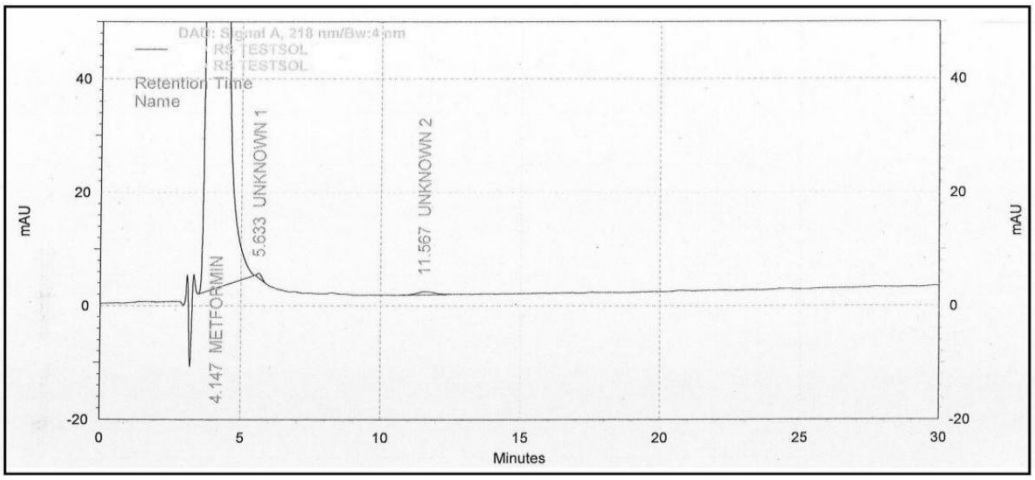

Fig 12: Related substance Test M3

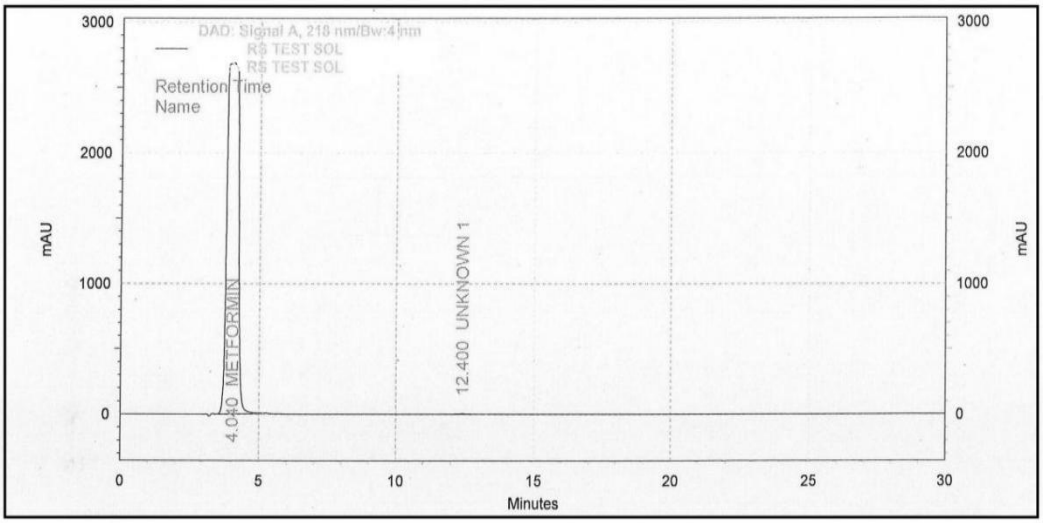

Fig 13: Reference Solution A M4

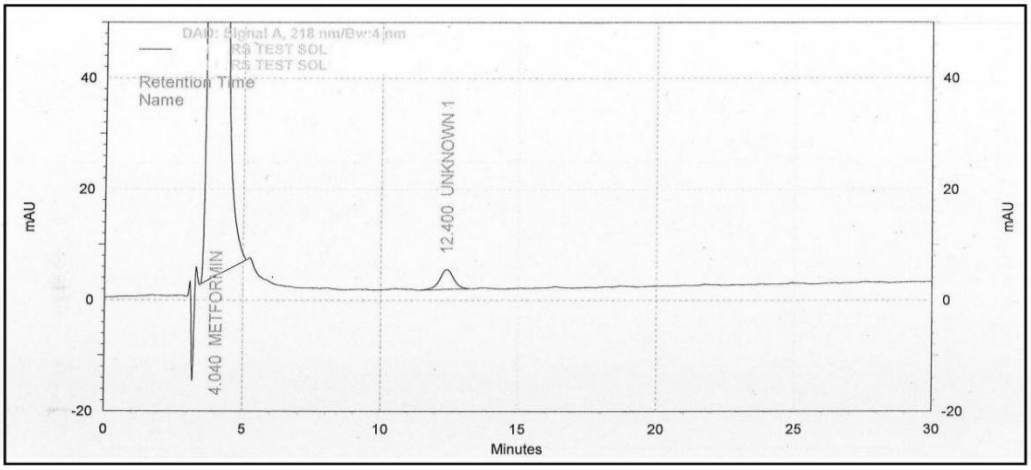

Fig 14: Related substance Test M4

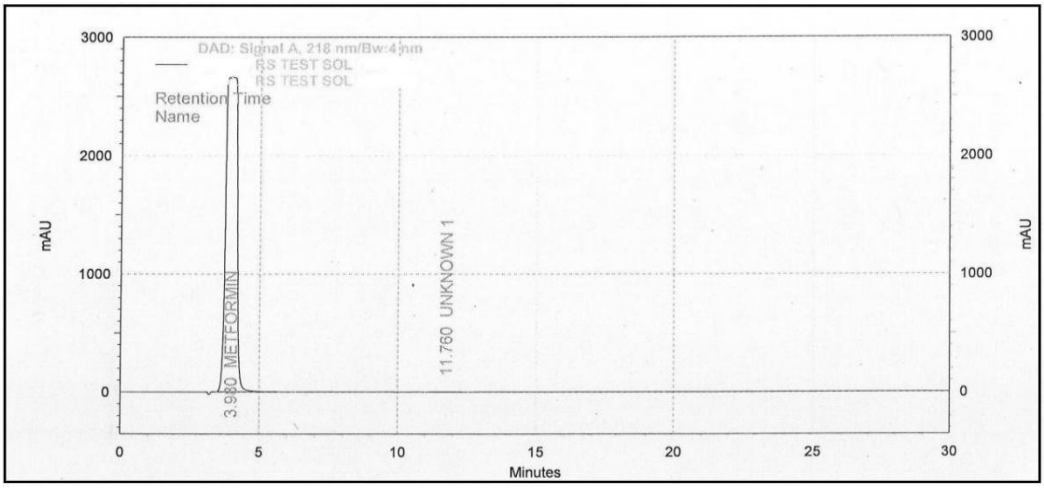

Fig 15: Reference solution A M5

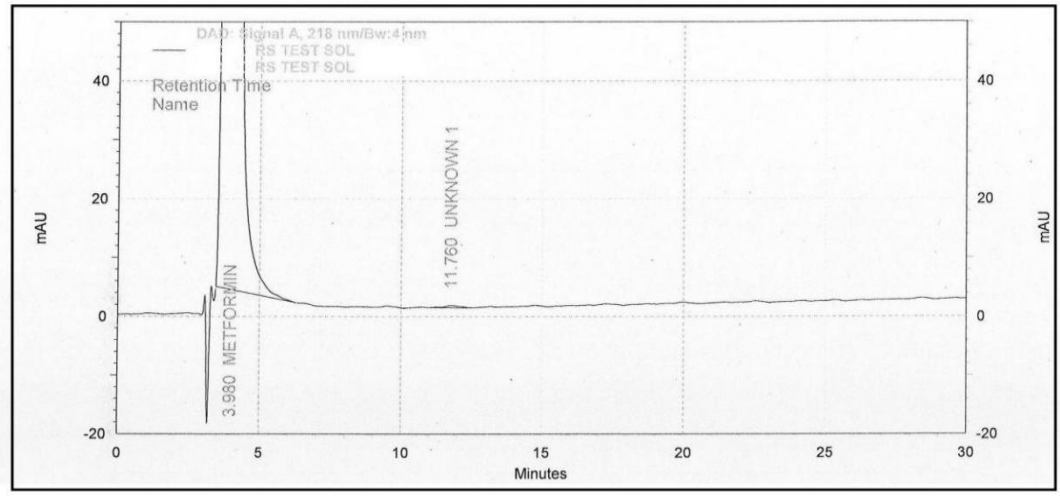

Fig 16: Related substance Test M5

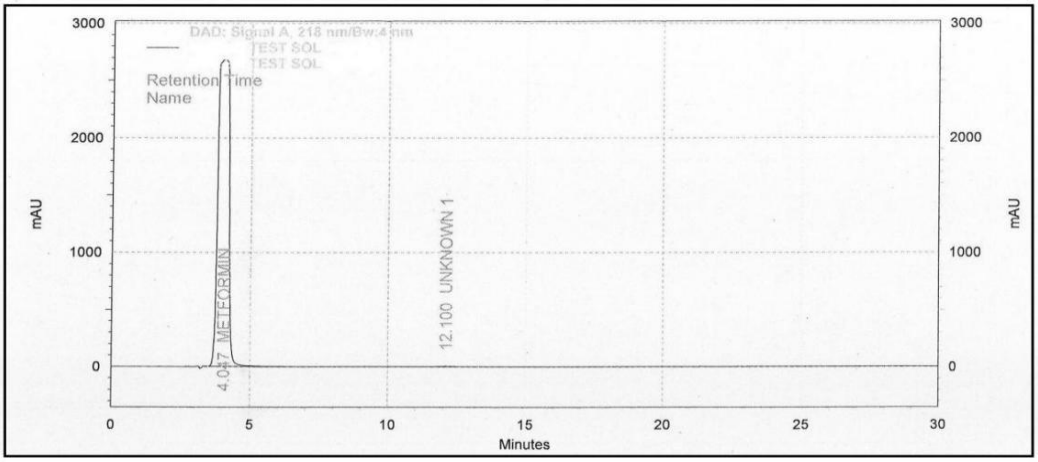

Fig 17: Reference solution A M6

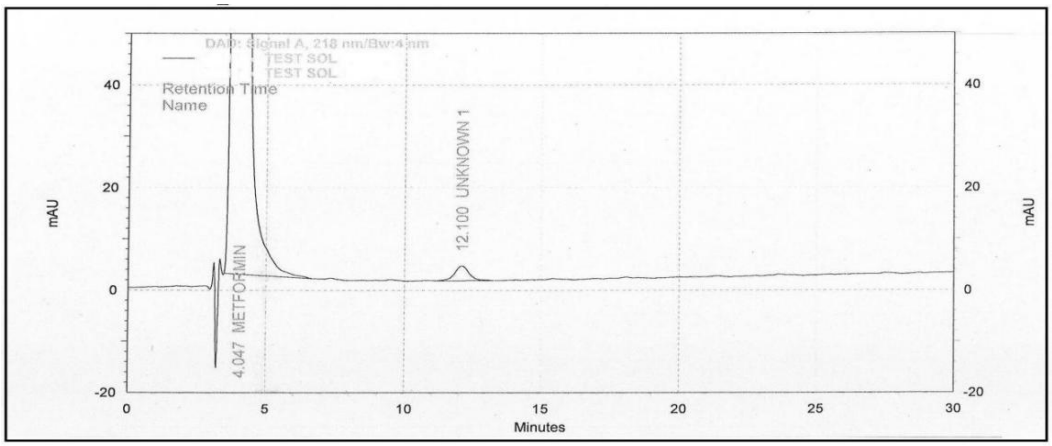

Fig 18: Related substance Test M6

IV. DISCUSSION

Ashenef et al. from Addis Ababa in 2019 [5] and Afifi et al. from Saudi Arabia in 2012 [6] performed comparative evaluation among six marketed samples of Metformin. These both studies concluded that all samples met the USP specifications except one brand. One brand failed in dissolution in both studies which can be correlated to lower bioavailability. Akinleye et al. from Nigeria in 2012 [7] performed the comparative study using eight brands of Metformin where only four brands passed all the tests and can be interchanged with innovator product. Sachan et al. from India in 2016 [8], Herath et al. from Sri Lanka in 2015 [9], Prithi et al. from Bangladesh in 2018 [10] and Labu et al. from Bangladesh in 2013[11] tested different brands of Metformin. These four studies concluded that all samples met the specified limits set in pharmacopoeia and no quality issue was found in reference of standard product. All samples were suitable to interchange with one another. The uniqueness of our present study is the involvement of generic drugs available under government supply and impurity determination. Impurity testing has not been done before in any study. Our present study is in congruence with the study of Sachan et al., Herath et al., Labu et al. and Prithi et al. as no sample in our study was out of the specified limits prescribed in Indian Pharmacopoeia 2018. No variation was found among generics Vs branded, generic Vs generic or branded Vs branded. No known impurity was detected in any sample and unknown impurities were within the limit. All samples are found to be pharmaceutically equivalent through assay and dissolution testing. This study had a limitation that *in-vitro* dissolution test was extrapolated to get insight in *in-vivo* bioavailability instead of actual *in-vivo* bioavailability data.

V. CONCLUSION

In the present study, it was concluded that all six samples of Metformin complied with the specifications given in Indian Pharmacopoeia 2018 in terms of hardness, friability, weight variation, disintegration and dissolution, label claim (assay) and related substances. Dissolution profile and assay confirmed that all samples are pharmaceutically equivalent and suitable for interchangeability. Comparison of results of branded drugs with generics point out to equivalence and proves that government supply generics are effective and of good quality. Results concluded that no variation was found amongst branded samples. Medical practitioners can prescribe the more economic brand of Cipla company as compared to other brands. During comparison of generics, all samples were equivalent proving the robustness of government system. No variation in results of assay was observed in terms of intraday, interday and inter-instrument. Both branded and generic samples were equally potent. All samples are safe to use in clinical practice.

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