World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

QUALIFICATION FOR BIOWAIVER AND BIOEQUIVALENCE STUDY ON SEVEN BRANDS OF METFORMIN HYDROCHLORIDE TABLETS EXISTING IN SUDAN MEDICINE MARKET

Dr. Abdrhman Mahmoud Gamil*[1] and Dr. Yuvaraj D. Mandavkar[2]

[1]Associate Professor of Pharmaceutics, Al-Neelain University, Sudan.
[2]M.Pharm, PhD, Executive Research and Development Manager, Sudan.

Corresponding Author: Dr. Abdrhman Mahmoud Gamil
Associate Professor of Pharmaceutics, Al-Neelain University, Sudan.

Article Received on 28/07/2020 **Article Revised on 18/08/2020** **Article Accepted on 08/09/2020**

ABSTRACT

Metformin HCl is the drug of choice for obese diabetic patients. It can be used alone with diet control or in combination with other antidiabetic remedies. It is one of the two antidiabetic medicines listed in the WHO list of essential medicines. Being classified in class 3 in the biopharmaceutical classification system, it is highly soluble with low permeability. Many Metformin brands exist in the Sudan Medicine market, some of them are locally manufactured and others are imported including the originator brand, Glucophage, of Merck Serono – France. To evaluate these brands in respect to the similarity with the originator and qualification for biowaiver study, seven brands were subjected to dissolution testing to study their dissolution profile using apparatus type 2 in a simulating intestinal medium using spectrophotometric method at 233 nm. One imported and two locally manufactured brands were found to show adequate similarity of the dissolution profile with the originator but they were disqualified for biowaiver study for class 3 of the BCS as they failed to exhibit 85% dissolution in 15 mins. The other two locally manufactured brands were dissimilar to the originator but they showed more than 85% dissolution in 15 mins which qualifies them for biowaiver study for class 3. One imported brand being dissimilar and disqualified for biowaiver study. It is recommended that DRA should set regulations for interchangeability and international harmonized specification for similarity, qualification for biowaiver study and the pharmacopeia specifications will be valuable.

KEYWORDS: Metformin, bioequivalence study, Biowaiver, Metformin in Sudan medicine Market.

INTRODUCTION

Metformin HCl is the only Biguanide drug for the treatment of diabetes. Chemically Metformin HCl is $C_4H_{11}N_5$.HCl is imidodicarbonimidic diamide, N, N-dimethyl monohydrochloride.

Metformin is used as an antidiabetic agent alone or in combination therapy because it acts by decreasing the gluconeogenesis and increasing the peripheral utilization of glucose. It acts only in presence of insulin so it is indicated for type 2 diabetes mellitus. It is the first choice for obese patients in whom diet control is not effective and when diabetes is not adequately controlled by sulphonyl urea products, thus combination is used. It is also used in polycystic ovary syndrome, weight reduction, normalization of menstrual cycle and hirsutism.[1] Metformin 500 mg and gliclazide CR 30, 60 and 80 mg are the two oral hypoglycemic drugs being listed in WHO essential medicinal list[2]. Metformin is freely soluble in water, pKa is 12.4. The pH of 1% aqueous solution is 6.68 and it was classified as Class 3 drug in BCS as high solubility, low permeability.[3]

The USFDA guidance for industry stated that the drug absorption from solid dosage form after oral administration depends on the release of drug from the tablets, the dissolution of drug in GIT fluid and the permeability across the GIT membrane. Because of the critical steps of release and dissolution, the *in vitro* dissolution may be relevant to the prediction of the *in vivo* performance. The approaches currently used as per guidance, is dissolution *in-vitro* test which is used to assess and ensure the quality and performance of product[4]. The dissolution profile is used for comparison of products for sameness, to waive bioequivalence, the office of generic drugs recommended dissolution profile every 15 mins using reference product and 12 units each. The dissolution profile maybe considered similar by virtue of the overall profile similarity and the similarity at each dissolution points. The similarity may be determined by model independent or model dependent

methods. The similarity model independent method use f_1 which is the difference factor that calculates the percent (%) between the two curves at each time point and is the measurement of the relative error between the two curves.[4]

$$f_1 = \{[\Sigma \mid R_t - T_t \mid] / \Sigma R_t\} \times 100 = 15.42$$

R_t= is the dissolution value % of the reference at time t, T_t= is the dissolution value % of the test at time t

The similarity factor f_2 is a logarithmic reciprocal square root transformation of the sum of squared error and is the measurement of the similarity percent (%) dissolution between the two curves.[4]

$$f_2 = 50 \log \left\{ \left[1 + \frac{1}{n} \sum_{n=1}^{n} (R_t - T_t)^2 \right]^{-0.5} * 100 \right\}$$

The similarity is used to compare brand of originator to the generic products. FDA defines generics as copies of brand –name drugs and are the same as those brand name drugs in dosage form, safety, strength, route of administration, quality, performance characteristic and intended use. This leads to interchangeability which had been adopted by European countries, USA, Korea and Australia.

Bioequivalence is the absence of significant differences of generic from the originator or where the extent of absorption does not show a significant differences and any difference in rate is intentional or not medically significant.

Metformin HCl could be absorbed from the whole intestine mainly at the duodenum. This concentration dependent permeability indicates that metformin is transported by both passive and active carrier-mediated saturable mechanism. The fraction absorbed is 74-80% along human intestine.[5] The difference in absorption in to the body between generic and originator is 3.5% as found by Irland study and it is comparable to different batches of originator drug.[6] This can generally occur between different batches of the same product. Metformin C_{max} is reached in 2.5 hours (t_{max}) and absolute bioavailability is 50-60%, where 20-30% goes into the faeces,[7] Steady plasma concentrations are reached in 24-48 hours and is generally less than 1 μg/ml.

There are many brands of metformin exists in the Sudan medicine market. The objective of this research is to determine the similarity of six generic brands; imported and locally manufactured in Sudan, with the originator brand of metformin HCl 500 mg tablets by studying the dissolution profile in simulated intestinal fluid (SIF). Specifically to determine where the pharmaceutical product stands based on the quality of the products.

MATERIAL AND METHODS

Materials

The product R -O is the reference product the originator brand, Glucophage 500 mg.
A-SD Metformin 500mg a locally manufactured product
B-SD Metformin 500mg a locally manufactured product
C-SD Metformin 500mg a locally manufactured product
D-SD Metformin 500mg a locally manufactured product
E-IM Metformin 500mg imported from Asia
F-IM Metformin 850mg imported from Arab country

Chemicals: Potassium dihydrogen orthophosphate, 1M Sodium Hydroxide, distilled water, absolute alcohol was used are analytical grade.

Equipment and Apparatus

Spectrophotometer UV-1700 Pharma speck, (Shimadzu); Disintegration apparatus (Electrolab); Dissolution tester ETC-1LX (Electrolab).

Test for linearity and range

Seven points calibration graphs were plotted, the linear curve was plotted against absorption verses drug concentration. Concentration range from 2.2µg/mL, 3.3 µg/mL, 4.4 µg/mL, 5.5 µg/mL, 6.6 µg/mL, 7.7 µg/mL, and 8.8 µg/mL it covers concentration from 40% to 160%. Three injections were performed and the mean of absorbance was obtained for each concentration. The standard deviation and the relative standard deviation were obtained and the slope of the curve and the coefficient R.

Dissolution[7]

Dissolution profile was performed using a medium of 900 mL of 0.68% w/v solution of potassium dihydrogen orthophosphate of adjusted to pH 6.8 by the addition of 1M sodium hydroxide and rotating the basket at 100 revolutions per minute. Withdraw a 10 mL sample at 10 min, 20 min, 30 min, 45 min and 60 min of the medium and at the same time 10 ml of medium was replaced. Filter, dilute 10 mL of the filtrate to 100 mL with water and dilute 10 mL of the resulting solution to 100 mL with water. Measured the absorbance of a layer of suitable thickness of the filtered sample, at the maximum at 233 nm. Calculate the total content of metformin hydrochloride, $C_4H_{11}N_5$,HC1, in the medium taking 806 as the value of A (1%, 1 cm) at the maximum at 233 nm.

(Abs/806) X (1/100) X (900/1) X 100/10) X 1000 X (100/500)

Similarity factor[4]

Determine the dissolution profile of two products 12 units each at each time interval. Use the mean for each point for each curve at each time interval and calculate f_1 and f_2. Difference factor f_1 should be close to zero and Similarity factor f_2 should be close to 100. Generally f_1 up to 15 and f_2 greater than 50 ensure sameness or equivalence[4]. This method is suitable when there are more dissolution time points are available. Time 10, 20,

30, 45 and 60 min are taken. At time 15 mins, the coefficient of variation % should not be more than 20% and at each time point should not be more than 10%.

Coefficient of Variation = (Standard Deviation / Mean) * 100.

In symbols: $CV = (SD/\bar{X}) * 100$

RESULTS

Test For linearity

Table (1): The test for linearity and range shows the results as tabulated in.

No. of sample	Conc. 40%	Conc. 60%	Conc. 80%	Conc. 100%	Conc. 120%	Conc. 140%	Conc. 160%
1	0.194	0.285	0.376	0.473	0.564	0.656	0.746
2	0.194	0.285	0.376	0.474	0.564	0.656	0.746
3	0.194	0.285	0.376	0.474	0.564	0.656	0.746
Mean	0.194	0.285	0.376	0.474	0.564	0.656	0.746
SD	00	00	00	00	00	00	00
RSD	00	00	00	0.12	00	00	00

The absorbance versus concentration is tabulated in table (2), figure 1.

Table (2): UV Absorbance versus concentration.

No. of inj	Conc. in ug/ml	absorbance
1	2.2	0.194
2	3.3	0.285
3	4.4	0.376
4	5.5	0.474
5	6.6	0.564
6	7.7	0.656
7	8.8	0.746
	slope	**0.083961**
	intercept	**0.008881**
	CORR.Coeff	**0.9999**

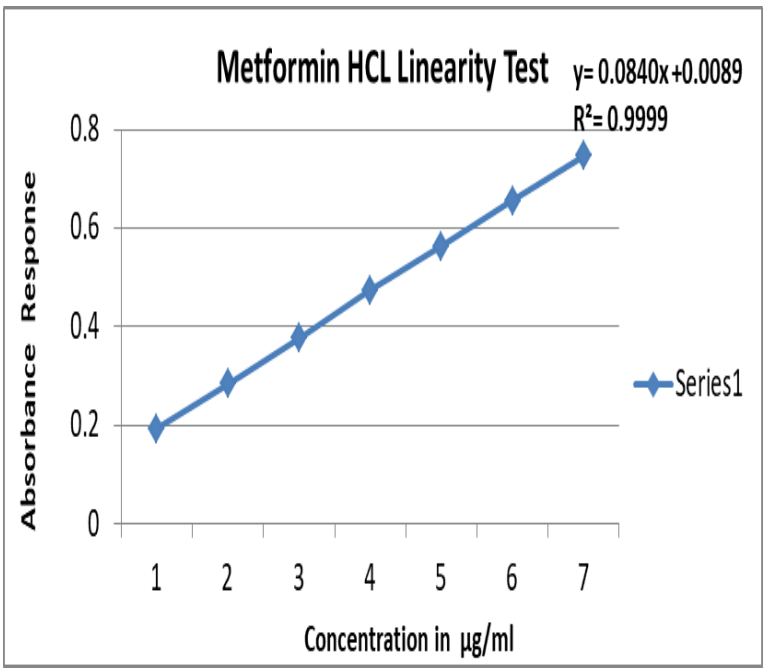

Figure 1: Linearity Graph for Metformin HCl.

12 tablets from each product were subjected to dissolution test, for time intervals 10, 20, 30, 45 and 60 mins. The absorption was measured at each time for 12 tablets. Then mean for every time for each product brand was obtained and the standard deviation was determined. Then the percentage release for each sample was calculated taking factor A as the value of 1% 806 as presented in table (3).

Table (3): Mean Absorbance, Percentage Release of Metformin HCl tablets.

	Time	10 min	20 min	30 min	45 min	60 min
R –O 500 mg tablets	**Mean abs**	0.191833	0.297083	0.365417	0.411167	0.42875
	STD-P	0.00783	0.022269	0.025529	0.00978	0.011656
	Result %	42.84	66.35	81.61	92.82	95.75%
	CoV	4.081557	7.495887	6.986342	2.378479	2.718519
A –SD 500 mg tablets	**Mean abs**	0.431667	0.42225	0.410333	0.416833	0.4265
	STD-P	0.009113	0.005356	0.003749	0.004723	0.011913
	Result %	96.4	94.3	91.64	93.09	92.25
	CoV	2.111231	1.26846	0.913665	1.133037	2.793172
B –SD 500 mg tablets	**Mean abs**	0.126583	0.190083	0.376917	0.39975	0.421917
	STD-P	0.015893	0.025867	0.02129	0.019245	0.013041
	Result %	28.12	42.12	84.17	89.27	94.22
	CoV	13.60798	5.648332	4.814156	3.090974	12.55509
C –SD 500 mg tablets	**Mean abs**	0.242417	0.384917	0.39375	0.419083	0.432333
	STD-P	0.014297	0.025336	0.014777	0.00877	0.026145
	Result %	54.14	85.96	87.93	93.59	94.69
	CoV	5.897776	6.582188	3.752841	2.092619	6.047393
D –SD 500 mg tablets	**Mean abs**	0.18	0.332583	0.38575	0.396167	#DIV/0!
	STD-P	0.017607	0.007794	0.011069	0.006866	#DIV/0!
	Result %	40.2	74.27	86.15	88.47	95.43
	CoV	9.781565	2.343408	2.869452	1.733053	
E –IM 500 mg tablets	**Mean abs**	0.22275	0.359167	0.40625	0.411667	0.427667
	STD-P	0.004585	0.012075	0.001588	0.00421	0.01519
	Result %	49.75	80.21	90.73	91.94	95.51
	CoV	2.058293	3.361948	0.390822	1.022618	3.551725
F-IM 850 mg tablets	**Mean abs**	0.21425	0.389583	0.502	0.65325	#DIV/0!
	STD-P	0.023819	0.040134	0.058184	0.043791	#DIV/0!
	Result %	28.15	51.18	65.95	85.82	96.7
	CoV	11.11748	10.30179	11.59036	6.703622	

CoV: Coefficients of variation

Table (4): Dissolution profile of the seven Products.

Time	R –O	A -SD	B -SD	C -SD	D -SD	E -IM	F –IM
0 min	00	00	00	00	00	00	00
10 min	42.84	96.4	28.12	54.14	40.2	49.75	28.15
20 min	66.35	94.3	42.12	85.96	74.27	80.21	51.18
30 min	81.61	91.64	84.17	87.93	86.15	90.73	65.95
45 min	92.82	93.09	89.27	93.59	88.47	91.94	85.82
60 min	95.75	92.25	94.22	94.96	95.43	91.32	96.5

Results are in mean of 12, in percentage release

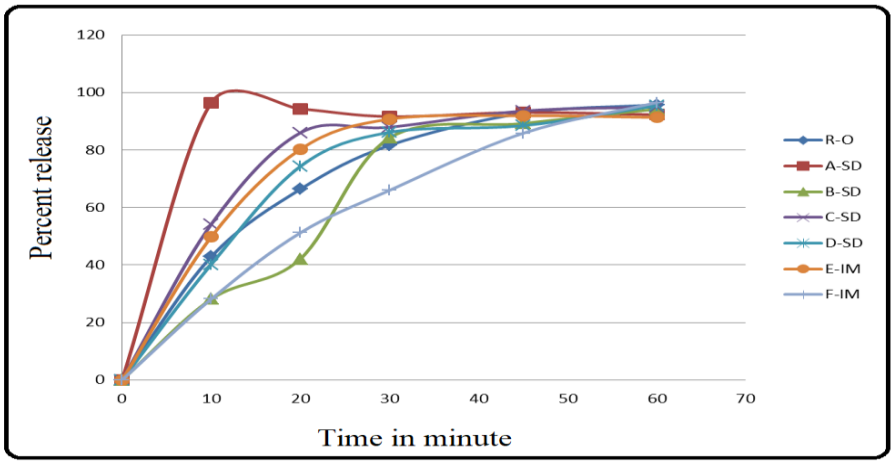

Figure 2: Dissolution Profile Curves of Metformin HCl Tablets, 7 Brands.

Table 5: Average weight of tablets and disintegration time.

Product	R - O	A -SD	B-SD	C -SD	D -SD	E -IM	F-IM/850
Average weight (mg)	530	585	550	650	530	600	925
Disintegration Time (mins)	14.13	3.30	7.30	9.0	9.30	10.15	13.0

DISCUSSION

Pharmacopoeial Specifications

Referring to the BP and USP acceptance criteria for dissolution of Metformin HCL tablets, using apparatus 2, USP specifies that 80% of Metformin should go onto dissolution at 30 mins for 500 mg and 75% for 850 mg. however, BP specifies a common method using apparatus 1 which pointed for 70% of the drug should go into dissolution within 45 mins.

Table (6): USP and BP Acceptance Criteria for Dissolution Metformin HCL Tablets.[8,9]

	USP 39				BP2016
	Common method for all strength		500 mg	850 and 1000mg	Common method for all strength
Test	1	3	2	2	1
Apparatus	1	1	2	2	1
rpm	100	100	50	75	100
Volume	1000	1000	1000	1000	900
Time	45	60	30	30	45
Acceptance	70%(Q)	70%(Q)	80% (Q)	75%(Q)	70%(Q)
Method	UV	HPLC	UV	UV	UV

According to USP specifications, all products comply except F-IM which shows 65.95% dissolution at 30 mins but it complies BP specification as it shows 85.82% dissolution at 45 mins.

Similarity to Originator

The US-FDA stated that on 12 tablets, taking the mean of each dissolution point, when f_1 difference factor is less than 15 and f_2 similarity factor greater than 50 ensures sameness or equivalence[4]. At time 15 mins the Coefficient of Variation % should not be more than 20% and at each time point should not be more than 10%. The use of f_2 to determine the similarity is recommended by FDA, EMA and WHO.

Taking product R-O Glucophage being developed by Merck Serono – France as a reference and comparing the other generic products with its dissolution profile, the similarity factors f_1 and f_2 can be calculated and presented in table (7)

Table (7): difference factor f_1 and Similarity factor f_2, n=5.

Sr. No.	Product	f_1	f_2
1	Glucophage	00	100
2	A –SD	25	28
3	B –SD	9	52
4	C –SD	11	49
5	D –SD	5	67
6	E –IM	8	55
7	F –IM	14	46

Although product A –SD and Product C –SD comply the USP dissolution test, they failed the similarity with the originator. Product F –SD failed the USP specification for dissolution and also shows a different dissolution profile which can be attributed to the difference in strength. Product A-SD showed a more fast dissolution than the originator at time 10 mins which is the cause of dissimilarity; however this may be attributed to the fast disintegration time 3.30 mins compared to the originator which is 14.13 mins Referring to the weight of tablet as it is a film coated tablets, it is 585 mg while the originator is 530 mg. this indicates that excessive excipient were added most probably due to excessive amount of disintegrant.

Product C –SD complied the USP dissolution acceptance test, however it shows a dissimilar dissolution profile with that of the originator. The source of dissimilarity is the same as that described for product A- SD. Products B- SD and D –SD and E –IM shows a similar dissolution profile as the originator.

Although the originator brand and its similar brands, B-SD, D-SD and E-IM, they were disqualified for biowaiver study of class 3 of the BCS because they didn't complied the requirement of >85% dissolution at 15 mins. Brands A-SD and C-SD inspite of being dissimilar to the originator brand but they fulfilled class 3 of BCS requirements for biowaiver study as they show more than 85% dissolution in 15 mins. This result is in conformance with Olubukolao. Oyetunde et al study at Logos- Nigeria whom they found that four brands and the innovator fail to achieve 85% dissolution in 15 mins and only one locally manufactured product achieved this level.[10] However, dissolution is not the absorption determinant step as the permeability is slow.

From these results, similarity of the generic products with the originator is possible and this is in conformance

with the result described by Ireland study which concluded that the difference in absorption between generic and originator is only 3.5% which may generally occur between different batches of the same product.[6] Also a study took place in Trinidad and Tobago found that only one generic of metformin HCl showed less than 85% release in 15 mins with f_2 value less than 50 based on that, WHO classified in BSC metformin as class 3 which must release 85% of its content in 15 min at 75 rpm. They found that six metformin formulations satisfy the WHO requirements with regard to in vitro dissolution behavior. Two brands of Glucophage inspite they are similar in biopharmaceutical quality but they are statistically different.[11]

A study held by Sheorey *et al* using specifications of 75% release in 45 mins concluded that only one metformin formulation did not fulfill the pharmacopeial requirements.[12] The same result was found by Zakeri-Milani et al whom they consider 80% release in 30 mins as an acceptance limit.[13] In Nigeria a study found that, only 4 brands out of 8 are biopharmaceutically and chemically equivalent.[14] In Ghana, Sougi et al concluded that not all brands are similar in dissolution profile as the originator.[15] In Saudi Arabia a study found that only one brand of Metformin HCl out of 5 is equivalent to Glucophage. In Jordan reached the same result.[16] In Malaysia a study concluded that the marketed tablets indicated that more than 80% of the drug is released within one hour, which complies with the Pharmacopoeial specifications.[17] A study in Egyptian marketed metformin, Hanan *et al.*, studied two reference products for control release and two generic products for immediate and concluded that these two generic products of 1000 mg immediate tablets which are generic brand can be interchangeable with the innovator.[18]

CONCLUSION

In vitro dissolution profile of a generic product and its similarity with the originator is an impressive practice to reflect the in vivo behavior of the drug product. The approval of the interchangeability practice for Metformin HCL should be based on successful similarity study. The seven brands of metformin HCL being studied for their dissolution profile in a simulating intestinal fluid, all brand complies the USP requirement. Four of them are locally manufactured indicating that the Sudanese pharmaceutical industry is of standard quality. On testing the similarity with the originator by calculating the similarity factors f_1 and f_2, it had been found that three products show dissimilarity. Two of them are Sudanese brand but the dissimilarity is that, the dissolution in 10 mins reached up to 96% in one product and more than 85% in 20 mins which are faster than the originator and they are the only brands that satisfy biowaiver study according to class 3 of the BCS. This rapid dissolution rate may be attributed to excessive disintegrant in the formulation.

It is recommended for the DRA to adopt interchangeability regulations based on the dissolution profile of generic products. It is recommended for the decision makers in a country suffering from economic crises to support and encourage production of generic products locally. It is also recommended that the International regulations should be harmonized.

REFERENCES

1. British National Formulary 69, Royal Pharmaceutical Society, September, published by BMJ group and Pharmaceutical Press, 1 Lambeth High Street, London, SE1 7JP, UK., 2015.
2. WHO Model List of Essential Medicines 21st, WHO, https://www.who.int., 2019.
3. Classification of Orally Administered Drugs on the World Health Organization Model List of Essential Medicines According to biopharmaceutics classification syste BCS, Eur. J. Pharma. Biopharm, 2004; 58(2): 265-278.
4. Guidance for Industry, Dissolution testing of immediate release solid dosage forms. US department of Health and Human Services, FDA, Centre for Drug Evaluation and Research, August, Rockville, MD 20857, 1997.
5. Nai-Ning Song, QuanLi and Chang-Xiao Liu, Intestinal Permeation of metformin using single-pass intestinal perfusion in rats, World J Gastroenterol, 2006; 12(25): 4064-70.
6. Suzanne Dunne, Bill Shannon, Colum Dunne and Walter Culen, A reviw of the differences and similarties between generic drugs and their originator, including economic benefits associated with usage of generic medicines, using Ireland as a case study. J Pharmacol Toxicol, 2013; 14(1). www.biomedcentral.com/2050 -6511/14/1.
7. Electronic medicine compendium. www.medicines.org.uk/emc/product 594/smpc.
8. USP, United states Pharmacopeia Convention, United States Pharmacopeia 39 & The National Formulary, 1/5/2016, 12601 Twinbrook Parkway, Rockville, MD 2052.
9. British Pharmacopoeia, 2016, British Pharmacopoeia Commission Office, MHRA, 151 Buckingham Palace Road, London SW1W 9SZ, publish by Stationary Office on behalf of MHRA. http://www.pharmacopoeia.com.
10. Olubukola O, Oyetunde, Fola Tayo, Moshood O Alinleye and Bolajoko A. Aina, In vitro Equivalence studies of generic metformin hydrochloride tablets and propranolol hydrochloride tablets under Biowaiver conditions in Lagos State, Nigeria, Dissolution Technologies, November 2012. Dox.doi.org/10.14227/DT 190412 P51.
11. A.Villarroel Stuart et al, Comparing dissolution profile of metformin formulations in simulated intestinal fluid, Dissolution Technologies, 2/2015. Dx.doi.org/10.14227/DT220115 P17.
12. Sheorey,SD; Hinge, M.A; Smgupta, R; Menon, B.V, Pharmaceutical equivelance between different

brands of metformin HCl tablets. J Pharm. Research, 2012.
13. Zakeri - Melani, Nayeri – Maleki, Ghanbarzadan S, Nemati M, Vaizableh H, In vitro bioequivalence study of 8 brands of metformin tablets in Iran market. J Appl Pharm Sci., 2012; 02(8): 194-7.
14. Olusola AM, Adekoya AI, Olanrewaju OJ. Comparative evaluation of physicochemical properties of some commercially available brands of metformin HCL tablets in Lagos, Nigeria. J Appl Pharm Sci., 2012; 2: 41-4.
15. Sougi A, Ofori Kwakye K, Kuntworbe N, Kipo SL, El Boakye Gyasi M. Evaluation of the physicochemical and in vitro dissolution properties of metformin hydrochloride tablet brands marketed in five cities in Ghana. Br J Pharm Res., 2016; 1: 1-14.
16. Hamdan II, Jaber AKB. Pharmaceutical evaluation of metformin HCl products available in the Jordanian market. Jordan J Pharm Sci., 2010; 3: 1-6.
17. Chandrasekaran Arcot Ravindran, Jia CY, Theng CS, Muniandy T, Muralidharan S, Dhanaraj SA. In vitro studies and evaluation of metformin marketed tablets-Malaysia. J Appl Pharm Sci., 2011; 3: 5-8.
18. Hanan M Hashem, Aya R. Abduo, Nadia Mursi, Lila H. Emara, Comparative in vitro dissolution study on Meformin Hcl products using different dissolution apparatuses, 8/2019, Int J Pharm Pharm Sci., 2019; 11(9): 65 – 72.