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Original Article

APPROACHES ON SURROGATE METHODS FOR *IN VIVO* BIOEQUIVALENCE STUDY OF FORMULATED BILAYER TABLETS OF DOMPERIDONE AND ITOPRIDE

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ABSTRACT

Objective: This study aims to provide a more efficient pathway for generic drug approval while maintaining the same level of therapeutic equivalence and safety as the reference product. This was based on the equivalence of *in vitro* evidence other than through expensive *in vivo* equivalence testing.

Methods: Biowaiver and IVIVC are surrogate methods for *in vivo* bioequivalence studies. The Biowaiver test was done according to WHO, TRS992, 2015 Annex 7, Appendix 1, the recommendation for conducting and assessing comparative dissolution. IVIVC was done by the level A Convolution method. Innovator product was used as Ganaton OD for Itopride and Motilium for Domperidone to perform the comparison testing.

Results: The similarity factor (F2) between the test and innovator product of Domperidone at pH 1.2 HCl, Acetate Buffer pH 4.5, and water was 79.51, 68.00, and 58.97 and the dissimilarity factor (F1) was 7.24, 8.05 and 11.01 respectively. From the IVIVC study by level A convolution method of C_{max}, AUC, T_{max} of Ganaton OD and formulated Itopride were found to be 409.16ng/ml, 5652.28 ngh/ml and 4h and 252.16ng/ml, 4601.12 ngh/ml and 12 h respectively.

Conclusion: The F2 limit is 50-100 and F1 is 15 mentioned as per guidelines followed for the biowaiver test, which means the formulated domperidone is deemed equivalent to (Motilium) innovator of domperidone. The predictive error on the AUC of Itopride formulated was found to be 18.59 % which was within the limit of±20 %, demonstrating the therapeutic range.

Keywords: Biopharmaceutics classification system, Bilayer tablets, Biowaiver, Dissolution, IVIVC

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INTRODUCTION

The term "bioequivalence" was used to refer to the drug substance achieving systemic circulation in two or more identical dosage forms at the same relative rate and to the same relative extent, i.e., their plasma concentration-time profiles will be identical without a visible statistical difference. *In vitro* and *in vivo* bioequivalence are the two types of bioequivalence. To establish that a generic drug product is pharmaceutically equivalent to the brand product and exhibits comparable bioavailability when provided at the same dose under comparable conditions, *in vivo* bioequivalence testing was carried out. Bioavailability study contains pharmacokinetic (Plasma level-time and Urinary Excretion studies) and pharmacodynamics (Acute pharmacological and therapeutic response) [1, 2].

In vitro studies i.e. dissolution studies, can be used instead of *in vivo* bioequivalence under certain circumstances called biowaiver. The Term "Biowaiver" refers to the removal of *in vivo* bioavailability of bioequivalence studies used in regulatory drug approval processes that are based on *in vitro* research where the study of *in vitro* dissolution is recognized as a surrogate for comparison of two medicine items [3, 4]. A pharmacological substance is categorized based on its water solubility, intestinal permeability, and rate of dissolution. A biowaiver study is conducted to assess the option of relinquishing *in vivo* bioequivalence studies for certain pharmaceutical products, usually generic drugs. The purpose is to determine whether *in vitro* studies can be used to support the bioequivalence claim. *In vitro In vivo* Correlation (IVIVC) can be applied as a predicting tool for *in vivo* bioequivalence if formulation, method, or production location changes, as well as to determine product dissolution specifications. The FDA guidelines list five different levels: Level A, Level B, Level C, Multiple Level C, and Level D for IVIVC [5-7]. Considering the pharmacokinetic properties of a test product, the convolution approach of Level A estimates blood drug levels from *in vitro* dissolution data. This is a straightforward procedure. Here, the plasma concentration-time profile is created using the *in vitro* dissolution profile (input). Convolutional models can be developed and maximum concentration ($C_{max,}$) times to reach maximum concentration (T_{max}), area under the concentration-time curve (AUC) values can be used for assessing drug concentration-time profiles produced from the results of dissolution [8-11].

Domperidone is a BCS Class II drug having a recommended dose of 10–40 mg in multiple doses. Its mechanism of action involves its ability to antagonize dopamine receptors in the gastrointestinal tract and the brain [12, 13]. Itopride is a prokinetic agent falls under BCS Class I with recommended dose of 150 mg/day in separated doses in adult patients. It functions as an acetylcholine esterase inhibitor as well as an antagonist of the dopamine D_2 receptor. It has an anti-emetic effect, speeds up gastric emptying, and enhances stomach tension and sensitivity [14]. Both domperidone and Itopride regulate gastric motility and hence can be used together for the treatment of Gastroesophageal Reflux Disorder. Domperidone was formulated as an immediate release for quick action, whereas Itopride was formulated as a gastroretentive tablet to increase bioavailability and for local action [15]. Treatment with Itopride and Domperidone may show superior results [16].

In the current study, a new delivery approach has been used by formulating a bilayer tablet, where one layer being immediate release and the other being gastro-retentive sustained release. This study aims to provide a more efficient pathway for generic drug approval while maintaining the same level of therapeutic equivalence and safety as the reference product. Bioequivalence test is a crucial aspect of drug development and regulatory approval, as it ensures that generic drugs perform equivalently to their corresponding brand counterparts. Biowaiver and IVIVC are related concepts in the pharmaceutical industry and regulatory science, particularly concerning drug development and the approval of generic medication. Biowaiver and IVIVC can be used the surrogate methods for the bioequivalence test.

MATERIALS AND METHODS

Materials

All the raw materials and reagents were procured and provided by Everest Pharmaceuticals Pvt. Ltd, Bhaktapur, Nepal. The Active

Pharmaceutical Ingredients, Itopride Hydrochloride (Amilife Science, Gujarat-India) and Domperidone maleate (Vasundhara, Hyderabad-India) were used. The excipients including Polyvinyl Pyrolidone (PVPk-30) (Boeinky Pharmaceutical, Jiaozuo-China), Barium sulphate (Lobachemie, Mumbai-India), Sunset yellow(Roha, Mumbai-India), Xanthum Gum (Deason Biochemical, Shandong-China) Carbomer 974 (Shreechem, Mumbai-India), Talcum (Neelkanth, Delhi-India), Microcrystalline Cellulose (MCC P102) grade, Magnesium Stearate and Sodium starch Glycolate (Prachin Chemical, Ahmedabad-India)Sodium Saccharine (Blue Jet Healthcare, Maharashtra-India), Hydroxyl Propyl Methyl Cellulose (HPMC K 100 M) (Nitika Pharmaceutical, Nagpur-India), Lactose anhydrous (Modern Diaries, Haryana-India). Additionally, HPLC water (Sartorius, HPLC Water System, Germany), Acetonitrile (Thermolab Fischer Scientific, India), Sodium Bicarbonate, Sodium Potassium dihydrogen phosphate, Dipotassium dihydrogen orthophosphate (Merck Life Science Pvt. Ltd, Mumbai-India), Sodium Phosphate dibasic anhydrous, Hydrochloric acid, Dimethyl Formamide (Loba Chemie Pvt. Ltd, Mumbai-India). All the chemicals used are lab or HPLC-grade materials. Ganaton OD 150 mg (B. No. IAH0025 MFD. Jun.22 EXP. MAY 2024) and Motilium M (B. No. N 166 MFD.08/21 EXP.07/23) were purchased from Bhatt Medicose Store-Dehradun.

Instruments

High-Performance Liquid Chromatography (Agilent 1260 Infinity II, Mumbai-India), UV Spectrophotometer (Agilent Cary 600, Mumbai-India), Disintegration apparatus (LAB India, Thane-India), Friability apparatus (Roche, India), Hardness Tester (Thermonik, Mumbai-India), Vernier Caliper (Mitutoyo, Japan), Dissolution apparatus (LAB India, Thane-India), Moisture balance (ADAM, AMB50), Differential Scanning Calorimeter (DSC-60 Plus–Shimadzu, Mumbai-India). Four-digit analytical balances (Sartorius, Germany), Fourier Transform Infrared (Cary-630 FTIR–Agilent, Germany), Double cone blender (R and D Multipurpose Equipment GMP Lab Model) and Double hopper double station Compression machine DRTM27STN GMP (Chamunda, Mumbai-India) were the instrument used.

Preparation of bilayer tablets

The bilayer tablet, consisting of Domperidone immediate release as a top layer and Itopride gastro-retentive as a basal layer, was formulated. For the Domperidone layer, the Domperidone maleate tablet was made by direct compression. All the excipients shown in table 1, were dried in a tray drier for 1 hr at 50 °C to have better flow properties except sodium bicarbonate and citric acid. Domperidone was passed through 60 mesh and others through 40 mesh. Domperidone maleate was geometrically mixed with MCCP 102 in a polybag for 5 min. After that all materials were mixed in a double cone blender 5 kg capacity for 10 min clockwise and 10 min anti-clockwise. The total compression weight of Domperidone was 100 mg. For the Itopride layer, Itopride and Citric acid were passed through 60 mesh and other excipients through 40 mesh shown in table 1. After passing all the material, they were mixed in a double cone blender (5 kg capacity) for 10 min clockwise and 10 min anti-clockwise. The total compression weight was 700 mg. Bilayer tablets were compressed in a 16 mm die punch following Duredas Trademark technology. The compression force was 5 tons, which provided immediate release from one layer and sustained release from another layer within the same tablet. Domperidone was placed in hopper A and Itopride was placed in hopper B. In-process quality parameters were checked and maintained within the limit of Indian Pharmacopoeia 2018.

Biowaiver study

The Biowaiver test was done according to WHO, TRS992, 2015 Annex 7, WHO Expert Committee on Specifications for Pharmaceutical Preparations Forty-ninth report, Appendix1, recommendation for conducting and assessing comparative dissolution. The dissolution test was done using UV-Spectrophotometry (Agilent Cary 600). Domperidone immediate release dissolution was compared with Motilium M manufactured by Johnson and Johnson Private Limited. The comparative dissolution studies were performed in three media at pH 1.2 Hydrochloric acid, pH 4.5 acetate buffer, and water instead of pH 6.9 phosphate buffer, respectively. The physicochemical properties of the Test and Innovator are shown in table 2. The dissolution measurements of the test (Domperidone formulated) and innovator (Motilium M) were done under the same test conditions. The sampling time points of the innovator and test product are the same, with a minimum of three time points (n=3) [17-20]. The dissimilarity factor (f1) and similarity factor (f2) were calculated to compare the dissolution profile by using the following formula:

$$\text{Similarity factor (f2)} = 50.\log\left\{\left[1+\frac{1}{n}\sum_{t=1}^{n}(Rt-Tt)^2\right]^{-0.5} X100\right\}$$

$$\text{Dissimilarity Factor (f1)} = \frac{\sum_{t=1}^{n}|Rt-Tt|}{\sum_{t=1}^{n}Rt} X\ 100$$

Where,

Rt = Average % release of innovator sample

Tt = Average % release of test sample

F1 = Dissimilarity factor

F2 = Similarity factor

N= number of points taken

The comparative dissolution study for Domperidone was conducted in a paddle apparatus with 900 ml of 0.1N Hydrochloric acid pH 1.2, acetate buffer pH 4.5, and water as dissolution medium used for three times, keeping other procedures constant. The paddles were rotated at 75 rpm and the temperature was maintained at 37 °C±0.5 °C. The samples were withdrawn at 5, 15, 30, and 45 min, respectively. The absorbance was taken at the wavelength of 286 nm. The cumulative percentage of drug release was calculated for data analysis.

The study of dissolution for Itopride Hydrochloride medium was conducted with 900 ml of 0.1 N HCl, the paddles were rotated at 75 rpm and the temperature was maintained at 37 °C±0.5 °C. One tablet/Capsule pellet in each dissolution vessel was placed and the apparatus was run, while as per the above condition, the sample solution was collected from each jar at the specified time. The absorbance on the UV spectrophotometer was 257 nm. Sampling were done in 1h, 2 h, 4 h, 6 h, 8 h, 16 h and 24 h. The cumulative percentage of drug release was calculated for data analysis.

Table 1: Composition of bilayer tablets for domperidone and itopride

Domperidone		**Itopride**	
Material name	**Quantity (mg)**	**Material name**	**Quantity (mg)**
Domperidone Maleate	12.72	Itopride Hydrochloride	150
MCC 102	26.28	PVPK-30	45
Sodium Starch Glycolate	8	Sunset Yellow Lake	2
Lactose	32	HPMC K 100M	109.64
Magnesium Stearate	5	Xanthum Gum	70
Talcum	5	Carbomer 974	35.03
Crosspovidone	6	Sodium Bicarbonate	100
Cross Carmellose Sodium	5	Citric Acid	70
-	-	MCC102	95.33
-	-	Talcum	23
Total weight	100		700

***In vitro in vivo* correlation (IVIVC) of Itopride**

The dissolution test was conducted for Ganaton OD capsules and Itopride-formulated tablets. *In vitro In vivo* correlation method was using the numerical convolution method. Using the pharmacokinetic characteristics of Ganaton, the convolution approach calculates blood drug levels from data on *in vitro* dissolution (6, 21-23). The pharmacokinetic parameters were obtained from published literature and the values reported are as follows:

Bioavailability, F = 60% =0.60; Body weight = 70 kg

$$\text{Half-life } (t_{1/2}) = 6 \text{ h.}$$

Elimination rate constant, k_e = 0.693/half-life = 0.693/6 = 0.1155 per h;

Apparent V_d= (Dose/Plasma Concentration) × Body weight = 50 X10000ng/303.72ng/ml= 1646.25 ml= 1.646L = 115.2L in 70 kg

The dissolution test was repeated three times (n=3) following the method mentioned in the above section and average data were converted into *in vivo* release. The step for the conversion of *in vitro* dissolution data into blood concentration profile is as follows:

Discrete amount released within sampling interval = Amount released at time (t_2)-Amount released at time (t_1).

$$\text{Amount Released} = \left(\frac{\%\text{ released X Dosage Strength}}{100\%}\right)$$

Percent dissolution at different times with corresponding amounts in mg obtained within the sampling interval. The average value of two data of discrete amount (mg) released (within sampling interval).

(b) The total amount of drug present in the blood at different times was calculated by adding all the calculated drug amounts for every time. Mathematically, such profiles may be described by an exponential equation such as:

$$C = C_0 \times e^{-ket}$$

Where C= Drug concentration at time t, k_e = elimination rate constant i.e., $0.693/t_{1/2}$ (Half-Life), C_0= drug concentration at time "zero". Sums of two or more exponential equations i.e. sum of two or more exponential components were done.

(c) The calculation of the blood concentration of the drug. This will provide the expected blood level profiles. This was done by dividing the blood amount at every time by volume of distribution and average body weight (70 kg).

Predicted Concentration (mcg/ml) at times = Predicted Total Blood Amount (mg) after Absorption *(F/Vd) *body weight.

d) Area under Curve (AUC) determined by

$$(C_2+C_1)/2 * (t_2-t_1)$$

Where C_1 and C_2 are the predicted amount of drug in blood at each time interval (t_1 and t_2).

e) Prediction Error (%)= [(actual-predicted)/actual] X100%

Where actual is of reference and predicted of test value (8, 9, 24-27).

RESULTS

Formulation of tablets

Domperidone immediate release tablet was formulated with 100 mg and Itopride gastro-retentive tablet with 700 mg with a total compression weight of 800 mg. Bilayer tablets were compressed without any compression defects like sticking, capping, weight variation, etc. The temperature was maintained at 25-29 °C and relative humidity at 45-55%, while the pressure difference was 5-15 Pascal between room and corridor. The immediate compression was done after the estimation of lubricated granules.

Biowaiver study

The physicochemical characteristics of the Test and Innovator of Domperidone were shown in table 2. Domperidone lies on BCS Class II with Low Solubility and High Permeability. The cumulative drug release of domperidone with test and innovator in three different mediums at different time intervals are shown in table 3. The Similarity factor (F2) between the test and the innovator product of Domperidone at pH 1.2 HCl, Acetate Buffer pH 4.5, and water was 79.51, 68.00, and 58.97 and the dissimilarity factor (F1) was 7.24, 8.05 and 11.01 respectively. The F2 limit was 50-100 and the F1 was 15 mentioned as per guidelines followed for the biowaiver test. From the above results, domperidone immediate release tablet can be marketed.

Dissolution of Ganaton 150 mg (Innovator) and Itopride (test) was performed. Ganaton drug releases at 1, 2, 4, 6, 8, 10, 12, 16 and 24 hr were 31.32±1.12, 46.81±1.47, 65.52±1.89, 73.03±1.69, 81.58±2.78, 87.53±1.56, 88.67±1.85, 90.85±2.36, and 95.19±2.48 %, respectively. The drug release for Itopride formulation at 1,2,4,6,8,10,12,16 and 24h were 9.78±1.23, 15.52±1.25, 25.17±1.78, 36.02±1.65, 42.89±1.26, 46.63±1.48, 61.16±1.89, 72.35±1.35, 97.69±1.58%, respectively.

Table 2: Comparison of domperidone and itopride with innovator

Formulation code	Physical appearance	Weight variation (mg)	Hardness (kg/cm²)*	Thickness (mm)*	Length (mm)*	Friability (%)	Disintegration (sec)*	Assay (%)*
Ganaton OD 150 mg	Red Cap Opaque body Hard gelatin capsule with size 2 with white pellets inside.	With Gelatin (64 mg)315±5.80	-	5.90±0.02	17.40±0.05	-	-	99.28±1.32
Domperidone and Itopride Bilayer Tablets	White Color Domperidone layer Sunset yellow Color Itopride layer Oblong biconvex plain tablet	803±9.23	26.23±3.28	5.22±0.23	16.8±0.32	0.18	-	Domperidone=100.28±0.92, Itopride=99.29±1.45
Motilium™ M	White color circular M embossed on One side and Breakline embossed on other side	111±3.2	4.82±0.48	2.86±0.23	6.5±0.02	0.23	180±8	102.3±1.22

*(All values are mean±SD; n=3)

IVIVC of itopride

In the convolution method, an *in vitro* dissolution study is carried out for 24 h and the results are converted to *in vivo* data (Plasma concentration-time profile) of the test and innovator. The dissolution method was followed the same as mentioned in Biowaiver for Itopride. Since the innovator of Itopride was in capsule dosage form pellets were withdrawn from gelatin and dissolution studies were conducted the same as the Itopride test product. From IVIVC by convolution method C_{max} (Maximum

Concentration), AUC (Area Under the Concentration-time curve), T_{max} (Maximum Time) were found to be (409.16 mg/ml, 5652.8ngh/ml, 4 h) and (252.16 mg/ml, 4601.12 ngh/ml and 12 h.) of Ganataon OD and Itopride formulated respectively. The AUC prediction error was 18.59% but the maximum concentration (C_{max}) does not lie within the limit of prediction error between the test and innovator. This may be due to variations in formulation, excipients selection, and manufacturing process. The calculated drug levels of the products in blood at various time intervals of innovator and test were shown in fig. 1a and 1b, respectively. As the calculation was based on the value given to the formula AUC, C_{max} and T_{max} may vary which was mentioned in the method for calculating IVIVC by convolution method. The calculation was shown in table 4-7 of the innovator and test in detail. The AUC prediction error was 18.59 shown in table 8, which was within the limit of±20 %, but C_{max} does not lie within the limit of prediction error between the test and innovator it may be due to variations in dosage form, excipients selection, and manufacturing process [9, 22, 24, 25].

Table 3: Cumulative *in vitro* release profiles of domperidone innovator and reference formulation

Dissolution medium	Tablet formulations	5 min (%)	15 min (%)	30 min (%)	45 min (%)
0.1 N Hydrochloric Acid (pH 1.2)	Innovator	99.17±2.89	101.86±1.42	103.25±1.69	103.30±1.45
	Test	99.40±1.69	99.10±2.89	99.70±1.79	100.04±2.86
Acetate Buffer (pH 4.5)	Innovator	93.45±1.85	98.71±3.14	102.80±2.61	103.66±2.47
	Test	97.36±2.47	99.30±2.15	99.41±2.79	100.78±3.48
Water	Innovator	65.32±2.75	80.51±2.13	89.90±2.45	95.93±3.14
	Test	74.69±2.68	80.76±2.16	88.72±3.14	96.50±2.14

(All values are mean±SD; n=3)

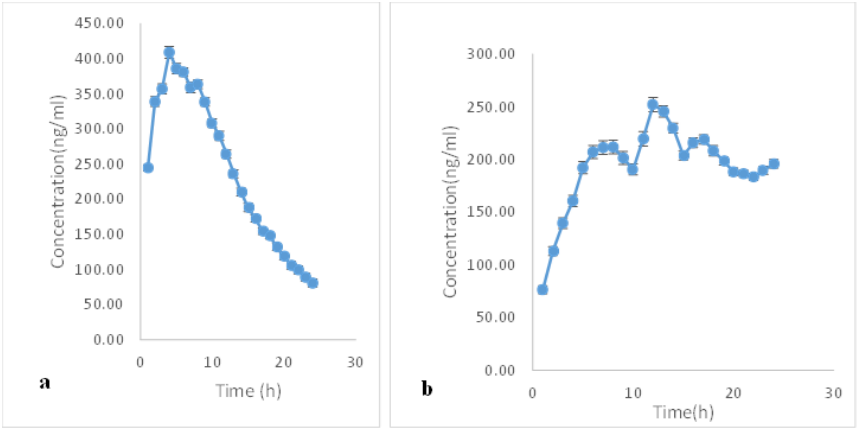

Fig. 1: Derived (calculated) dissolution profile (a) Ganaton (b) Itopride

Table 4: The predicted amount of drug released in the body based on dissolution release of (Innovator) Ganaton OD

Time (h)	% Released (cumulative)	% Released (within sampling interval)	Amt (mg) Released (within sampling interval)
0	0	0	0
1	31.32±2.31	31.32±1.12	46.98±1.36
2	46.81±1.45	15.49±1.25	23.24±2.39
3	53.84±2.14	7.03±1.45	10.55±2.48
4	65.52±3.15	11.68±0.15	17.52±1.15
5	68.26±2.12	2.74±0.89	4.11±0.85
6	73.03±1.12	4.77±0.45	7.15±0.48
7	75.69±1.25	2.66±0.78	3.99±0.69
8	81.58±1.65	5.89±0.12	8.84±0.47
9	83.85±2.85	2.27±0.23	3.40±0.25
10	85.12±2.35	1.27±0.09	1.91±0.45
11	87.53±1.26	2.41±0.08	3.61±0.14
12	88.67±3.15	1.14±0.08	1.71±0.78
13	89.21±1.45	0.54±0.09	0.81±0.14
14	89.52±1.78	0.31±0.08	0.47±0.21
15	89.95±1.98	0.43±0.06	0.65±0.41
16	90.85±2.35	0.9±0.05	1.35±0.12
17	91.25±1.89	0.4±0.1	0.60±0.26
18	92.85±1.36	1.6±0.01	2.40±0.25
19	93.12±1.89	0.27±0.07	0.41±0.64
20	93.52±4.15	0.4±0.04	0.60±0.31
21	93.76±3.15	0.24±0.04	0.36±0.36
22	94.62±4.15	0.86±0.03	1.29±0.36
23	94.84±3.25	0.22±0.02	0.33±0.35
24	95.19±1.86	0.35±0.03	0.52±0.05

(All values are mean±SD; n=3)

Table 5: Predicted drug levels at different times following absorption of drug released *in vitro* during sampling intervals of (Innovator) ganaton OD

Dissolution sampling time (h)	**1**	**2**	**3**	**4**	**-**	**12**	**-**	**24**			
Amount (mg) equivalent	**46.98**	**23.23**	**10.55**	**17.52**	**-**	**1.71**	**-**	**0.52**			
Time after absorption (h)	**Blood amount after absorption**								**Total blood amt (mg)**	**Conc (ng\ml)**	**AUC**
0	0								0	0	0
1	46.98								46.98	244.62	291.76
2	41.86	23.23							65.09	338.90	347.88
3	37.29	20.70	10.55						68.54	356.87	383.02
4	33.22	18.44	9.40	17.52					78.58	409.16	397.55
-	-	-	-	-	-	-		-	-	-	-
12	13.19	7.32	3.73	6.95	-	1.71			50.72	264.10	250.22
-	-	-	-	-	-	-	-	-	-	-	-
24	3.30	1.83	0.93	1.74	-	0.27		0.52	15.45	80.44	40.22 5652.28

All values are mean

Table 6: Predicted amount of drug released in the body based on dissolution release of (test) Itopride

Time (h)	**% Released (cumulative)**	**% Released (within sampling interval)**	**Amt (mg) Released (within sampling interval)**
0	0	0	0
1	9.78±2.23	9.78±1.85	14.67±1.78
2	15.52±1.25	5.74±1.46	8.61±1.47
3	20.5±2.98	4.98±1.15	7.47±1.96
4	25.17±1.78	4.67±1.36	7.00±1.47
5	31.43±3.56	6.26±1.48	9.39±1.89
6	36.02±1.65	4.59±1.15	6.88±2.12
7	39.62±1.14	3.6±1.78	5.40±1.85
8	42.89±2.26	3.27±1.69	4.90±1.15
9	44.85±1.36	1.96±1.48	2.94±0.97
10	46.63±3.48	1.78±1.78	2.67±1.48
11	53.38±0.98	6.75±1.35	10.125±1.79
12	61.16±1.89	7.78±1.95	11.67±1.56
13	64.58±1.64	3.42±1.34	5.13±1.26
14	66.73±1.48	2.15±1.15	3.225±1.45
15	67.31±3.12	0.58±1.38	0.87±1.48
16	72.35±1.35	5.04±1.15	7.56±1.79
17	76.48±1.98	4.13±1.34	6.195±1.68
18	78.92±4.35	2.44±0.85	3.66±0.97
19	81.32±2.34	2.4±1.97	3.6±1.45
20	83.48±1.48	2.16±2.14	3.24±1.16
21	86.56±1.15	3.08±1.85	4.62±1.63
22	89.47±2.01	2.91±1.48	4.365±1.53
23	93.52±2.47	4.05±1.16	6.075±1.78
24	97.69±1.58	4.17±1.78	6.255±1.15

(All values are mean±SD; n=3)

Table 7: Predicted drug levels at different times following absorption of drug released *in vitro* during Sampling intervals of test (Itopride)

Dissolution sampling time (h)	**1**	**2**	**3**	**4**	**-**	**12**	**-**	**24**			
Amount (mg) equivalent	**14.7**	**8.61**	**7.47**	**7**	**-**	**11.67**	**-**	**6.25**			
Time after absorption (h)	**Blood amount after absorption**								**Total blood amt (mg)**	**Conc (ng\ml)**	**AUC**
0	0								0	0	0
1	14.7								14.67	76.38	94.63
2	13.1	8.61							21.68	112.88	126.17
3	11.6	7.67	7.47						26.78	139.46	150.1
4	10.4	6.83	6.65	7.01					30.86	160.73	176.41
-	-	-	-	-	-	-		-	-	-	-
12	4.11	2.71	2.64	2.78	-	11.67			48.42	252.16	248.89
-	-	-	-	-	-	-	-	-	-	-	-
24	1.02974	0.678361	0.66060117	0.69533	-	1.81		6.25	37.595	195.75	97.87 4601

All values are mean

Table 8: Prediction error

S. No.	Parameters	Predicted value of ganataon OD*	Observed value of Itopride formulated*	% prediction error
1	C_{max} (ng/ml)	409.16±10.54	252.16±9.85	38.37%
2	AUC (ng. hr/ml)	5652.28±16.84	4601±10.76	18.59%

(All values are mean±SD; n=3)

DISCUSSION

Through anti-dopaminergic and anti-acetylcholine stearic activities, the bilayer tablet of domperidone immediate release and itopride gastro retentive formulation promotes the synergistic effect of gastric motility.

Biowaiver and IVIVC are tools used in pharmaceutical and regulatory fields that play a crucial role in the drug development and approval process while guaranteeing the efficacy and safety of the generic drug. BCS Classification influences the potential for biowaiver and IVIVC. Biowaiver was considered for BCS Class I and III drugs and WHO has extended to BCS Class II. Domperidone lies in BCS Class II (Low Solubility, High Permeability). IVIVC was a consideration for BCS Class I and II drugs. Itopride lies in BCS Class I (High Solubility, High Permeability). The key considerations for granting a biowaiver include comparable dissolution profiles between the test and reference products, ensuring that the drug is released at a similar rate. Water was considered as an additional medium in the biowaiver study of domperidone because both the innovator and domperidone (test) were unstable on pH 6.8 phosphate buffer, which was confirmed by dissolution release of domperidone below 70 % at 45 min. Domperidone's immediate release of the test product has passed all the specifications recommended for conducting and assessing comparative dissolution profiles for biowaiver from conditions of grant for BCS based biowaiver [28].

Since drugs released for the innovator and test products of Itopride are different, dissolution comparative study cannot be performed. Itopride(test) was formulated as gastro retentive tablet and Ganaton OD (innovator) as hard gelatin capsules. Itopride test product was formulated as gastro retentive tablets to increase bioavailability whereas Ganaton was formulated with coated pellets filled in hard gelatin capsule size 2 red cap and transparent clear gelatin. Sustained release pellets are made by coating with water-insoluble Ethyl Cellulose (EC N 50) and Hydrophilic Polymer Hydroxyl Methyl Cellulose (HPMC E5) [29, 30]. The selection of dosage form i.e. capsule to tablet was different and on the other hand polymer selection was different than the innovator product.

IVIVC is to predict how changes in the formulation or manufacturing process of a drug product might impact its *in vivo* performance without the need for conducting extensive and costly clinical trials. Since the AUC_{24} result of Itopride formulated drug (4601.12 ngh/ml) was within the predictive error of±20% limit was 18.59%, which shows the therapeutic range as innovator Ganaton OD (5652.8 ngh/ml). The % prediction error was less than ±20%, which indicates the similarity of *in vivo* performance in comparison with reference product according to bioequivalence practice. The drug concentration-time profiles obtained from dissolution results may be evaluated using criteria for *in vivo* bioequivalence assessment based on C_{max} and AUC parameters. Since IVIVC by convolution method is dependent on pharmacokinetic parameters the value of AUC may be different depending upon the data available. This can be further discussed with an example, the AUC_{inf} and C_{max} of domperidone by Amirbandeep Bose et. al were 579.61 ngh/ml, 76.09ng/ml, and by A. Khan et. al was 168.7 ngh/ml, 24ng/ml respectively [8, 10, 31]. Yehia SA et. al found C_{max} of selected microcapsule formulation of Itopride 1624 ng/ml and AUC_{inf} 83835 ngh/ml and ganaton tablet was 1518 ng/ml and 9476 ngh/ml, respectively [32]. The same dosage form or by optimizing and selecting different polymers for drug release same as Ganaton another formulation should be made in the future by researchers and industrialists to have *in vivo* bioequivalence.

CONCLUSION

A formulated bilayer tablet consisting of 10 mg domperidone immediate release and 150 mg Itopride gastroretentive sustained release was showed bioequivalence through biowaiver and IVIVC by Convolution method, respectively. Biowaiver and IVIVC enhance the efficiency and reliability of drug development and regulatory processes. Domperidone (BCS Class II) shows a drug similar dissolution result to Motilium; WHO has given an extension of Biowaiver to BCS Class II. Itopride (BCS Class I) has the possibility of predicting IVIVC from dissolution Data. The AUC_{24} of the both itopride test and innovator were within the predictive error of ±20 %, which shows the therapeutic range of the test was within the limit. However, C_{max} was out of the limit, which may be because Itopride has been formulated with gastroretentive sustained-release tablets but the innovator was formulated in hard gelatin capsules with pellets inside. Hence surrogate methods like biowaiver and IVIVC can be used instead of expensive and time-consuming *in vivo* bioequivalence testing for drug development.

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CONFLICTS OF INTERESTS

The authors declared no conflict of interest.

AUTHORS CONTRIBUTIONS

All authors are contributed equally.

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