Original Research Article

In vitro-In vivo Bio-equivalence Correlation Study of Metronidazole, and its Brands of Immediate Release tablet under bio-waiver conditions

ABSTRACT:

The aim of present study is to examine the in-vitro in-vivo correlation (IVIVC) of immediate release product. Metronidazole 500mg and its brands of immediate release dosage forms. Metronidazole is clearly classified into BCS class I, and could be evaluated under bio waiver conditions. The in vitro parameters employed were hardness, weight uniformity, friability, disintegration time, absolute drug content , dissolution rate (in 0.1 N Hydrochloric acid, phosphate buffer and acetate buffer at 37ºC),and dissolution efficiencies were also analyzed.

The in-vitro dissolution study was performed on the brands, according to FDA,USP dissolution profile in three different PH (1.2),(4.5), and (6.8) at37ºC ,using the USP apparatus II, then f1 ,f2 were determined for the time intervals of 10, 15, 30, 45 and 60 minutes, and dissolution efficiencies were calculated. MINITAB 14 statistical programused for in vitro in vivo correlation, level A was done for reference product. A non linear relation was established which is typical for immediate release formulation, of class 1.

Key words: *bioavailability, bioequivalence, biopharmaceutical classification system,Bio-waiver correlation.*

INTRODUCTUON

Bio-equivalence:

Is defined as “the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study”.

If two medicines are bioequivalent there is no clinically significant difference in their bioavailability.

In vitro testing, preferably based on a documented "in-vitro/in-vivo correlation". May sometimes provide the same indication of bioequivalence between two pharmaceuticals.

Bioequivalence is determined based on the relative bioavailability of the innovator medicine versus the generic medicine. It is measured by comparing the ratio of the pharmacokinetic variables for the innovator versus the generic medicine where equality is 1.

Bioequivalence studies focus on the release of drug from dosage form, formulation and subsequent absorption into the systemic circulation. Bio-equivalence studies may involve both in-vivo and in-vitro studies.

In Vivo in Vitro Correlations

Development and optimization of formulation is an integral part of manufacturing and marketing of any therapeutic agent which is indeed a time consuming and costly process. Optimization process may require alteration in formulation composition, manufacturing process, equipment and batch sizes. If these types of changes are applied to a formulation, studies in human healthy volunteers may be required to prove that the new formulation is bioequivalent with the old one. Certainly, implementation of these requirements not only halts the marketing of the new

formulation but also increases the cost of the optimization processes. It would be, desirable, therefore, to develop in vitro tests that reflect bioavailability data. A regulatory guidance for both immediate- and modified-release dosage forms has been, therefore, developed by the FDA to minimize the need for bioavailability studies as part of the formulation design and optimization (Amidon, et.al, 1995).

IVIVC can be used in the development of new pharmaceuticals to reduce the number of human studies during the formulation development.

Correlation Definitions:

The term correlation is frequently employed within the pharmaceutical and related sciences to describe the relationship that exists between variables. Mathematically, the term correlation means interdependence between quantitative or qualitative data or relationship between measurable variables and ranks (Blaskovich, et.al, 2003). From biopharmaceutical standpoint, correlation could be referred to as the relationship between appropriate in vitro release characteristics and in vivo bioavailability parameters, Two definitions of ivivc have been proposed by the USP and by the FDA (Chen, et.al, 2005),(Jayaprakasam, et.al, 2003).

MATERIALS AND METHODS:

Physical Test:

Uniformity of Weight Test.

i. 20 randomly selected tablets were weighed. The average weights were determined.

ii. The tablets were weighed individually and the percentage of deviation of its weight from the average weight was determined for each tablet .

iii. The deviation of individual weight from the average weight should not exceed the limit given in table 3:

Hardness Test.

The hardness of 10 tablets randomly selected from each batch weredetermined on an automatic tablet hardness tester. The crushing strength of uncoated tablets is accepted within 4-8 kg/cm2

Friability Test

20 tablets previously freed of dust were weighed together beforetransferring to a frabilator set to run for 4 min at 25 r.p.m. Thereafter they were removed, dusted and reweighed:

% Friability = [(Wi – Wf)/ Wi] x 100, (should be less than 1%)

Where;

Wi is the initial weight and Wf the final weight of the tablets.

Disintegration Time Test.

According to official monograph determination of disintegration time for uncoated tablets was adopted using a disintegrating apparatus and the medium was distilled water at 37±1^{o}C.six tablets were used for the determination. Accepted range for the uncoated tablet up to 30 mi

Absolute drug content

Five pre-weighed tablets were crushed; the equivalent weight of a tablet was weighed out and dissolved in 500 ml of 0.1M NaoH in a volumetric flask, and filtered. The absorbance reading was determined using UV-visible spectrophotometer at 319nm.

In Vitro Dissolution Test

Volume of 900 ml of each buffer was employed. Dissolution testing was performed using Tablet Dissolution Tester (USP Apparatus 2) at 75 rpm for class I, test and reference products, temperature will be adjusted to 37°C ± 0.5 C .Twelve dosage units of each product test and reference were evaluated in the three media. Sample aliquots of 10 ml were taken manually with

syringes. Samples were withdrawn at specified time intervals (10, 15, 30, 45, and 60 min) and replaced with 10 ml of appropriate medium. With drawn samples were filtered using 0.45-µm Millipore Filters, then 5 ml taken after filtration by volumetric pipette (3ml taken when use HCL buffer solution, and 1ml taken in case of acetate and phosphate buffer, and diluted to 50 ml). A uv–visible spectrophotometer was used to analyze dissolved drug in dissolution testing. Scanning of wavelength done in each buffer, and spectrum recorded between 200---800nm, and percentage % of drug dissolved calculated.

Buffers Preparation:

Simulated gastric fluid (SGF), simulated intestinal fluid (SIF), and acetate buffer pH (4.5) were prepared according to instructions in USP test solution. All media were prepared without enzymes, as follow:

a- Simulated Gastric Fluid (SGF) pH (1.2):

To prepare hydrochloric acid 0.1N, 8.5 ml was taken from concentrated HCL (37%) and volume completed to 1000 ml by distilled water.

b- Simulated Intestinal Fluid (SIF) pH (6.8):

Potassium phosphate monobasic KH_2PO_4 0.2 M was prepared by dissolving 27.22 g in water, and volume diluted to 1000 ml by distilled water. Then sodium hydroxide 0.2 M prepared by dissolving 8g in water and volume diluted to 1000 ml by distilled water. 250 ml from Potassium phosphate monobasic KH2PO4 0.2 M was placed into 200 ml volumetric flask, also 112 ml taken from sodium hydroxide 0.2M and volume completed to 1000 ml with distilled water.

c- Acetate Buffer pH (4.5):

Firstly acetic acid 0.2N was prepared from concentrated acetic acid 99.93%. 116 ml was taken and diluted with distilled water. Then 2.99 g of sodium acetate ($NaC_2H_3O_2$) taken, and placed in 1000 ml volumetric flask,14ml from acetic acid was added and volume completed to 1000 ml by distilled water.

Preparation of Standard Stock Solutions:

Standard stock solutions of Metronidazole in HCL, phosphate and acetate buffers were prepared by dissolving 500 mg of standard in 100 ml volumetric flask using HCL, acetate and phosphate buffers as solvents to give concentration of 5 mg/ml, one ml taken by volumetric pipette in 100 ml volumetric flask to give concentration of 50µg /ml, using 50 ml volumetric flask to give serial concentration of standard curve.

Data Analysis:

All dissolution data evaluated using Excel spread sheet, andthe results will be plotted for each brand. (Raimar, et.al, 2012).Average of % content of active pharmaceutical ingredient (API) dissolved in each media of 12 tablets will be taken and a plot of % of (API) dissolved against time will be drawn to represent the dissolution profile .The dissolution profile for local brand will be compared to that of the reference drug.

If they are similar the similarity factor, f2 equal to or more than 50.This means that they are equivalent, if it's less than 50 they are not equivalent.

$$f1 = \{[3t=1n \mid Rt - Tt\mid]/[3t=1n\ Rt\ ]\}C\ldots\ldots\ (1)$$

$$f2 = 50\ C\ log\ \{[1+(1/n)3t=1n\ (\ Rt - Tt\ )2\ ]\ -0.5C\ 100\}\ldots\ldots\ (2)$$

Similarity factor f2 has been adopted by FDA and the European Agency for the Evaluation of Medicinal Products (EMEA) by the Committee for Proprietary Medicinal Products (CPMP) as a criterion to compare the similarity of two or more dissolution profiles. Similarity factor f2 is

included by the Centre for Drug Evaluation and Research (CDER) intheir guidelines such as guidance on dissolution testing of immediate release solid oral dosage forms (FDA, 1997) and guidance on Waiver of In-Vivo Bioavailability and Bioequivalence Studies for Immediate Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System (FDA, 2000).The area under the dissolution-time curve method was used in calculating the dissolution efficiency (DE), and this was calculated at 30 min .The higher the dissolution efficiency (DE) is, the better the release efficiency of the tablets' active ingredient, according to equation (3):

$$DE = \left\{ \left[\int_{t_1}^{t_2} \%D_t \cdot dt \right] / \left[\%D_{max} \cdot (t_2 - t_1) \right] \right\} \cdot 100 = \left[AUC_{0-T} / \%D_{max} \cdot T \right] \cdot 100$$

……. (3)

Where %D is the percentage dissolved at time t, % D (max) is the maximum dissolved at the final time T, and AUC(0-T)is the area under the curve from zero to time T(Anderson, et.al, 1998).

Correlation calculation will carried on using MINITAB14 specific statistical program.

RESULTS:

Correlation Results:

In vitro - In vivo relationship Determination of Level A correlation.

In vivo percent absorbed of reference product was calculated from equation (4):

$$\frac{A_t}{A_0} = \frac{C_t + K_{el} \bullet AUC_0^t}{K_{el} \bullet AUC_0^\infty}$$

……. (4)

where, $\frac{At}{A0}$denotes the fraction of drug absorbed at time t, Ct is the plasma drug concentration at time t, Kel is elimination rate constant, AUC0-t and AUC0-∞ are the area under the plasma concentration– time profile curve at time t and ∞ respectively.

Then the values of percent of drug released were plotted against the percent of drug absorbed for reference products of Metronidazole using **MINITAB14 analysis program**to find out the relationship between data (correlation).

Amount of drug released in the three different pH was plotted against amount of drug absorbed.

DISCUSSION:

A summary of the results of weight uniformity, hardness, friability, disintegration and assay are shown in Table 4. Weight uniformity may serve as a pointer to amount of the active pharmaceutical ingredient (API) contained in the formulation. All the brands complied with the compendial specification for weight uniformity.

Hardness is referred to as non-compendial test. The hardness or crushing strength assesses the ability of dosage form to withstand handling without fracturing or chipping , It can also influence other parameters such as friability and disintegration. Hence, the dosage formsof all brands were satisfactory for hardness.

Friability **test** is used to evaluate the tablets resistance to abrasion. Friability is now included in the United States Pharmacopeia (USP, 1995) as a compendia test. The compendial specification for friability is less or equal to 1%. Friability for all brands of Metronidazole were below 1%. Disintegration is the process of breaking of tablets in the liquid. Disintegration is a crucial step for immediate release dosage forms because the rate of disintegration affects the dissolution and subsequently the therapeutic efficacy of the medicine. A drug will be released rapidly as the dosage forms disintegrate. British Pharmacopeia specifies that uncoated tablets should disintegrate within 15 min and film coated tablet disintegrate within 30 min while USP specification for disintegration is 30 min forbothuncoated and film coated tablets. All the brands were complied with both BP and USP specifications for disintegration as maximum disintegration time.

Potency is the average amount of the active ingredient present per tablet. All the brands complied both BP and USP specification, as USP specification is that the content of active ingredient should not be less than 90% and not more than 110% while BP specifies that the content should not be less than 95% and not more than 105%.

The results of dissolution studies are graphically represented in the dissolution profile figures. All dissolution data are based on the actual drug content of the test dosage form as calculated from the assay results. All the Metronidazole brands released > 90% drug in acidic media (pH 1.2) within 30 min, and PH (4.5). Amount released in phosphate buffer PH (6.8) were about 84% for reference drug and 91.4%,86.5% for test brands, This may be due to the pH depended solubility of metronidazole.

Analysis of Dissolution Data: To compare the dissolution profiles of the brands, a model independent approach of difference factor f1 and similarity factor f2 were employed. Difference factor f1 is the percentage difference between two curves at each point and is a measurement of the relative error between the two curves. The similarity factor (f2) is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent (%) dissolution between two curves. Two dissolution profiles to be considered similar and bioequivalent, f1 should be between 0 and 15 while f2 should be between 50 and 100 (FDA, 1997). All the values for f2 and f1shown in tables 29 for metronidazole, as mentioned in previous tables, all brands f2 values were more than 50 and f1 values were less than 15. Which mean that all brands are equivalent with the innovator brand.

In-vitro AUC in three PH (1.2),(4.5),(6.8) for class I product were found three times in vivo bioequivalence AUC calculated before, which is acceptable result because the in-vitro dissolution studies were carried out in ideal conditions without any factors that could affect their performance, such as volunteers internal biological inconsistency.

Dissolution efficiency (DE) was also employed to compare the drugrelease from various brands. The reference and the test product can said to beequivalent if the difference between their dissolutionefficiencies is within appropriate limits (± 10%, which isoften used) (Anderson NH, et, al 1998). Dissolution efficiency of all the brands (class I) differed by less than 10% from the innovator brand. So, we can say that all the brands are pharmaceutically equivalent with the innovator brand.

In Vivo in Vitro Correlation Data Analysis:

As ivivc is a predictive mathematical model describing the relationship between variables (an in vitro property of a dosage form and a relevant in vivo response).

According to MINITAM 14 statistical program, there was significant relationship between in vitro and in vivo data of reference metronidazole product, Correlation and distribution of data with correlation coefficient (r= 0.724, 0.837, 0.707), non linear relationship with p-value

(>0.05)= (0.167, 0.098 , 0.182), there is no out lines, no lake of fits at P-Values = 0.0040, 006, 0.026. By analysis of variance (ANOVA) the data points have significant relationship with p-value (> 0.05) for the three pH (1.2), (4.5), (6.8) respectively.

Estimating the uncertainty in predicted correlation between in vitro and in vivo data was also performed. The interval is represented by the curved lines on either side of the regression line and gives an indication of the range within which the 'true' line might lie. Note that the confidence interval is narrowest near the center (the point x, y) and less certain near the extremes.

Using MINITAM 14 statistical program, there was significant relationship between in vitro and in vivo data of reference Atenolol product, Correlation and distribution of data with correlation coefficient (r= 0.798, 0.815, 0.967), non linear relationship with p-value (>0.05) = (0.106 , 0.93 , 0.009), there is no out lines, no lake of fits at P-Values = 0.106, 0.040, 0.056 (>0.05) for the three pH (1.2,4.5,6.8) respectively.

Estimating the uncertainty in predicted correlation between vitro and vivo data. The interval is represented by the curved lines on either side of the regression line and gives an indication of the range within which the 'true' line might lie. Note that the confidence interval is narrowest near the center (the point x, y) and less certain near the extremes.

By applying analysis of variance (ANOVA) for thedissolutiondata using MINITAB 14 we concluded that the test products are bioequivalent to reference products of metronidazole and atenolol and could be interchangeable.

CONCLUSION:

The bio waiver study has emphasized that pharmaceutical equivalence indicate that product have same drug molecule with approximately same pattern of dissolution release profile. By making fine turning in bioequivalent study we can reduce the time, cost, avoid Ethical, Ethnical consideration by unnecessary exposure of healthy subjects to medicines and finally to market the quality generic drug product. By applying level A in-vivo in-vitro correlation, we might concluded that there is no linear correlation between percent of drug released and percent of drug absorbed ,this may be due to uncontrollable gastric emptying rate for class one Metronidazole.

Metronidazole is an immediate release formulations. As dissolution is not a rate-limiting step in IR products, the fraction of drug absorbed against the fraction of drug released profile would be non-linear type which was obtained in our present study. So it may be concluded that theIn vitro - In vivo correlation is well established and justified for reference formulation by level A correlation.

By applying analysis of variance (ANOVA) for thedissolution data using MINITAB 14 we concluded that the test products are bioequivalent to reference products of metronidazole and could be interchangeable.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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Table (1): Materials

Name	Manufacture	Batch number	Production date	Expiry date
Metronidazole (standard)	AzalPharma Industries CO.LTD-Sudan	3020142	2/2013	2/2018
Negazole500 mg	Julphar-Gulf pharmaceutical industries, Ras Al Khaima,U.S.E	0155	4/ 2017	1/ 2019
Nilozol 500 mg	Blue Nile Pharmaceutical industries –Khartoum	170103	8/2017	8/2019
Metrodex 500mg	Consolidated pharmaceutical industries Khartoum-sudan	TMF076	5/2016	5/2018

Table (2) : Instruments

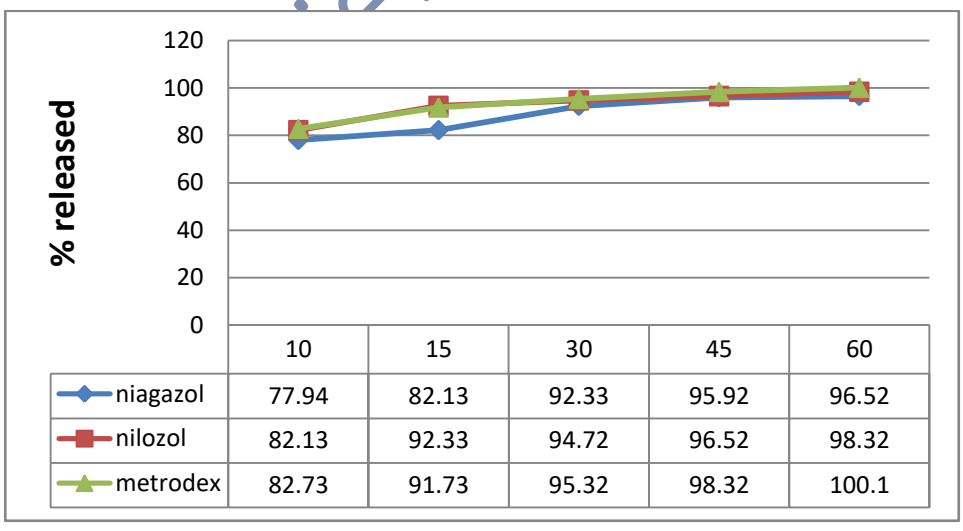
Instrument	Production Country	Name	Serial Number
Sensitive balance	Switzerland	Metter instrument AG	AE 260-5 SNR K 3L1360
Automatic Tablet hardness tester		Tianjin guoming medical equipment	0415021320
Friability tester	Germany	D-63150 Heusentamm	123320.06 ad
Dissolution tester	Germany	Erweka type DT 800 low head Heusrstarmm	
Disintegration tester	germany	erweka, TA120, ,Heusrstarmm	
PH Metter	Switzerland	PH lab , 827, metrohm	
UV spectrophotometer	Shimadzu, Japan	UV min 1240,	

Table (3):Weight uniformity of atenolol tablets

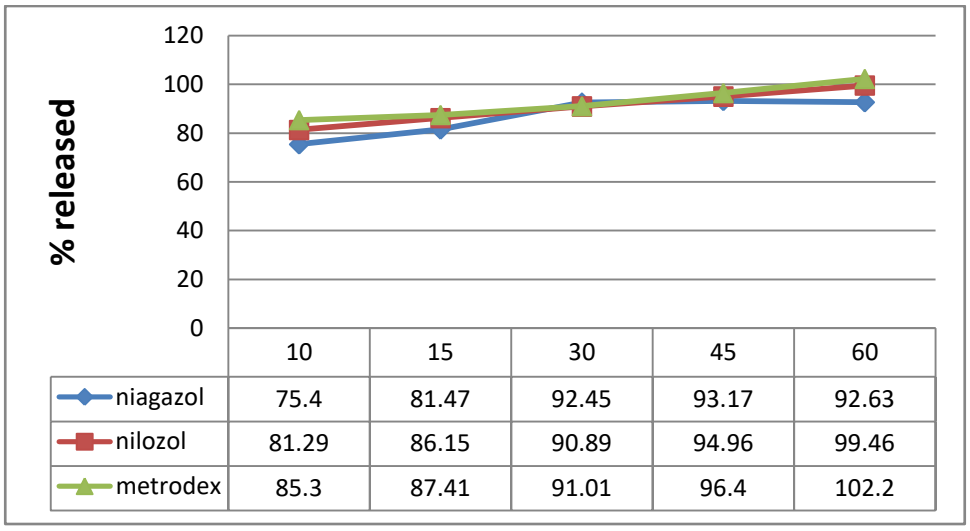
Average weight of tablets	Deviation (%)	Number of tablets
Less than 80 mg	± 10.0 ± 20.0	**Minimum 18** **Maximum 20**
80mg to 250mg	± 7.5 ± 15.0	**Minimum 18** **Maximum 20**
More than 250mg	± 5.0 ± 10.0	**Minimum 18** **Maximum 20**

Table (4): Quality control results of Metronidazole

Brands	Hardness (Kg/cm)	Weight variation (RSD)	Disintegration Time(min)	friability %	Assay %
Sample (A)	**12.0**	**0.00386**	**8:27**	**0.01158**	**99.88**
Sample (B)	**12.5**	**0.0419**	**2:22**	**0.1843**	**98.75**
Sample (C)	**10.7**	**0.0243**	**3:20**	**0.0184**	**99.97**

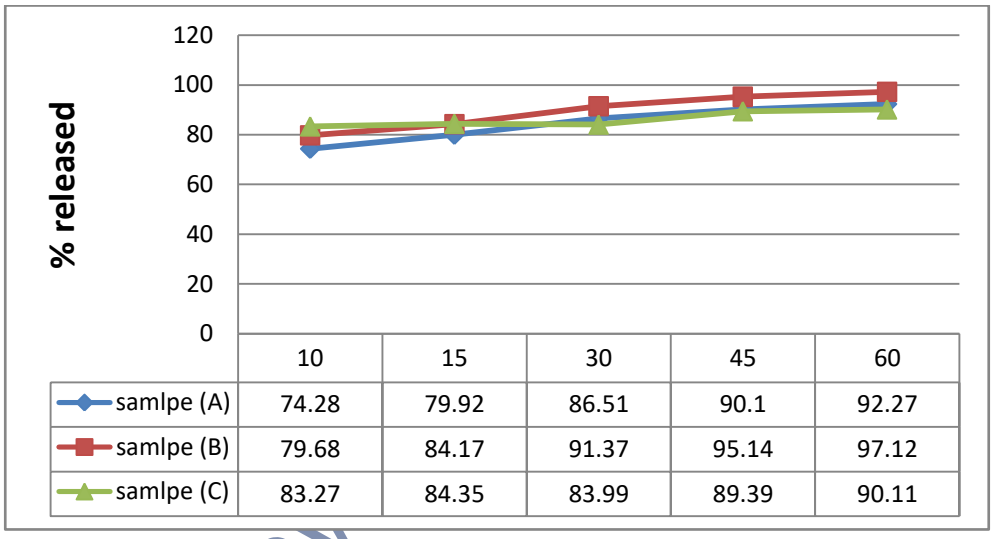


Dissolution profile of Metronidazole in pH (1.2)



	10	15	30	45	60
niagazol	75.4	81.47	92.45	93.17	92.63
nilozol	81.29	86.15	90.89	94.96	99.46
metrodex	85.3	87.41	91.01	96.4	102.2

Dissolution profile of metronidazole in pH (4.5)



	10	15	30	45	60
samlpe (A)	74.28	79.92	86.51	90.1	92.27
samlpe (B)	79.68	84.17	91.37	95.14	97.12
samlpe (C)	83.27	84.35	83.99	89.39	90.11

Dissolution profile of metronidazole in pH (6.8)

Table (5) : F1 and f2 Values:

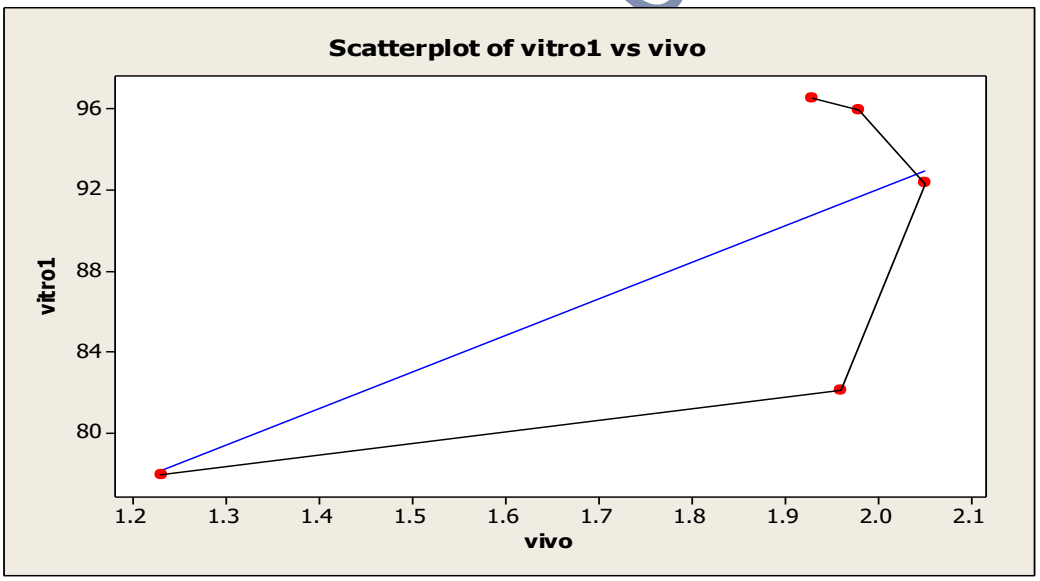
Samples	1.2		4.5		6.8	
	F1	F2	F1	F2	F1	F2
sample (B)	4	64	5	66	6	63
sample (C)	5	63	7	58	4	66

Table (6): Dissolution efficiency for Metronidazole brands:

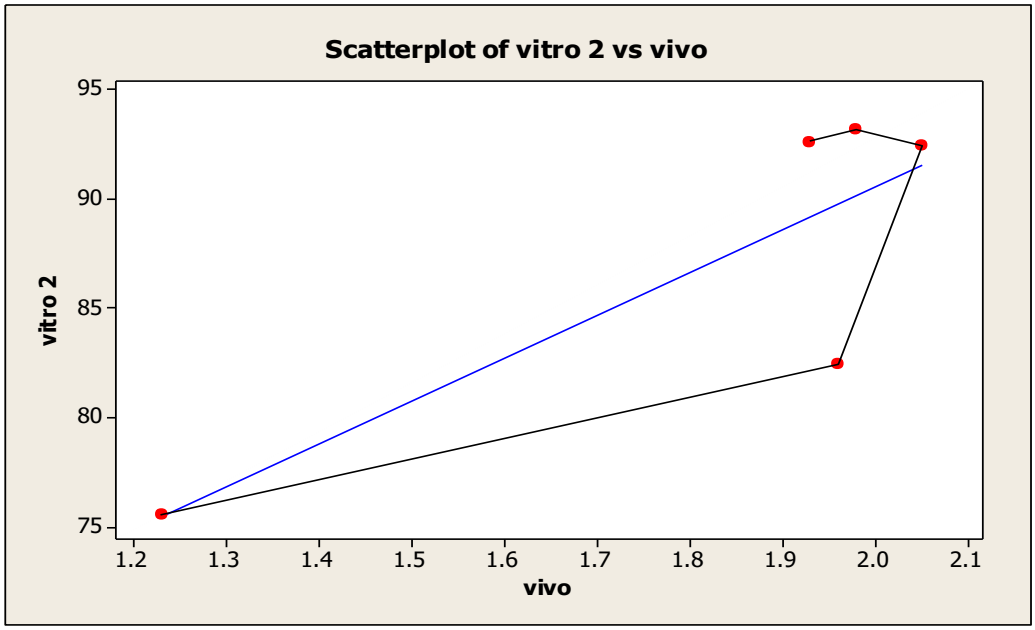
Samples	1.2		4.5		6.8	
	AUC	Difference with reference	AUC	Difference with reference	AUC	Difference with reference
Sample (A)	356.37	-	361.96	-	357.84	-
Sample (B)	364.14	-7.77	350.14	11.82	355.03	-3.19
Sample (C)	361.02	-4.65	345.85	16.11	364.51	-12.73

Table (7): Relative dissolution efficiency of Metronidazole brands:

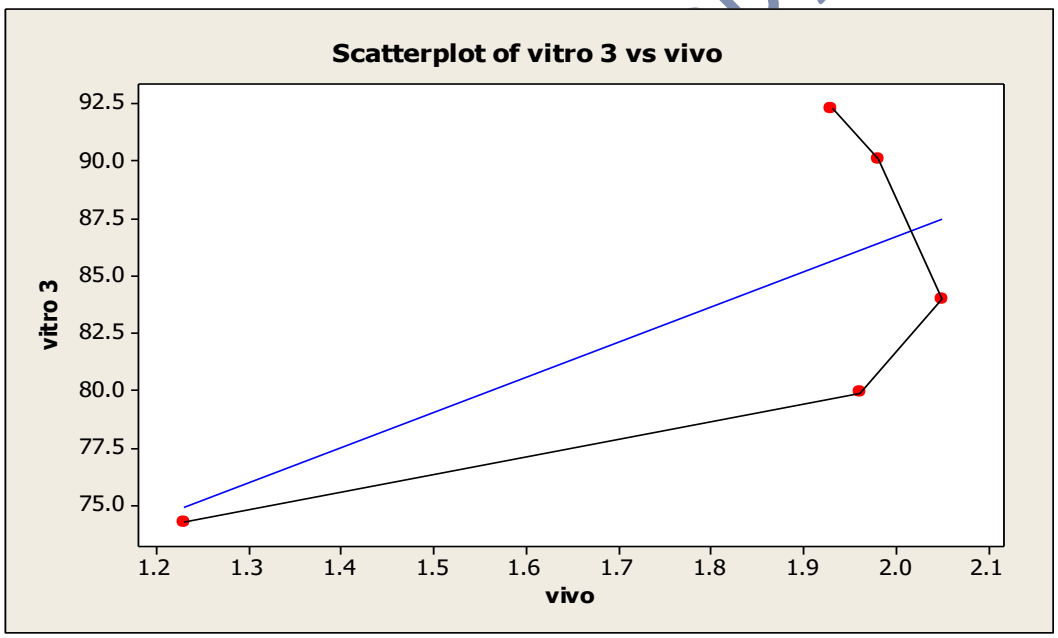
PH	1.2	4.5	6.8
Brand (B)	102.18%	96.73%	**100.91%**
Brand (C)	101.30%	95.45%	**103.62%**



Metronidazole correlation in pH (1.2)



Metronidazole correlation in pH (4.5)



Metronidazole correlation in pH (6.8)