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"MANOVA OVER ANOVA"- A BETTER OBJECTIVE IN BIOEQUIVALENCE STUDY

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ABSTRACT: Bioequivalence studies should be conducted for two products marketed by different licensees, containing same active ingredient(s), must be shown to be therapeutically equivalent to one another order to be considered interchangeable. The bioequivalence of two formulations of the same drug can be determined based on the absence of significant differences in primary pharmacokinetic properties of bioavailability, such as pharmacokinetic parameters like C_{max}, T_{max}, $AUC_{0-t,}$ and $AUC_{0-\infty}$. The pharmacokinetic parameters derived from the plasma concentration-time curve are subjected to ANOVA. So we need to check ANOVAs for all pharmacokinetic parameters. Instead of that we can use multivariate analysis of variance (MANOVA) as it contains ANOVA results and further give more information regarding significance. From the results we can see that we get the same values like ANOVA and additionally we get 4 different tests for significance. Wilk's Lambda shows that 6.9%, 14.1% and 20% of the variance of the dependent variable (C_{max}, T_{max}, $AUC_{0-t,}$ and $AUC_{0-\infty}$) is accounted for by the differences between drugs, phase and interaction respectively. Pillai's Trace, Hotelling's Trace and Roy's largest root says that the data lead to statistical insignificance. So from these results we can suggest MANOVA instead of ANOVA in bioequivalence and control the increase risk of Type I error.

INTRODUCTION: Bioequivalence studies should be conducted for the comparison of two medicinal products containing the same active substance, also compare the expected in vivo biological equivalence of two formulations of a drug [1–4].

The studies should provide an objective means of critically assessing the possibility of alternative use of them. Two products marketed by different licensees, containing same active ingredient(s), must be shown to be therapeutically equivalent to one another order to be considered interchangeable [5]. The bioequivalence of two formulations of the same drug can be determined based on the absence of significant differences in primary pharmacokinetic properties of bioavailability, such as the rates of absorption and elimination, the extent of absorption or total amount of drug absorbed in the body [6].

Pharmacokinetic parameters and Statistical Analysis: C_{max} (This is the maximum drug concentration achieved in systemic circulation following drug administration.), T_{max} (It is the time required to achieve maximum drug concentration in systemic circulation.), AUC_{0-t} (Area under the plasma concentration-time curve from 0 to the last quantifiable concentration to be calculated using the trapezoidal rule.), $AUC_{0-\infty}$ (Area under the plasma concentration-time curve, from zero to infinity to be calculated as the sum of AUC_{0-t} plus the ratio of the

last measurable concentration to the elimination rate constant). Maximal plasma concentration (C_{max}) and time to reach the peak concentration (T_{max}) were obtained directly by the visual inspection of each subject's plasma concentration-time profile. The AUC_{0-t} from time zero to the last quantifiable point (Ct) was calculated using the trapezoidal rule and the extrapolated AUC from Ct to infinity ($AUC_{0-\infty}$) was determined as Ct/K_{el}. The area under the plasma concentration-time from 0 to infinity ($AUC_{0-\infty}$) was calculated as the sum of the AUC_{0-t} plus the ratio of the last measurable concentration to the elimination rate constant. To test the bioequivalence of the test and reference formulations, analysis of variance (ANOVA) for the crossover design was conducted on log-transformed C_{max}, $T_{max,}$ AUC_{0-t}, and $AUC_{0-\infty}$. The pharmacokinetic parameters derived from the plasma concentration-time curve are subjected to ANOVA in which the variance is partitioned into components due to subjects, periods and treatments. In ANOVA null hypothesis is of equal means, test and reference are equivalent (i.e. H_0: $\mu_T = \mu_R$), where μ_T and μ_R represents the expected mean bioavailabilities of the test and reference formulations, respectively. The alternate hypothesis is test and reference is bioinequivalent. (i.e. H_0: $\mu_T \neq \mu_R$). For a crossover trial with n subjects and t treatments, the ANOVA takes the form as shown in **Table 1** [3].

TABLE 1: ANALYSIS OF VARIANCE (ANOVA) TABLE FOR t-PERIOD, T-TREATMENT CROSSOVER DESIGN

Source of variation	Degree of freedom (DF)	Sum of Squares (SS)	Mean sum of squares (MS)	F statistic
Treatment	t^a-1	SST	MST = SST/ t^a-1	MST/MSE
Subject	n^b-1	SSS	MSS=SSS/ n^b-1	MSS/MSE
Period	t-1	SSP	MSP=SSP/ t-1	MSP/MSE
Error	(t-1)(n-2)	SSE	MSE=SSE/(t-1)(n-2)	
Total	tn-1			

[a]t is number of treatments; [b]n is number of subjects

SST-Sum of squares due to treatments; SSS-Sum of squares due to subjects; SSP-Sum of squares due to period; SSE- Sum of squares due to error, MST- Mean sum of squares due to treatments; MSS- Mean sum of squares due to subjects; MSP- Mean sum of squares due to period; MES- Mean sum of squares due to error

ANOVA is to be used to identify the source contributions by factors including subjects, period, formulation and potential interactions. The geometric mean ratio together with the ANOVA residual mean error term, are used to identify the statistical basis for the 90% confidence interval for the ratio of the population means (New Formulation/Original Formulation). The products were considered bioequivalent if the difference between the two compared parameters was statistically insignificant (P >0.05).

Why MANOVA over ANOVA: MANOVA is used under the same circumstances as ANOVA but when there are multiple dependent variables as well as independent variables within the model which we wish to test. MANOVA is considered as a valid alternative to the repeated measures ANOVA when sphericity is violated. In bioequivalence study we need to examine different ANOVAs for each pharmacokinetic parameter. However, since the pharmacokinetic parameters are related, the results from separate ANOVAs would not be independent. Using multiple ANOVAs would increase the risk of Type I error (rejecting the null hypothesis when it is true).

MANOVA deals with the multiple dependent variables by combining them in a linear manner to produce a combination which best separates the independent variable groups. An ANOVA is then performed on the newly developed dependent variable. In MANOVA, the independent variables relevant to each main effect are weighted to give them priority in the calculations performed. In interactions the independent variables are equally weighted to determine whether or not they have an additive effect in terms of the combined variance they account for in the dependent variables.

Like an ANOVA, MANOVA examines the degree of variance within the independent variables and determines whether it is smaller than the degree of variance between the independent variables. If the within subjects variance is smaller than the between subjects variance it means the independent variable has had a significant effect on the dependent variables.

There are two main differences between MANOVAs and ANOVAs. The first is that MANOVAs are able to take into account multiple independent and multiple dependent variables within the same model, permitting greater complexity. Secondly, rather than using the F value as the indicator of significance a number of multivariate measures (Wilks' lambda, Pillai's trace, Hotelling trace and Roy's largest root) are used. The difference between the four measures is the ways in which they combine the dependent variables in order examine the amount of variance in the data.

Wilks' lambda: Wilks' lambda demonstrates the amount of variance accounted for in the dependent variable by the independent variable; the smaller the value, the larger the difference between the groups being analyzed. 1 minus Wilks' lambda indicates the amount of variance in the dependent variables accounted for by the independent variables.

Pillai's trace: Pillai's trace is considered the most reliable of the multivariate measures and offers the greatest protection against Type I errors with small sample sizes. Pillai's trace is the sum of the variance which can be explained by the calculation of discriminant variables. It calculates the amount of variance in the dependent variable which is accounted for by the greatest separation of the independent variables.

Hotelling-Lawley trace: The Hotelling-Lawley trace is generally converted to the Hotelling's T-square. Hotelling's T is used when the independent variable forms two groups and represents the most significant linear combination of the dependent variables.

Roy's largest root: Roy's largest root, also known as Roy's largest eigenvalue, is calculated in a similar fashion to Pillai's trace except it only considers the largest eigenvalue (i.e. the largest loading onto a vector). As the sample sizes increase the values produced by Pillai's trace, Hotelling-Lawley trace and Roy's largest root become similar. Wilks' lambda is the easiest to understand and therefore the most frequently used measure.

DESCRIPTION OF THE STUDY: Fenofibrate is a lipid-lowering agent introduced internationally in 1975 and now used in >80 countries. It has become one of the world's most widely prescribed pharmacologic treatments for hypercholesterolemia, combined dyslipidemia, remnant hyperlipidemia, endogenous hyperlipemia (hypertriglyceridemia), and mixed hyperlipemia (Frederickson types IIa, IIb, III, IV and V dyslipidemia, respectively) [7, 8].

Fenofibrate is a prodrug [9, 10]. After oral administration, it is rapidly converted through hydrolysis of the ester bond to its active form and major metabolite, fenofibric acid. Plasma levels of fenofibric acid peak 6 to 8 hours after oral administration, and food enhances its absorption [11-13]. The extent of absorption of fenofibrate tablets is increased approximately 35% under fed as compared to fasting conditions [8].

Steady-state plasma levels are reached within 5 days of dosing, and no accumulation has been observed in healthy volunteers following multiple doses [9]. Fenofibric acid is metabolized by the hepatic cytochrome P (CYP)-450 3A4 isozyme and has a half-life ($t_{1/2}$) of 20 hours, which allows once-daily administration. Fenofibrate is mainly excreted in urine as metabolites, primarily fenofibric acid and fenofibric acid glucuronide.

Since fenofibrate was first made commercially available, its main drawback has been the low bioavailability of the active metabolite, fenofibric acid, when the prodrug is taken orally on an empty stomach [8, 12-18]. Fenofibrate is virtually insoluble in water and is highly lipophilic, hence it is poorly absorbed when taken orally, especially under fasting conditions [2, 9].

In contrast, its absorption is substantially increased in the presence of food [7, 14, 18]. Therefore, product labeling of formulations marketed to date have mandated administering the drug with meals, even for newer fenofibrate formulations such as micronized capsule and a micro coated tablet, that were introduced to improve bioavailability [7,14,12].

MATERIAL AND METHODS: The study was carried out at the B. V. Patel Pharmaceutical Education and Research Development centre, Ahmedabad. 18 subjects provided written informed consent to participate in the study prior to enrolment and were free to withdraw at any time during the study. The study was approved by the institutional ethics committee and was conducted in accordance with good clinical practice and the declaration of Helsinki.

Study Subjects: The study population consisted of 18, adult, male healthy Indian subjects with mean BMI 21.7 (range 19.14 – 24.21), a mean age of 32.2 years (range 25 - 44), mean weight of 59.8 kg (range 48 - 69) and a mean height of 165.6 cm. (range 154 - 177)

Design: The study was designed as Single labeled, Balanced, Randomized, Two- Treatment, Two-Sequence, Two Period, Single Dose, Crossover Bioequivalence study with a 14 days washout period. The volunteers were administered one of the two study drugs after standardized meal. The dose administration was performed as per the randomization schedule generated at B.V. Patel PERD Centre, Ahmedabad. Subjects received single oral doses of the test formulation (fenofibrate 145 mg) and reference formulation (fenofibrate 145 mg).

Blood sampling: A total of 16 blood samples were collected during each period. Blood samples were collected through an indwelling cannula placed in the forearm vein using disposable syringe or with disposable syringes and needles. 6 mL of blood samples (including 0.2 mL discarded heparinised blood) were withdrawn at pre-dose and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0, 14.0, 24.0, 36.0, 48.0, 72.0 and 96.0 hrs following drug administration in each period. After centrifugation, plasma separated from blood samples and was stored at –20 ± 5°C for interim storage and then at –80 ± 4°C until analysis.

Safety and Tolerability: General clinical safety was assessed *via* physical examinations and vital signs conducted at screening and at the end of the study. Clinical laboratory tests and ECGs were also conducted at screening, before dosing within each treatment period, and at the end of the study. Adverse events were assessed for severity and relationship to treatment throughout the study.

Pharmacokinetic data of the study: Table 2 contains the each individual pharmacokinetic parameters of the test and reference formulation of Fenofibrate.

TABLE 2: DATA SHOWS THE PHARMACOKINETIC PARAMETERS FOR THE TEST AND REFERENCE DRUG.

Subject	A = Reference Formulation				B = Test Formulation			
	C_{max} (µg/ml)	T_{max} (h)	AUC_{0-t} (µg.h/ml)	$AUC_{0-\infty}$ (µg.h/ml)	C_{max} (µg/ml)	T_{max} (h)	AUC_{0-t} (µg.h/ml)	$AUC_{0-\infty}$ (µg.h/ml)
1	9.52	6	238.98	245.35	6.16	5	153.9	158.75
2	10.31	5	222.33	232.95	5.82	4	185.2	199.26
3	5.17	4	104.47	107.20	5.68	4	96.8	99.21
4	6.73	4	79.12	79.55	6.98	4	89.4	90.46
5	5.33	3	124.01	127.02	5.47	3	112.1	117.15
6	5.07	4	152.3	170.99	7.30	7	212.4	229.11
7	7.30	3	136	139.49	7.06	6	130.6	133.26
8	6.24	4	105.6	108.50	5.5	4	105.4	114.82
9	8.37	5	192.8	208.00	5.10	5	149.4	161.50
10	8.52	4	194.8	208.43	10.88	3	202.6	207.92
11	10.16	5	154.6	161.77	8.32	5	152.1	159.84
12	6.15	3	145.9	152.12	4.38	4	146.4	155.31
13	8.18	4	105.7	106.62	5.24	5	78.7	79.39
14	3.15	4	103.4	107.98	3.61	3	86.9	90.21
15	3.41	4	55.3	55.74	5.25	4	83.3	83.84
16	6.56	4	206.9	225.37	5.50	5	172.6	189.00
17	12.13	7	275.6	294.93	10.60	4	175.5	194.86
18	5.84	5	165.2	170.52	6.35	4	179.1	191.00

To run MANOVA in SPSS 16.0 software dependent variables are pharmacokinetic parameters (C_{max}, T_{max}, AUC_{0-t} and $AUC_{0-\infty}$) and independent (fixed) factors are drug (test/reference) and phases (phase I/phase II).

RESULTS:

ANOVA for C_{max}:

TABLE 3: DESCRIPTIVE STATISTICS OF C_{max}

Drug	Phase	Mean	Std. Deviation	N
Reference	Phase I	6.7300	1.86439	9
	Phase II	7.5078	2.93641	9
	Total	7.1189	2.41940	18
Test	Phase I	6.8200	2.52322	9
	Phase II	5.9800	1.03035	9
	Total	6.4000	1.91896	18
Total	Phase I	6.7750	2.15266	18
	Phase II	6.7439	2.27488	18
	Total	6.7594	2.18280	36

Dependent Variable: C_{max}

TABLE 4: LEVENE'S TEST OF EQUALITY OF ERROR VARIANCE[a] FOR C_{max}

F	df1	df2	Sig.
3.781	3	32	0.020

Dependent Variable: C_{max}; Tests the null hypothesis that the error variance of the dependent variable is equal across groups. [a]. Design: Intercept + Drug + Phase + Drug * Phase

TABLE 5: TESTS OF BETWEEN-SUBJECTS EFFECTS FOR C_{max}

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	10.549[a]	3	3.516	0.720	0.547
Intercept	1644.843	1	1644.843	336.942	0.000
Drug	4.651	1	4.651	0.953	0.336
Phase	0.009	1	0.009	0.002	0.967
Drug * Phase	5.889	1	5.889	1.206	0.280
Error	156.214	32	4.882		
Total	1811.605	36			
Corrected Total	166.762	35			

Dependent Variable:C_{max}; [a.] R Squared = 0.063 (Adjusted R Squared = -0.025)

ANOVA for T_{max}:

TABLE 6: DESCRIPTIVE STATISTICS OF T_{max}

Drug	Phase	Mean	Std. Deviation	N
Reference	Phase I	4.2222	.97183	9
	Phase II	4.4444	1.13039	9
	Total	4.3333	1.02899	18
Test	Phase I	3.8889	0.78174	9
	Phase II	4.8889	1.05409	9
	Total	4.3889	1.03690	18
Total	Phase I	4.0556	0.87260	18
	Phase II	4.6667	1.08465	18
	Total	4.3611	1.01848	36

Dependent Variable: T_{max}

TABLE 7: LEVENE'S TEST OF EQUALITY OF ERROR VARIANCE[a] FOR T_{max}

F	df1	df2	Sig.
0.241	3	32	0.867

Dependent Variable:T_{max}, Tests the null hypothesis that the error variance of the dependent variable is equal across groups. [a.] Design: Intercept + Drug + Phase + Drug * Phase

TABLE 8: TESTS OF BETWEEN-SUBJECTS EFFECTS FOR T_{max}

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4.750[a]	3	1.583	1.606	0.207
Intercept	684.694	1	684.694	694.338	0.000
Drug	0.028	1	0.028	0.028	0.868
Phase	3.361	1	3.361	3.408	0.074
Drug * Phase	1.361	1	1.361	1.380	0.249
Error	31.556	32	0.986		
Total	721.000	36			
Corrected Total	36.306	35			

Dependent Variable: T_{max}, [a.] R Squared = 0.131 (Adjusted R Squared = 0.049)

ANOVA for AUC_{0-t}:

TABLE 9: DESCRIPTIVE STATISTICS OF AUC_{0-t}

Drug	Phase	Mean	Std. Deviation	N
Reference	Phase I	1.4126E2	56.42855	9
	Phase II	1.6574E2	62.18373	9
	Total	1.5350E2	58.96459	18
Test	Phase I	1.4323E2	43.27250	9
	Phase II	1.3591E2	45.50615	9
	Total	1.3957E2	43.24195	18
Total	Phase I	1.4225E2	48.79195	18
	Phase II	1.5083E2	55.04277	18
	Total	1.4654E2	51.44736	36

Dependent Variable: AUC_{0-t}

TABLE 10: LEVENE'S TEST OF EQUALITY OF ERROR VARIANCE[a] FOR AUC_{0-t}

F	df1	df2	Sig.
0.241	3	32	0.867

Dependent Variable: AUC_{0-t}, Tests the null hypothesis that the error variance of the dependent variable is equal across groups. a. Design: Intercept + Drug + Phase + Drug * Phase

TABLE 11: TESTS OF BETWEEN-SUBJECTS EFFECTS FOR AUC_{0-t}

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4684.541[a]	3	1561.514	0.568	0.640
Intercept	773023.559	1	773023.559	281.245	0.000
Drug	1745.366	1	1745.366	0.635	0.431
Phase	662.938	1	662.938	0.241	0.627
Drug * Phase	2276.236	1	2276.236	0.828	0.370
Error	87954.526	32	2748.579		
Total	865662.625	36			
Corrected Total	92639.066	35			

Dependent Variable: AUC_{0-t}, [a.] R Squared = 0.051 (Adjusted R Squared = -0.038).

ANOVA for $AUC_{0-\infty}$

TABLE 12: DESCRIPTIVE STATISTICS OF $AUC_{0-\infty}$

Drug	Phase	Mean	Std. Deviation	N
Reference	Phase I	1.4760E2	60.32269	9
	Phase II	1.7490E2	68.35799	9
	Total	1.6125E2	64.09958	18
Test	Phase I	1.5247E2	47.20704	9
	Phase II	1.4251E2	51.06314	9
	Total	1.4749E2	47.97921	18
Total	Phase I	1.5004E2	52.60603	18
	Phase II	1.5871E2	60.85827	18
	Total	1.5437E2	56.23577	36

Dependent Variable: $AUC_{0-\infty}$

TABLE 13: LEVENE'S TEST OF EQUALITY OF ERROR VARIANCE[a] FOR $AUC_{0-\infty}$

F	df1	df2	Sig.
0.752	3	32	0.529

Dependent Variable:$AUC_{0-\infty}$. Tests the null hypothesis that the error variance of the dependent variable is equal across groups. [a.] Design: Intercept + Drug + Phase + Drug * Phase

TABLE 14: TESTS OF BETWEEN-SUBJECTS EFFECTS FOR $AUC_{0-\infty}$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5505.433[a]	3	1835.144	0.558	0.646
Intercept	857913.705	1	857913.705	261.010	0.000
Drug	1703.228	1	1703.228	0.518	0.477
Phase	677.060	1	677.060	0.206	0.653
Drug * Phase	3125.146	1	3125.146	0.951	0.337
Error	105180.721	32	3286.898		
Total	968599.860	36			
Corrected Total	110686.154	35			

Dependent Variable: $AUC_{0-\infty}$. [a]. R Squared = 0.050 (Adjusted R Squared = -0.039)

MANOVA for all pharmacokinetic parameters:

TABLE 15: DESCRIPTIVE STATISTICS OF ALL PHARMACOKINETIC PARAMETERS

	Drug	Phase	Mean	Std. Deviation	N
$AUC_{0-\infty}$	Reference	Phase I	1.4760E2	60.32269	9
		Phase II	1.7490E2	68.35799	9
		Total	1.6125E2	64.09958	18
	Test	Phase I	1.5247E2	47.20704	9
		Phase II	1.4251E2	51.06314	9
		Total	1.4749E2	47.97921	18
	Total	Phase I	1.5004E2	52.60603	18
		Phase II	1.5871E2	60.85827	18
		Total	1.5437E2	56.23577	36
C_{max}	Reference	Phase I	6.7299	1.86638	9
		Phase II	7.5064	2.93711	9
		Total	7.1182	2.42042	18
	Test	Phase I	6.8178	2.52154	9
		Phase II	5.9778	1.03011	9
		Total	6.3978	1.91786	18
	Total	Phase I	6.7738	2.15252	18
		Phase II	6.7421	2.27541	18
		Total	6.7580	2.18301	36
AUC_{0-t}	Reference	Phase I	1.4126E2	56.42855	9
		Phase II	1.6574E2	62.18373	9
		Total	1.5350E2	58.96459	18
	Test	Phase I	1.4323E2	43.27250	9
		Phase II	1.3591E2	45.50615	9
		Total	1.3957E2	43.24195	18
	Total	Phase I	1.4225E2	48.79195	18
		Phase II	1.5083E2	55.04277	18
		Total	1.4654E2	51.44736	36
T_{max}	Reference	Phase I	4.2222	.97183	9
		Phase II	4.4444	1.13039	9
		Total	4.3333	1.02899	18
	Test	Phase I	3.8889	.78174	9
		Phase II	4.8889	1.05409	9
		Total	4.3889	1.03690	18
	Total	Phase I	4.0556	.87260	18
		Phase II	4.6667	1.08465	18
		Total	4.3611	1.01848	36

TABLE 16: BOX'S TEST OF EQUALITY OF COVARIANCE MATRICES[a] FOR ALL PHARMACOKINETIC PARAMETERS

Box's M	32.217
F	0.822
df1	30
df2	2.815E3
Sig.	0.741

Tests the null hypothesis that the observed covariance matrices of the dependent variables are equal across groups. [a]. Design: Intercept + Drug + Phase + Drug * Phase

TABLE 17: MULTIVARIATE TESTS[c] FOR ALL PHARMACOKINETIC PARAMETERS

	Effect	Value	F	Hypothesis df	Error df	Sig.	Noncent. Parameter	Observed Power[b]
Intercept	Pillai's Trace	0.961	1.765E2[a]	4.000	29.000	0.000	705.805	1.000
	Wilks' Lambda	0.039	1.765E2[a]	4.000	29.000	0.000	705.805	1.000
	Hotelling's Trace	24.338	1.765E2[a]	4.000	29.000	0.000	705.805	1.000
	Roy's Largest Root	24.338	1.765E2[a]	4.000	29.000	0.000	705.805	1.000
Drug	Pillai's Trace	0.069	0.541[a]	4.000	29.000	0.707	2.163	0.161
	Wilks' Lambda	0.931	0.541[a]	4.000	29.000	0.707	2.163	0.161
	Hotelling's Trace	0.075	0.541[a]	4.000	29.000	0.707	2.163	0.161
	Roy's Largest Root	0.075	0.541[a]	4.000	29.000	0.707	2.163	0.161
Phase	Pillai's Trace	0.141	1.192[a]	4.000	29.000	0.335	4.769	0.325
	Wilks' Lambda	0.859	1.192[a]	4.000	29.000	0.335	4.769	0.325
	Hotelling's Trace	0.164	1.192[a]	4.000	29.000	0.335	4.769	0.325
	Roy's Largest Root	0.164	1.192[a]	4.000	29.000	0.335	4.769	0.325
Drug * Phase	Pillai's Trace	0.200	1.811[a]	4.000	29.000	0.154	7.244	0.483
	Wilks' Lambda	0.800	1.811[a]	4.000	29.000	0.154	7.244	0.483
	Hotelling's Trace	0.250	1.811[a]	4.000	29.000	0.154	7.244	0.483
	Roy's Largest Root	0.250	1.811[a]	4.000	29.000	0.154	7.244	0.483

[a.] Exact Statistic. [b.] Computed using alpha = 0.05. [c.] Design: Intercept + Drug + Phase + Drug*phase

TABLE 18: LEVENE'S TEST OF EQUALITY OF ERROR VARIANCES[a] FOR ALL PHARMACOKINETIC PARAMETERS

	F	df1	df2	Sig.
$AUC_{0-\infty}$	0.752	3	32	0.529
C_{max}	3.774	3	32	0.020
AUC_{0-t}	0.700	3	32	0.559
T_{max}	0.241	3	32	0.867

Tests the null hypothesis that the error variance of the dependent variable is equal across groups. [a.] Design: Intercept + Drug + Phase + Drug * Phase

TABLE 19: TESTS OF BETWEEN-SUBJECTS EFFECTS FOR ALL PHARMACOKINETIC PARAMETERS

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power[b]
Corrected Model	$AUC_{0-\infty}$	5505.433[a]	3	1835.144	0.558	0.646	1.675	0.152
	C_{max}	10.560[c]	3	3.520	0.721	0.547	2.163	0.186
	AUC_{0-t}	4684.541[d]	3	1561.514	0.568	0.640	1.704	0.154
	T_{max}	4.750[e]	3	1.583	1.606	0.207	4.817	0.381
Intercept	$AUC_{0-\infty}$	857913.705	1	857913.705	261.010	0.000	261.010	1.000
	C_{max}	1644.127	1	1644.127	336.751	0.000	336.751	1.000
	AUC_{0-t}	773023.559	1	773023.559	281.245	0.000	281.245	1.000
	T_{max}	684.694	1	684.694	694.338	0.000	694.338	1.000
Drug	$AUC_{0-\infty}$	1703.228	1	1703.228	0.518	0.477	0.518	0.107
	C_{max}	4.671	1	4.671	0.957	0.335	0.957	0.158

	AUC_{0-t}	1745.366	1	1745.366	0.635	0.431	0.635	0.121
	T_{max}	.028	1	0.028	0.028	0.868	0.028	0.053
Phase	$AUC_{0-\infty}$	677.060	1	677.060	0.206	0.653	0.206	0.072
	C_{max}	.009	1	0.009	0.002	0.966	0.002	0.050
	AUC_{0-t}	662.938	1	662.938	0.241	0.627	0.241	0.076
	T_{max}	3.361	1	3.361	3.408	0.074	3.408	0.433
Drug * Phase	$AUC_{0-\infty}$	3125.146	1	3125.146	0.951	0.337	0.951	0.157
	C_{max}	5.880	1	5.880	1.204	0.281	1.204	0.187
	AUC_{0-t}	2276.236	1	2276.236	0.828	0.370	0.828	0.143
	T_{max}	1.361	1	1.361	1.380	0.249	1.380	0.207
Error	$AUC_{0-\infty}$	105180.721	32	3286.898				
	C_{max}	156.234	32	4.882				
	AUC_{0-t}	87954.526	32	2748.579				
	T_{max}	31.556	32	0.986				
Total	$AUC_{0-\infty}$	968599.860	36					
	C_{max}	1810.920	36					
	AUC_{0-t}	865662.625	36					
	T_{max}	721.000	36					
d	$AUC_{0-\infty}$	110686.154	35					
	C_{max}	166.794	35					
	AUC_{0-t}	92639.066	35					
	T_{max}	36.306	35					

[a.] R squared = 0.050 (Adjusted R squared = 0.039). [b.] Computed using alpha = 0.05. [c.] R squared = 0.063 (Adjusted R squared = -0.025). [d.] R squared = 0.051 (Adjusted R squared = -0.038). [e.] R squared = 0.131 (Adjusted R squared = 0.049)

DISCUSSION: Table 3, 6, 9, 12, 15 provides the mean and standard deviation for the groups that have been split by both independent variables. In addition, the tables also provide "total" rows, which allow means and standard deviations for groups only split by one independent variable for all dependent variables.

ANOVA-MANOVA comparison: Table 4, 7, 10, 13 and 18 shows Levene's test of equality of error variances of all dependent variables. Levene's test and Box's M test are almost same but the only difference is this test is concern about variance only. From table 4 we can see that we have homogeneity of variances of the dependent variables across groups. Here Sig. = 0.020 < 0.05 (level of alpha), so from this we can say that the variance across groups was significantly different for dependent variables. From table 7, 10 and 13 we have sig. = 0.867 > 0.05, sig. = 0. 559 > 0.05 and sig. = 0.529 > 0.05, so we can say that the variance across groups was not significantly different for dependent variable T_{max}, AUC_{o-t}, $AUC_{o-\infty}$. Same values we have in MANOVA analysis table 18 for all the dependent variables.

In bioequivalence study, instead of doing different ANOVAs for pharmacokinetic parameters we can do MANOVA and have the same results like ANOVA. Further MANOVA has four tests, from that we can interpret more our data instead of ANOVA. **Table 5, 8, 11, 14** shows the test of between-subject effects (ANOVA) and Table no. 19 shows the ANOVA results from MANOVA analysis for all dependent variables. Table 5, 8, 11, 14 indicate that whether significant mean differences between groups for two independent variables (drug and phase) and for their interaction (drug*phase) for all dependent variables. From Table 5, 8, 11 and 14 we can say that drug*phase interaction have a statistically significant interaction at the p=0.280 level, p = 0.249, p = 0.370 and p = 0.337 respectively.

We can say from tables, there was no significant difference in dependent variables between two drugs (p = 0.336>0.05), (p = 0.868>0.05), (p = 0.431>0.05) and (p = 0.477>0.05) respectively and similarly for phases (p = 0.967>0.05), (p = 0.074>0.05), (p = 0.627>0.05) and (p = 0.653>0.05). From **Table 19** tests between-subjects effect for all pharmacokinetic parameters we can see four dependent variables, F column shows the value of F ratio and Sig. column shows the significance of that F ratio. So comparing this table with the Table no. 5, 8, 11 and 14 we have the same results.

Multivariate Tests Analysis: Table 16 shows Box's Test of equality of covariance matrices. This test in effect asks whether the correlations between the dependent variables and the standard deviations are similar over groups.

In this table the Box's test is not significant, so the variance-covariance matrices can be pooled without any concern. Here sig. = 0.741>0.05. Therefore, the variance-covariance matrices are equal.

Table 17 shows Multivariate test of the analysis. All 4 tests explore whether the means for phases and drugs are the same or not. Among 4 tests the most commonly used and accepted statistic is Wilk's Lambda. It is a statistics to test whether there are differences between the means of identified groups of subjects on a combination of dependent variables. T-test, Hotelling's T and F-test are special cases of Wilk's Lambda.

It is a measure of the percent of variance in the dependent variables that is not explained by differences in the level of the independent variables (drug and phase).

Here we have λ=0.931, F (4, 29) = 0.541, P (0.707)>0.001 for drug, for phase λ=0.859, F (4, 29) = 1.192, P (0.335)>0.001, and for interaction term (drug*phase) we have λ=0.800, F (4, 29) = 1.811, P (0.154)>0.001. Therefore, from this result we can say that 6.9%, 14.1% and 20% of the variance of the dependent variable is accounted for by the differences between drugs, phase and interaction respectively.

The value of Pillai's Trace is a positive valued statistic and it shows the proportion of variance in the dependent variables which is accounted for by variation in the independent variables. Here we have 0.069, 0.141 and 0.200 which is very small value that lead to statistical insignificance.

Hotelling's Trace is the sum of the eigen values of the test matrix and it is a positive valued statistic for which increasing values indicate effects that contribute more to the model. Roy's largest root is similar to the Pillai's trace but is based only on the first root. It is less robust than the other tests in the face of violations of the assumptions of multivariate normality.

Same like other tests larger the root, the more that effect contributes to the model. Here we have Hotelling's trace and Roy's largest root's values are 0.075, 0.164 and 0.250 for drug, phase and interaction respectively. This shows smaller values that lead to statistical insignificance.

CONCLUSION: The concept of BE has been accepted worldwide by the pharmaceutical industry and national regulatory authorities for over 20 years and is applied to new as well as generic products. As a result, thousands of high-quality generic drugs at reduced costs have become available in every corner of the globe.

The assessment of BE is not a simple issue, however, and much of the research has been done in recent years to develop new and more effective approaches to the assessment of BE. Statistical analysis is a part of BE and we need to abridge it in such a way that it involve less time and more construal from the data.

The essential feature of doing MANVOA is we have complete ANOVA results and adding the multivariate results. So from that we can check the significance of the dependent variables.

Additionally we get multivariate analysis. The value of Wilk's Lambda shows the proportion of the total variance of the dependent variable which is not accounted for by the independent variables. Therefore, smaller the value of Lambda corresponds to larger differences between groups (or strong associations between the dependent variables and numeric independent variables). Here we have larger values of Wilks lambda that shows minimum differences between groups or we can say weak association between the dependent variables and independent variables.

If Pillai's Trace has large value then the more the given effect contributes to the model. In other words same like Hotelling's trace and Roy's largest root, increasing values of the statistic indicate effects that contribute more to the model. So here we have small value of all the three tests shows statistical insignificance to the model.

So now we can say that we can use MANOVA instead of doing separate ANOVA. And we can control the increase the risk of Type I error.

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