

## **Analysis of pharmacokinetic data by wilk's lambda (An important tool of manova)**

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**ABSTRACT:** *In the present research article, we applied Wilks Lambda on two different pharmacokinetic studies. In the first study we check the bioavailability of the three different formulations (Dynapar QPS). We check the effectiveness of these formulations using Wilks Lambda. We found no significant difference in the effectiveness as measured by pharmacokinetic parameters. For the second study, we test the significance effects of the two drugs (reference and test), of three different formulations and with their interaction affects using Wilks Lambda. We found no significant difference on pharmacokinetic parameters (Cmax, Tmax), formulations and interaction.*

**Keywords:** *Bioavailability, Formulation, MANOVA, Pharmacokinetic*

### **I. INTRODUCTION**

Multivariate analysis of variance (MANOVA) is simply an ANOVA with several dependent variables. The MANOVA is a type of multivariate analysis used to analyze data that involves more than one dependent variable at a time. Like ANOVA, MANOVA has variations. For example, the one-way MANOVA contains a single factor (independent variable) distinguishing participants into groups and two or more quantitative dependent variables. For example, one could do three separate one-way ANOVAs; however, using MANOVA, we can see how the combination of the three variables distinguishes the groups, in one analysis. There is a two-way or two-factor MANOVA that has two independent variables and two or more quantitative dependent variables. A doubly multivariate or mixed MANOVA has a between groups independent variable and a repeated measures (within groups) independent variable and two or more quantitative dependent variables [1]. MANOVA allows us to test hypotheses regarding the effect of one or more independent variables on two or more dependent variables. A MANOVA analysis generates a p-value that is used to determine whether or not the null hypothesis can be rejected.

As with ANOVA, the independent variables for a MANOVA are factors, and each factor has two or more levels. Unlike ANOVA, MANOVA includes multiple dependent variables rather than a single dependent variable. MANOVA evaluates whether the population means on a set of dependent variables vary across the levels of a factor or factors. That is, a one-way MANOVA tests the hypothesis that the population means for the dependent variables are the same for all levels of a factor (across all groups). If the population means of the dependent variables are equal for all groups, the population means for any linear combination of these dependent variables are also equal for all groups. Consequently, a one-way MANOVA evaluates a hypothesis that includes not only equality among groups on the dependent variable, but also equality among groups on linear combinations of these dependent variables.

The multivariate analysis of variance (MANOVA) is a complex statistic similar to ANOVA but with multiple dependent variables analyzed together. That is, the MANOVA is a multivariate extension of ANOVA. The dependent variables should be related conceptually, and they should be correlated with one another at a low to moderate level. If they are highly correlated, one runs the risk of multicollinearity. If they are uncorrelated, there is usually no reason to analyze them together. [2]

The following is a short list of some of the popularly reported test statistics for MANOVA:

- **Wilks's lambda** : pooled ratio of error variances to effect variance plus error variance
- Pillai's trace : pooled effect variances
- Lawley–Hotelling trace : pooled ratio of effect variance to error variance
- Roy's largest root : largest eigen value

### **II. METHODOLOGY OF WILKS LAMBDA**

The most widely used of the available test statistics is Wilk's lambda [3, 4]. Wilk's lambda is widely used in pharmaceutical industry and medical research [5]. Which is based on three matrices W (the within group matrix of sums of squares and products), T (the total matrix of sums of squares and cross-products) and B (the between group matrix of sums of squares and cross-products), defined as follows:

$$T = \sum_{i=1}^g \sum_{j=1}^{n_i} (X_{ij} - \bar{X})(X_{ij} - \bar{X})$$

$$W = \sum_{i=1}^g \sum_{j=1}^{n_i} (X_{ij} - \bar{X}_i)(X_{ij} - \bar{X}_i)$$

$$B = \sum_{i=1}^g n_i (X_i - \bar{X})(X_i - \bar{X})$$

Where  $X_{ij}$ ,  $i=1, \dots, g$ ,  $j=1, \dots, n_i$  represent the  $j^{\text{th}}$  multivariate observation in the  $i^{\text{th}}$  group,  $g$  is the number of groups and  $n_i$  is the number of observations in the  $i^{\text{th}}$  group. The mean vector of the  $i^{\text{th}}$  group is represented by  $(\bar{X}_i)$  and the mean vector of all the observations by  $(\bar{X})$ . These matrices satisfy the equation  $T = W + B$ . Wilk's lambda is given by the ratio of the determinants of W and T, i.e.

$$\Lambda = \frac{|W|}{|T|} = \frac{|W|}{|W + B|}$$

The statistic  $\Lambda$  can be transformed to give an F-test to assess the null hypothesis of the equality of the population mean vectors. Wilks lambda ranges from 0 – 1 and the lower the Wilks lambda, the larger the between group dispersion. A small (close to 0) value of Wilks' lambda means that the groups are well separated. A large (close to 1) value of Wilks' lambda means that the groups are poorly separated.

Wilks' lambda statistic can be transformed (mathematically adjusted) to a statistic which has approximately F distribution. This makes it easier to calculate the P-value. Often authors will present the F-value and degrees of freedom rather than giving the actual value of Wilks' lambda. Wilks Lambda has the virtue of being convenient and related to the likelihood ratio criterion. The exact distribution of Wilks lambda can be derived for the special cases listed in table 1[6].

**TABLE: 1 Distribution of Wilks Lambda.**

No. of Variables	No. of Groups	Sampling Distribution for Multivariate Normal Data
$P = 1$	$g \geq 2$	$\left(\frac{\sum n_i - g}{g - 1}\right) \left(\frac{1 - \Lambda}{\Lambda}\right) \sim F_{g-1, \sum n_i - g}$
$P = 2$	$g \geq 2$	$\left(\frac{\sum n_i - g - 1}{g - 1}\right) \left(\frac{1 - \sqrt{\Lambda}}{\sqrt{\Lambda}}\right) \sim F_{2(g-1), 2(\sum n_i - g - 1)}$
$P \leq 1$	$g = 2$	$\left(\frac{\sum n_i - p - 1}{p}\right) \left(\frac{1 - \Lambda}{\Lambda}\right) \sim F_{p, \sum n_i - p - 1}$
$P \leq 1$	$g = 3$	$\left(\frac{\sum n_i - p - 2}{p}\right) \left(\frac{1 - \sqrt{\Lambda}}{\sqrt{\Lambda}}\right) \sim F_{2p, 2(\sum n_i - p - 1)}$

**2.1 Assumptions**

Assumptions underlying the One-Way MANOVA

Normal Distribution: - The dependent variable should be normally distributed within groups. Overall, the F test is robust to non-normality, if the non-normality is caused by skewness rather than by outliers. Tests for outliers should be run before performing a MANOVA, and outliers should be transformed or removed.

Linearity - MANOVA assumes that there are linear relationships among all pairs of dependent variables, all pairs of covariates, and all dependent variable-covariate pairs in each cell. Therefore, when the relationship deviates from linearity, the power of the analysis will be compromised.

Homogeneity of Variances: - Homogeneity of variances assumes that the dependent variables exhibit equal levels of variance across the range of predictor variables. Remember that the error variance is computed (SS error) by adding up the sums of squares within each group. If the variances in the two groups are different from each other, then adding the two together is not appropriate, and will not yield an estimate of the common

within-group variance. Homoscedasticity can be examined graphically or by means of a number of statistical tests.

Homogeneity of Variances and Covariances: - In multivariate designs, with multiple dependent measures, the homogeneity of variances assumption described earlier also applies. However, since there are multiple dependent variables, it is also required that their intercorrelations (covariances) are homogeneous across the cells of the design. There are various specific tests of this assumption. [7]

## 2.2 Advantages

- Repeated measures are a subset of MANOVA.
- If you use multiple one way ANOVAS to try to do this, you will raise the probability of a Type I error too high. MANOVA controls the experiment-wide error rate. Lots of ANOVAS raise power, but that is spurious because the Type I error rate goes up as well.
- MANOVA is a "gateway". Look at the multivariate F. If it is significant, then it allows you to look at the individual univariate analyses (rather than with ANOVA, having to run a separate post hoc test.)
- You can use it with assorted dependent variables, or with repeated measures. This is important because you'd usually use it when you can't collapse the measures into a few factors because they're all different.
- When there is multicollinearity (you can make a linear combination out of the dependent variables), MANOVA may detect combined differences not found in the univariate tests.
- You gain power over separate ANOVAs.
- The more DVs you have, the more you need MANOVA.

## 2.3 Limitations

- The number of people in the smallest cell should be larger than the total number of dependent variables.
- It can be very sensitive to outliers, for small N.
- It assumes a linear relationship (some sort of correlation) between the dependent variables.
- MANOVA won't give you the interaction effects between the main effect and the repeated factor.

Use Wilks' Lambda because it is the most commonly available and reported, however Pillai's criterion is more robust and therefore more appropriate when there are small or unequal sample sizes.

## III. STUDY DESCRIPTION

### 3.1 Discussion of study design

This study was an Open label, balanced, randomized, Two -treatment, Two-sequence, Two-period, single dose, crossover bioequivalence study with 07 days washout period between each drug administration. Subjects were admitted and housed in the clinical facility from at least 10-12 hours before administration of the dose and were discharged after collection of the 16 hours post dose sample. Subjects were fasting overnight for at least 10 hours prior to dosing and till around 4 hours after dosing and drinking water was not allowed from one hour before dosing and three hours after dosing unless and until clinically needed. Lunch was served at 4 hours post dose respectively in each period.

### 3.2 Study population

Five healthy, adult, male subjects belonging to the middle class socioeconomic strata were dosed in this trial. The subjects were randomized to receive formulations 'A', 'B' and 'C' in each period in order to eliminate any bias factors.

### 3.3 Blinding

The study was an open label study. The clinical investigators as well as the subjects were aware of the allocation of the treatment. Allocation of treatments to subjects was done using random numbers generated by computer.

### 3.4 Blood sampling

Following administration of formulation in each period, a total of 16 blood samples of 6 ml each were collected at pre-dose and at 10, 20, 30, 40, and 50 min, and 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5 and 6 h following drug administration.

During each session, an indwelling catheter was inserted into a forearm vein. Samples were collected in tubes containing lithium heparinate and immediately centrifuged at +4°C. Plasma was separated and frozen at -20°C for analysis.

### 3.5 Study period

Five eligible male subjects were admitted in the study after they were found to meet the selection criteria for the study. Each study period was of 16.0-hour (post dose) duration with a washout period of 07 days. Subjects were admitted in clinical facility at B. V. Patel PERD Centre for Period-I and after 7 days for Period-II and then for period III. Eighteen blood samples (6ml each) were collected from each subject during each period.

### 3.6 Methodology

This study was an Open label, balanced, randomized, two -treatment, two-sequence, two-period, single dose, crossover bioequivalence study with 07 days washout period between each drug administration. Subjects were informed about this study during screening procedure. All study related procedures, restrictions, duration, dates and timings, information on the study formulation and confidentiality of subject were explained clearly to the subjects by trained clinical assistants.

Subjects who signed the consent form and showed their willingness to participate in the study were enrolled. Subjects with normal physical and clinically acceptable lab findings and those who satisfied the inclusion and exclusion criteria were considered eligible to be admitted into the study. They reported to the study site on the day prior to the study day. All subjects were ensured for a minimum of 10 hours pre-dose fasting.

Next morning, they were dosed as per the randomization schedule. Study restrictions with respect to posture, smoking, fluid intake and physical activity were implemented throughout their stay in the clinical site. Plasma samples were stored in deep freezer at  $-20 \pm 5$  °C for interim storage and finally stored at  $-70 \pm 5$  °C for analytical analysis.

### 3.7 Drug

Dynapar QPS contains Diclofenac Sodium. Diclofenac Sodium is a nonsteroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. The mode of action is not fully known but it does not act through the pituitary-adrenal axis. Diclofenac inhibits prostaglandin synthesis by interfering with the action of prostaglandin synthetases. This inhibitory effect may partially explain its action. Diclofenac has been marketed since 1973 [8]. It has been approved in the United States [9].

### 3.8 Pharmacokinetics

In humans, orally administered Diclofenac Sodium is rapidly and almost completely absorbed and distributed to blood, liver and kidneys. The plasma concentration show a linear relationship to the amount of the drug administered. No accumulation occurs provided the recommended dosage intervals are observed [10]. Administration with food slows the rate but does not alter the extent of absorption. There is a substantial first pass effect such that only about 50% of Diclofenac is available systemically. The drug is extensively bound to the plasma protein (99%).

### 3.9 Pharmacokinetic Analysis

Maximal plasma concentration (C<sub>max</sub>) and time to reach the peak concentration (T<sub>max</sub>) were obtained directly by the visual inspection of each subject's plasma concentration-time profile. The AUC<sub>0-t</sub> from time zero to the last quantifiable point (C<sub>t</sub>) was calculated using the trapezoidal rule that called AUC [11]. So we get the data of C<sub>max</sub> and AUC. The data in table 2 shows the pharmacokinetic parameters of the three different formulations of DYNAPAR QPS. (Supplementary material)

## IV. PHARMACOKINETIC STUDIES

### 4.1 Study 1

**Table 2 pharmacokinetic data of three different formulations of dynapar qps.**

Formulation I		Formulation II		Formulation III	
C <sub>max</sub> (X1) (mg/ml)	AUC (X2) (mg.h/ml)	C <sub>max</sub> (X1) (mg/ml)	AUC (X2) (mg.h/ml)	C <sub>max</sub> (X1) (mg/ml)	AUC (X2) (mg.h/ml)
0.342	2.100	0.169	1.097	0.091	0.724
0.110	0.747	0.295	1.760	0.264	1.538
0.279	1.833	0.381	2.294	0.463	2.417
0.200	1.320	0.173	1.024	0.190	1.379
0.207	1.245	0.370	2.384	0.101	0.737

Ho: There is no significance difference in the effectiveness as measured by C<sub>max</sub> and AUC among three different formulations.

#### 4.1 Results and Discussion

Total sum of squares and cross products matrix

$$T = \begin{pmatrix} 0.175 & 0.922 \\ 0.922 & 5.043 \end{pmatrix} \quad d.f.T = 14$$

Between sample sum of squares and cross products:

$$B = \begin{pmatrix} 0.009 & 0.055 \\ 0.055 & 0.336 \end{pmatrix} \quad d.f.B = 2$$

$$T = \begin{pmatrix} 0.175 & 0.922 \\ 0.922 & 5.043 \end{pmatrix} \quad d.f.T = 14$$

$$W = T - B$$

$$= \begin{pmatrix} 0.166 & 0.867 \\ 0.867 & 4.707 \end{pmatrix} \quad d.f.W = 12$$

#### 4.2 The Wilk's Lambda statistics

$$|T| = \begin{vmatrix} 0.175 & 0.922 \\ 0.922 & 5.043 \end{vmatrix} = 0.033$$

$$|W| = |E| = \begin{vmatrix} 0.166 & 0.867 \\ 0.867 & 4.707 \end{vmatrix} = 0.029$$

$$\Lambda = \frac{|E|}{|T|} = 0.879$$

Now, F statistics is

$$F = \frac{1 - \sqrt{\Lambda}}{\sqrt{\Lambda}} \frac{15 - 3 - 1}{3 - 1} = 0.367$$

$$df1 = 4$$

$$df2 = 24$$

Now, F tabulated value is  $F_{0.01, 4, 24} = 0.8297$

F calculated = 0.367 < F tabulated = 0.8297

Therefore, we accept our Null hypothesis. (Appendix I)

There is no significance difference in the effectiveness as measured by Cmax and AUC among the three different formulations at 5% level of significance.

For this analysis we take only two pharmacokinetic parameters like Cmax and AUC but there are other pharmacokinetic parameters so we can check for that also but the main purpose of these two parameter is that, Cmax and AUC are the main pharmacokinetic parameter as Cmax consider the maximum plasma concentration and AUC contains area under the curve for the study. Here we analyze the effectiveness as measured by the pharmacokinetic parameters (Cmax and AUC) among the three different formulations of at the 5% level of significance. Form the F statistics we have calculated value is 0.367 and F tabulated value is 0.8297. So our calculated value is less than our tabulated value so that we can say that we accept our null hypothesis that there is no difference in the effectiveness as measured by Cmax and AUC among the three different formulations at 5% level of significance. So from our study we can say that the three Dynapar QPS formulation have same effect based on the pharmacokinetic parameters.

#### 4.2 Study: 2 (Two-Way Classification of Measurements with Replication)

The study has been done at B.V. Patel PERD Centre with three different formulations of Diclofenac QPS with test and reference (already available in market). After HPLC which is an analytical method for plasma analysis we get the pharmacokinetic data from statistical analysis (Supplementary material). Table 3 shows the data of reference and test drug or three different formulations.

**Table: 3 pharmacokinetic parameters of the three different formulations of test and reference drug.**

	A1= Reference Drug		A2 = Test Drug	
	Cmax (Y1) (µg/ml)	Tmax (Y2)(h)	Cmax (Y1) (µg/ml)	Tmax (Y2) (h)
<b>Formulation 1</b>	0.091	7	0.121	9
	0.174	4	0.067	6
	0.199	4	0.074	6
	0.113	7	0.129	8
	0.295	6	0.070	7
<b>Formulation 2</b>	0.168	6	0.179	6
	0.295	6	0.096	7
	0.381	4	0.078	8
	0.173	5	0.119	6
	0.369	6	0.121	7
<b>Formulation 3</b>	0.091	8	0.048	8
	0.264	4	0.118	7
	0.463	4	0.419	4
	0.189	6	0.131	6
	0.101	7	0.097	7

Ho: There is no significance difference in the effectiveness as measured for pharmacokinetic parameters (Cmax and Tmax), formulations and their interaction.

**V. RESULTS AND DISCUSSION**

**Table: 3 summary of pharmacokinetic parameters and formulations.**

Totals For (Y1) Cmax (µg/ml)			
	A1	A2	Total
<b>For 1</b>	0.872	0.461	1.333
<b>For 2</b>	1.386	0.593	1.979
<b>For 3</b>	1.108	0.813	1.921
	3.366	1.867	5.233
Totals For (Y2) Tmax (h)			
	A1	A2	
<b>For 1</b>	28	36	64
<b>For 2</b>	27	34	61
<b>For 3</b>	29	32	61
	84	102	186

**Table 3 contains summary of pharmacokinetic parameters of three different formulations.**

Now using computational forms for  $h_{A_{rr}}$  the (1, 1) element of  $H_A$  (corresponding to  $Y_1$ ) is given by

$$h_{A_{11}} = 0.128$$

For the (2, 2) element of  $H_A$  (corresponding to  $y_2$ ) we have

$$h_{A_{22}} = 18$$

For the (1, 2) element of  $H_A$  (corresponding to  $y_1y_2$ ) we use for  $h_{A_{rs}}$  to obtain

$$h_{A_{12}} = -1.495$$

$$\text{Thus, } H_A = \begin{pmatrix} 0.128 & -1.495 \\ -1.495 & 18 \end{pmatrix}$$

We obtain  $H_B$  similarly,

$$H_B = \begin{pmatrix} 0.043 & -0.204 \\ -0.204 & 1 \end{pmatrix}$$

For  $H_{AB}$  we have

$$H_{AB} = \begin{pmatrix} 0.019 & -0.125 \\ -0.125 & 2.33 \end{pmatrix}$$

The error matrix E is obtained using the computational forms given for  $e_{rr}$  and  $e_{rs}$

$$e_{rr} = \sum_{ijk} y_{ijkr}^2 - \frac{y_{...r}^2}{nab} - h_{Arr} - h_{Brr} - h_{ABrr}$$

$$e_{rs} = \sum_{ijk} y_{ijkr} y_{ijks} - \frac{y_{...r} y_{...s}}{nab} - h_{Ars} - h_{Brs} - h_{ABrs}$$

Proceeding in this fashion, we obtain

$$E = \begin{pmatrix} -0.238 & -22.96 \\ -22.96 & -733.33 \end{pmatrix}$$

With  $vE = ab(n-1) = 24$

To test the main effect of A with wilk's lambda 'Λ' we compute

$$\Lambda_A = \frac{|E|}{|E + H_A|}$$

$$\Lambda_A = \frac{|E|}{|E + H_A|} = 0.678$$

$$> \Lambda_{0.05,2,1,24} = 0.771$$

And we conclude that pharmacokinetic parameters (Cmax and Tmax) have not significant effect on formulations.

For the B main effect, we have

$$E + H_B = -393.77$$

$$\Lambda_B = \frac{|E|}{|E + H_B|} = 0.896$$

$$> \Lambda_{0.05,2,3,24} = 0.591$$

We conclude that the effect of formulations is not significant.

For the AB interaction, we obtain

$$E + H_{AB} = -373.06$$

$$\Lambda_{AB} = \frac{|E|}{|E + H_{AB}|} = 0.945$$

$$> \Lambda_{0.05,2,3,24} = 0.591$$

Hence, we conclude that the interaction effect is not significant.

Here we analyze the data with two way classification of measurement with replication because the interaction between the test and reference drugs and the formulations is important for the analysis. Hence, with the help of Wilks lambda in MANOVA we found that for the main effect of pharmacokinetic parameters Wilks Lambda calculated is 0.678 and the tabulated value is 0.771. So from that we can say that the calculated value is greater than tabulated value so we conclude that the pharmacokinetic parameters (Cmax and Tmax) have no significant effect on formulations. For the three different formulations, the Wilks Lambda calculated is 0.896 and tabulated is 0.591. So our calculated value is greater than tabulated value. So we can conclude that the effect of three different formulations is not significant. And for the interaction point of view the calculated value is 0.945 and our tabulated value is 0.591 so it is greater than that so we can say that the interaction effect is again not significant.

## VI. CONCLUSION

From the first study we can say that there is no significance difference in the effectiveness among three different formulations and from the second study we can say for formulations, pharmacokinetic parameters and interaction are not significant. A large (close to 1) value of Wilks' lambda means that the groups have smaller differences. From our two data we have large value of Wilks lambda and therefore we can say that between-group dispersion is smaller. So, from the both the examples we can say that the Wilks lambda the estimator of MANOVA is most powerful among others.

MANOVA has wider application in pharmaceutical industry but in bioavailability studies ANOVA is generally used for pharmacokinetic analysis. Based on these results we conclude that, for doing separate ANOVA for each pharmacokinetic parameter now we can apply Wilks Lambda for test the significance. As per our knowledge MANOVA has been used first time in statistical analysis of data of pharmacokinetic parameters generated in bioavailability study.

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**Supplementary Material**

**Study 1**

Total sum of squares and cross products matrix

$$T = \begin{pmatrix} t_{11} & t_{12} & \dots & \dots & t_{1p} \\ t_{21} & t_{22} & \dots & \dots & t_{2p} \\ t_{31} & t_{32} & \dots & \dots & t_{3p} \\ \dots & \dots & \dots & \dots & \dots \\ t_{p1} & t_{p2} & \dots & \dots & t_{pp} \end{pmatrix} \quad d.f.T = n - 1$$

$$T = \begin{pmatrix} 0.175 & 0.922 \\ 0.922 & 5.043 \end{pmatrix} \quad d.f.T = 15 - 1 = 14$$

Between sample sum of squares and cross products:

$$B = \begin{pmatrix} 0.009 & 0.055 \\ 0.055 & 0.336 \end{pmatrix} \quad d.f.B = 3 - 1 = 2$$

$$T = \begin{pmatrix} 0.175 & 0.922 \\ 0.922 & 5.043 \end{pmatrix} \quad d.f.T = 14$$

$$W = T - B$$

$$= \begin{pmatrix} 0.166 & 0.867 \\ 0.867 & 4.707 \end{pmatrix} \quad d.f.W = 14 - 2 = 12$$

**The Wilk's Lambda statistics**

$$|T| = \begin{vmatrix} 0.175 & 0.922 \\ 0.922 & 5.043 \end{vmatrix} = 0.033$$

$$|W| = |E| = \begin{vmatrix} 0.166 & 0.867 \\ 0.867 & 4.707 \end{vmatrix} = 0.029$$

$$\Lambda = \frac{|E|}{|T|} = 0.879$$

Now, F statistics is

$$F = \frac{1 - \sqrt{\Lambda}}{\sqrt{\Lambda}} \frac{15 - 3 - 1}{3 - 1} = 0.367$$

$$df1 = 4$$

$$df2 = 24$$

Now, F tabulated value is  $F_{0.01, 4, 24} = 0.8297$

**Study 2**

Now using computational forms for  $h_{A11}$  the (1, 1) element of  $H_A$  (corresponding to  $Y_1$ ) is given by

$$h_{A11} = \frac{(3.366)^2 + (1.867)^2}{(3)(3)} - \frac{(5.233)^2}{(3)(3)(2)}$$

$$= 0.128$$

For the (2, 2) element of  $H_A$  (corresponding to  $y_2$ ) we have

$$h_{A22} = \frac{(84)^2 + (102)^2}{(3)(3)} - \frac{(186)^2}{(3)(3)(2)}$$

$$= 18$$

For the (1, 2) element of  $H_A$  (corresponding to  $y_{1y_2}$ ) we use for  $h_{Ars}$  to obtain

$$h_{A12} = \frac{(3.366)(84) + (1.867)(102) - (5.233)(186)}{9} - \frac{(5.233)(186)}{18}$$

$$= -1.495$$

$$\text{Thus, } H_A = \begin{pmatrix} 0.128 & -1.495 \\ -1.495 & 18 \end{pmatrix}$$

We obtain  $H_B$  similarly,

$$H_B = \begin{pmatrix} 0.043 & -0.204 \\ -0.204 & 1 \end{pmatrix}$$

For  $H_{AB}$  we have

$$H_{AB} = \begin{pmatrix} 0.019 & -0.125 \\ -0.125 & 2.33 \end{pmatrix}$$

The error matrix E is obtained using the computational forms given for  $e_{rr}$  and  $e_{rs}$

$$e_{rr} = \sum_{ijk} y_{ijk}^2 - \frac{y_{...r}^2}{nab} - h_{Arr} - h_{Brr} - h_{ABrr}$$

$$e_{rs} = \sum_{ijk} y_{ijk} y_{ijks} - \frac{y_{...r} y_{...s}}{nab} - h_{Ars} - h_{Brs} - h_{ABrs}$$

Proceeding in this fashion, we obtain

$$E = \begin{pmatrix} -0.238 & -22.96 \\ -22.96 & -733.33 \end{pmatrix}$$

With  $vE = ab(n-1) = 2(3)(5-1) = 24$

To test the main effect of A with wilk's lambda ' $\Lambda$ ' we compute

$$\Lambda_A = \frac{|E|}{|E + H_A|}$$

$$E + H_A = -519.6$$

For the B main effect, we have

$$E + H_B = -393.77$$

$$\Lambda_B = \frac{|E|}{|E + H_B|} = \frac{-352.63}{-393.77} = 0.896$$

For the AB interaction, we obtain

$$E + H_{AB} = -373.06$$

$$\Lambda_{AB} = \frac{|E|}{|E + H_{AB}|} = \frac{-352.63}{-373.06} = 0.945$$