

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**50-818**

**STATISTICAL REVIEW(S)**

## **STATISTICAL REVIEW**

April 3, 2008

### **NDA 50-818**

**Drug Product:** Tobramycin 0.3% and Dexamethasone 0.05%  
**Sponsor:** Alcon Research, Ltd.  
**Reference Product:** Tobradex<sup>®</sup> (Tobramycin 0.3%/Dexamethasone 0.1% Ophthalmic Suspension)  
**Study Number:** C-06-37  
**Study Title:** A Double-Masked, Parallel-group, Randomized, Single-Dose Bioequivalence Study of Tobradex AF Suspension and Tobradex Ophthalmic Suspension  
**OCP Reviewer:** Kimberly Bergman, Pharm. D.  
**Statistical Reviewer:** Meiyu Shen, Ph.D.

### **Objectives of the study**

The primary objective of this study was to demonstrate that the prednisolone concentration in the aqueous humor, as assayed in this study, for the corticosteroid component of the corticosteroid/antibacterial drug product, Test Product (Dexamethasone 0.05% and Tobramycin 0.3%) ophthalmic suspension) was bioequivalent to the currently marketed corticosteroid, Reference Product (Dexamethasone 0.1% and Tobramycin 0.3% ).

### **Study Design**

This was a multi-center, randomized, double-masked, parallel-group, single-dose study to evaluate the bioequivalence of Tob 0.3%/Dex 0.05% and TOBRADEX<sup>®</sup> by measuring concentrations of dexamethasone in the aqueous humor of cataract surgery patients following a single topical ocular dose of the Tob 0.3% / Dex 0.05% formulation or TOBRADEX. Nine hundred eighty-seven male and female patients 18 years of age and older, of any race, who required cataract surgery, were enrolled to be able to collect pharmacokinetic data for 75 patients for each of the 5 post-dose time points per treatment.

The two treatments are:

- Test Product (Dexamethasone 0.05% and Tobramycin 0.3%) ophthalmic suspension (Lot #: 06-500836-1, Formulation identification #: 109442)
- Reference Product (Dexamethasone 0.1% and Tobramycin 0.3%) ophthalmic suspension (Lot #: 06-500809-1, Formulation identification #: 10611)

### **Efficacy Evaluations**

There was no placebo control treatment in this study. Efficacy evaluation was not performed.

### **Bioequivalence Evaluation**

#### **Sponsor's primary pharmacokinetic variable**

The primary pharmacokinetic variable was the area under the concentration-time curve up to the last measured concentration (AUC<sub>0-5</sub>). The pharmacokinetic variable was estimated from the mean aqueous humor drug concentrations of dexamethasone at each of the five sparse sampling time points (0.5, 1, 2, 3 and 5 hours). The area under the curve was estimated using a method appropriate for sparse sampling (Nedelman JR, Gibiansky E, Lau DTW. Applying Bailer's method for AUC confidence intervals to sparse sampling. Pharm Res 1995; 12(1):124-128.).

The maximum mean concentration ( $C_{max}$ ) is estimated directly from the observed concentrations. That is,  $C_{max} = \max$  expected value  $\{C_0, C_1, \dots, C_k\}$ , where subscript  $k$  represents the number of sampling time points.

On the basis of the trapezoidal rule, the  $AUC_{0-t_j}$ , the area under the concentration time profile from zero to the time,  $t_j$  ( $0 < t_1 < t_2 < t_3, \dots, < t_j$ ), is computed as

$$AUC_{0-t_j} = t_1 * \bar{x}_{t_1} / 2 + \sum_{i=1}^{j-1} (\bar{x}_{t_i} + \bar{x}_{t_{i+1}}) \cdot (t_{i+1} - t_i) / 2 \quad (1)$$

Let  $x_{qr}$  represent the response of the  $r^{\text{th}}$  individual at the  $q^{\text{th}}$  sampling time point ( $q=1, \dots, k$ ). The sponsor defined the sample mean at time  $t_k$  in any given group to be:

$$\bar{x}_q = \frac{1}{n_q} \sum_{r=1}^{n_q} x_{qr}$$

The AUC from time zero to time  $t_k$ , denoted by  $AUC(0-t_k)$ , was approximated by the sponsor by

$$AUC(0-t_k) = \sum_{q=1}^{n_q} c_q \bar{x}_q$$

Where  $c_q = \begin{cases} \frac{1}{2} \Delta_2 & \text{for } q = 1 \\ \frac{1}{2} (\Delta_q) + (\Delta_{q+1}) & \text{for } q = 2, \dots, k-1 \\ \frac{1}{2} \Delta_k & \text{for } q = k \end{cases} \quad (2)$ 

for  $\Delta_q = t_q - t_{q-1}$ ,  $q = 2, \dots, k$

Note that the correct definition for  $c_q$  is:

$$c_q = \begin{cases} \frac{1}{2} \Delta_2 & \text{for } q = 1 \\ \frac{1}{2} [(\Delta_q) + (\Delta_{q+1})] & \text{for } q = 2, \dots, k-1 \\ \frac{1}{2} \Delta_k & \text{for } q = k \end{cases}$$

for  $\Delta_q = t_q - t_{q-1}$ ,  $q = 2, \dots, k$

We believe the sponsor made a typing error in (2). This is supported by our replication of their results, listed in Table 4.

### Measurement time

Aqueous humor samples were obtained using a sparse sampling scheme, whereby the time of sample collection will either be 0.5 hours ( $\pm 5$  min.), 1 hour ( $\pm 5$  min.), 2 hours ( $\pm 10$  min.), 3 hours ( $\pm 10$  min.), or 5 hours ( $\pm 20$  min.) following a single pre-operative dose of test article on the day of surgery.

### **Sponsor's analysis populations**

The sponsor's safety population included all patients who received study medication.

The sponsor's Intent-to-Treat (ITT) population included all patients who received study medication, had an aqueous humor sample collected, and for whom adequate pharmacokinetic data were collected and available.

The primary analysis of the ITT data set was based on samples obtained at the closest nominal time of actual sample collection. The sponsor said "for example, if the sample for a planned 30-minute time point is actually taken within 1-hour time window then the sample was analyzed as part of the nominal 1-hour data. If the actual sample time did not fall within one of the protocol time windows, then the sample was analyzed as part of the closest nominal time point. If the sampling time is equidistant between two time points then it was analyzed with the planned time point (or the first of the two if between two time points, neither of which was planned)."

The sponsor's Per Protocol (PP) population included all patients who received study medication, satisfied pre-randomization protocol inclusion/exclusion criteria that were relevant to the assessment of pharmacokinetic parameters, had an aqueous humor sample collected, and for whom adequate pharmacokinetic data were collected and available.

In the per protocol analysis, only data from patients for whom aqueous humor samples were collected within the protocol defined window for their assigned time were included.

Table 1 lists disposition and evaluability of patients. Table 2 presents the number of aqueous humor samples, by time point, included in the intent-to-treat and per protocol analyses. In accordance with the analysis plan, the intent-to-treat analysis was performed on some samples collected outside the time window specified in the clinical protocol (TDOC-0005200). Hence, time points were reassigned to the closest nominal time point of actual sample collection. The per protocol analysis was performed only on samples collected within the protocol defined window for the assigned randomized time point.

Table 1. Disposition and evaluability of patients

Population		Total number of patients enrolled: 987	
		ITT	PP
Number of patients		957	886
Number of patients excluded	Not receiving test article	2	2
	No aqueous humor sample collected	2	2
	Inadequate sample (<25 µl)	7	7
	Contaminated sample	11	11
	Thawed sample	6	6
	Sample collected outside of defined window		27
	Concomitant medications administered within 20 minutes of test article dosing		9
	Concomitant disease		26
	Concomitant medication		1
	Dosing with wrong test article		4
	Issues with test article dosing		2
	Unconfirmed sample collection time		1
	Possible sample contamination with vitreous		1

Table 2. Number of Pharmacokinetic Samples Analyzed

Treatment Assignment	Time Point Assignment	Intent-to-Treat Analysis (N)*	Per Protocol Analysis (N)**
Tob 0.3% / Dex 0.05%	0.5 Hours	98	91
	1 Hour	94	85
	2 Hours	96	91
	3 Hours	97	86
	5 Hours	95	88
TOBRADEX	0.5 Hours	94	87
	1 Hour	98	90
	2 Hours	97	92
	3 Hours	96	90
	5 Hours	92	86

Tob 0.3% / Dex 0.05% = Tobramycin 0.3% / Dexamethasone 0.05% Ophthalmic Suspension

TOBRADEX = Tobramycin 0.3% / Dexamethasone 0.1% Ophthalmic Suspension

\*Closest nominal time point to actual sample collection

\*\*Actual randomized time point

**Sponsor's analysis for primary endpoint**

The ratio of AUC<sub>0-5</sub> (Tob 0.3% / Dex 0.05% to TOBRADEX®) and the 90% confidence intervals surrounding the ratio (calculated using Fieller's method and Bootstrap method) were determined. The sponsor used the per protocol population for the primary analysis. Dexamethasone concentrations that were below the lower limit of quantitation (1.00 ng/mL) were replaced with one-half the lower limit of quantitation. Additional data analyses utilized imputation methods where BLQ values were analyzed as missing or zero.

**Data**

Each subject contributed one concentration value. Figure 1 shows the distribution of the dexamethasone concentrations versus time for the PP population and Figure 2 shows those for the dexamethasone concentrations versus time for ITT population.

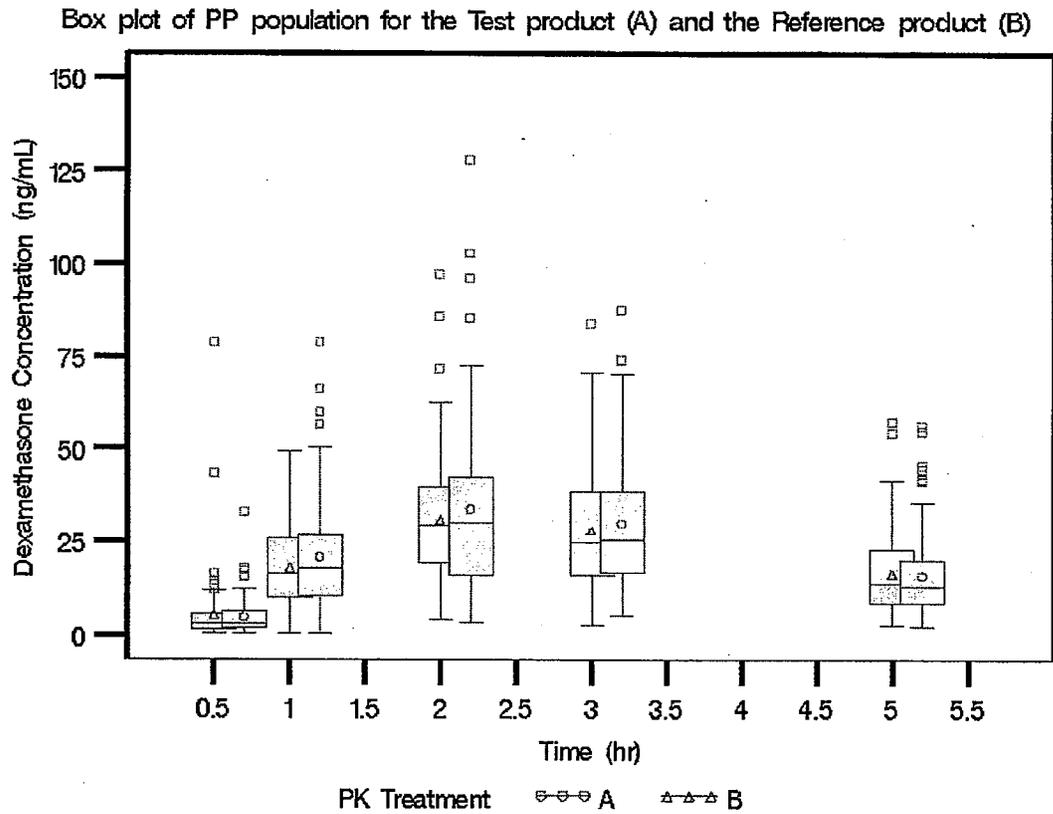


Figure 1. Box plot of concentration versus time for PP population: 0.2 is added to the variable Time of the test product such that two box plots can be displayed side by side.

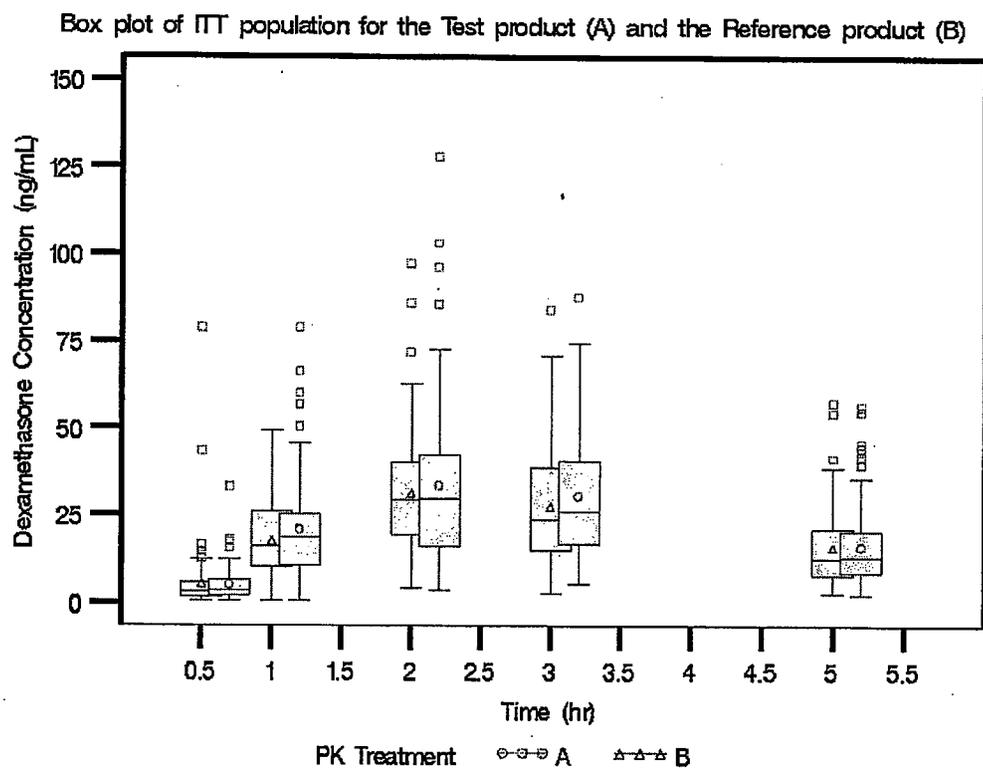


Figure 2. Box plot of concentration versus time for ITT population: 0.2 is added to time for the test product such that two box plots can be displayed side by side.

**The sponsor's analysis**

The sponsor used both the Fieller's method and the bootstrap method to estimate the 90% confidence interval for the ratio of  $AUC_{0-5}$  of the test product versus the reference product for PP and ITT populations.

Table 3. 90% Confidence Intervals Surrounding the Ratio of Dexamethasone  $AUC_{0-5}$  Values for Tob 0.3% / Dex 0.05% to TOBRADEX (BLQ Replaced with One-Half the Limit of Quantitation)

Method	Population	5% percentile	Ratio of $AUC_{0-5}$ for test over reference	95% percentile
Fieller's method	PP	0.983	1.07	1.16
Bootstrap	PP	0.996	1.07	1.19
Fieller's method	ITT	1.01	1.09	1.18
Bootstrap	ITT	1.01	1.09	1.20

**This statistical reviewer’s analysis:**

**(1) Comparison of the sponsor’s AUC and this reviewer’s AUC**

In order to evaluate the sponsor’s equation for calculating AUC, I compared the sponsor’s AUC and my calculated AUC in Table 4. The results are identical, but would not have been had Equation (2) been followed. This is why I believe the sponsor did the correct AUC calculation, but made a typing error in (2).

Table 4. Comparison of the sponsor’s calculated AUC and this reviewer’s calculated AUC for PP population with below the limit of quantitation (BLQ) replaced with one-half the limit of Quantitation

Treatment	The sponsor’s calculation		This reviewer’s calculation	
	AUC	SE	AUC	SE
Tob 0.3%/Dex 0.05%	112	4.08	112.2	4.08
Tobradex (Tob 0.3%/Dex 0.1%)	105	3.57	105.2	3.57

**(2) Bootstrap method**

The bootstrap method for estimating 90% confidence interval is illustrated with  $AUC_{0.5}$ . To estimate 90% confidence interval for  $AUC_{0.3}$ ,  $AUC_{0.2}$ , and  $AUC_{0.1}$ , we followed the same steps.

- (a) Estimation of 90% confidence intervals for  $AUC_{0.5}$ ,  $AUC_{0.3}$ ,  $AUC_{0.2}$ , and  $AUC_{0.1}$  via bootstrapping the data from the 886 PP patients, receiving both products and having adequate humor, with replacement**

First, bootstrap all 886 PP patients to select 886 with replacement repeatedly 5,000 times. Second, for each bootstrap sample, compute  $AUC_{0.5}$  for the test product and the reference product, separately.

Third, for each bootstrap sample, compute the ratio of  $AUC_{0.5}$  for the test product over  $AUC_{0.5}$  for the reference product. The 5<sup>th</sup> percentile and 95<sup>th</sup> percentile of the ratio of  $AUC_{0.5}$  for the test over  $AUC_{0.5}$  for reference product comprise the 90% confidence interval.

Fourth, 90% Confidence Intervals for ratio of  $AUC_{0.5}$  for the test product and the reference product is obtained as just described in the third step. The results are listed in Table 6.

Table 6. The 90% confidence intervals for ratio of  $AUC_{0.5}$ ,  $AUC_{0.3}$ ,  $AUC_{0.2}$ , and  $AUC_{0.1}$  for the test product versus the reference product (BLQ Replaced with One-Half the Limit of Quantitation)

	Population	5% percentile	Ratio of AUC for test over reference	95% percentile
$AUC_{0.5}$	PP	0.983	1.069	1.159
$AUC_{0.3}$	PP	0.997	1.095	1.197
$AUC_{0.2}$	PP	0.995	1.110	1.235
$AUC_{0.1}$	PP	0.906	1.079	1.268

This reviewer also calculated the 90% confidence intervals for ratio of  $AUC_{0.5}$ ,  $AUC_{0.3}$ ,  $AUC_{0.2}$ , and  $AUC_{0.1}$  for the test product versus the reference product if BLQ was replaced by missing or by zero. The results listed in the Table 7 are similar among three methods handling BLQ data.

Table 7. The 90% confidence intervals for ratio of  $AUC_{0.5}$ ,  $AUC_{0.3}$ ,  $AUC_{0.2}$ , and  $AUC_{0.1}$  for the test product versus the reference product

	BLQ method	Population	5% percentile	Ratio of AUC for test over reference	95% percentile
$AUC_{0.5}$	Missing	PP	0.982	1.067	1.159
$AUC_{0.3}$	Missing	PP	0.998	1.095	1.201
$AUC_{0.2}$	Missing	PP	0.996	1.110	1.235
$AUC_{0.1}$	Missing	PP	0.884	1.063	1.251
$AUC_{0.5}$	Zero	PP	0.981	1.067	1.156
$AUC_{0.3}$	Zero	PP	0.998	1.095	1.197
$AUC_{0.2}$	Zero	PP	0.996	1.112	1.232
$AUC_{0.1}$	Zero	PP	0.904	1.089	1.278

**(b). Estimation of 90% confidence intervals for  $AUC_{0.5}$ ,  $AUC_{0.3}$ ,  $AUC_{0.2}$ , and  $AUC_{0.1}$  via bootstrapping the data from the 957 ITT patients, receiving both products and having adequate humor, with replacement**

First, bootstrap 957 ITT patients to select 957 with replacement repeatedly 5,000 times.

Repeat Steps 2 to 4 in the above Section (a). The obtained results are listed in Table 8.

Table 8. The 90% confidence intervals for ratio of  $AUC_{0.5}$ ,  $AUC_{0.3}$ ,  $AUC_{0.2}$ , and  $AUC_{0.1}$  for the test product versus the reference product (BLQ Replaced with One-Half the Limit of Quantitation)

	Population	5% percentile	Ratio of AUC for test over reference	95% percentile
$AUC_{0.5}$	ITT	1.009	1.093	1.182
$AUC_{0.3}$	ITT	1.010	1.101	1.120
$AUC_{0.2}$	ITT	1.000	1.112	1.229
$AUC_{0.1}$	ITT	0.934	1.103	1.285

**(3) Fieller's method for the estimation of 90% confidence intervals**

The method for estimating 90% confidence interval is illustrated with  $AUC_{0.5}$ . To estimate 90% confidence interval for  $AUC_{0.3}$ ,  $AUC_{0.2}$ , and  $AUC_{0.1}$ , we repeat the following steps.

First, compute  $AUC_{0.5}$  using formula (1) for the test and reference products.

Second, compute the standard error (SE) for each  $AUC_{0.5}$ .

Third, using the Fieller's method to compute the 90% confidence interval for the ratio of the ( $AUC_{0.5}$ ) of the test versus ( $AUC_{0.5}$ ) of the reference.

Table 9. The 90% confidence intervals for ratio of AUC<sub>0.5</sub>, AUC<sub>0.3</sub>, AUC<sub>0.2</sub>, and AUC<sub>0.1</sub> for the test product versus the reference product (BLQ Replaced with One-Half the Limit of Quantitation)

Parameter	Population	5% percentile	Ratio of AUC for test over reference	95% percentile
AUC <sub>0.5</sub>	PP	0.983	1.067	1.158
	ITT	1.008	1.091	1.180
AUC <sub>0.3</sub>	PP	0.995	1.092	1.197
	ITT	1.005	1.099	1.200
AUC <sub>0.2</sub>	PP	0.993	1.106	1.230
	ITT	0.999	1.106	1.223
AUC <sub>0.1</sub>	PP	0.901	1.069	1.274
	ITT	0.929	1.090	1.283

**(4) Stratified bootstrap at each time point**

A refinement to the simple bootstrap method is to bootstrap the data using stratification by time point. This method is illustrated for PP with AUC<sub>0.5</sub>. To estimate 90% confidence interval for PP for AUC<sub>0.3</sub>, AUC<sub>0.2</sub>, and AUC<sub>0.1</sub>, we followed the same steps. We used the same procedures for ITT population.

First, bootstrap all number of PP patients at 0.5 hour (say n1) to select n1 with replacement repeatedly 5,000 times. We repeated this for other time points. Then we combined these bootstrap samples at all time points.

Second, for each bootstrap sample, compute AUC<sub>0.5</sub> for the test product and the reference product, separately.

Third, for each bootstrap sample, compute the ratio of AUC<sub>0.5</sub> for the test product over AUC<sub>0.5</sub> for the reference product. The 5<sup>th</sup> percentile and 95<sup>th</sup> percentile of the ratio of AUC<sub>0.5</sub> for the test over AUC<sub>0.5</sub> for reference product comprise the 90% confidence interval.

Fourth, 90% Confidence Intervals for ratio of AUC<sub>0.5</sub> for the test product and the reference product is obtained as just described in the third step. The results are listed in Table 10.

Table 10. The 90% confidence intervals for ratio of AUC<sub>0.5</sub>, AUC<sub>0.3</sub>, AUC<sub>0.2</sub>, and AUC<sub>0.1</sub> for the test product versus the reference product (BLQ Replaced with One-Half the Limit of Quantitation)

	Population	5% percentile	Ratio of AUC for test over reference	95% percentile
AUC <sub>0.5</sub>	PP	0.981	1.065	1.153
AUC <sub>0.3</sub>	PP	1.000	1.095	1.194
AUC <sub>0.2</sub>	PP	1.007	1.116	1.230
AUC <sub>0.1</sub>	PP	0.905	1.090	1.282
AUC <sub>0.5</sub>	ITT	1.010	1.089	1.172
AUC <sub>0.3</sub>	ITT	0.999	1.101	1.209
AUC <sub>0.2</sub>	ITT	1.014	1.115	1.219
AUC <sub>0.1</sub>	ITT	0.939	1.108	1.296

## Review Conclusion

- Bootstrap method:
  - 886 PP patients,
    - the 90% confidence limits of the ratios of the test product versus the reference product for  $AUC_{0-5}$ ,  $AUC_{0-3}$ , and  $AUC_{0-2}$  for PP population lie in the interval (0.8, 1.25) and the point estimates of the ratios are in the range of (1.069, 1.110) if BLQ was replaced with One-Half the Limit of Quantitation;
    - the 90% confidence limits of the ratios of the test product versus the reference product for  $AUC_{0-1}$  do not lie in the interval (0.8, 1.25) and the point estimate of the ratios is 1.079 for the PP population if BLQ was replaced with One-Half the Limit of Quantitation.
    - If BLQ was replaced by missing or by zero, the 90% confidence intervals for ratio of  $AUC_{0-5}$ ,  $AUC_{0-3}$ ,  $AUC_{0-2}$ , and  $AUC_{0-1}$  for the test product versus the reference product are similar to those obtained if BLQ were replaced with One-Half the Limit of Quantitation.
  - 957 ITT patients,
    - the 90% confidence limits of the ratios of the test product versus the reference product for  $AUC_{0-5}$ ,  $AUC_{0-3}$ , and  $AUC_{0-2}$  for ITT population lie in the interval (0.8, 1.25) and the point estimates of the ratios are in the range of (1.093, 1.112);
    - the 90% confidence limits of the ratios of the test product versus the reference product for  $AUC_{0-1}$  do not lie in the interval (0.8, 1.25) and the point estimate of the ratio is 1.103 for ITT population.
- Fieller's method:
  - 866 PP patients and 957 ITT patients
    - the 90% confidence limits of the ratios of the test product versus the reference product for  $AUC_{0-5}$ ,  $AUC_{0-3}$ , and  $AUC_{0-2}$  for PP population as well as for ITT population lie in the interval (0.8, 1.25) and the point estimates of the ratios are in the range of (1.067, 1.106).
    - the 90% confidence limits of the ratios of the test product versus the reference product for  $AUC_{0-1}$  do not lie in the interval (0.8, 1.25) and the point estimates of the ratios are 1.069 for PP population, and 1.090 for ITT population.

Using the stratified bootstrap method, we got almost the same results as when we used the unstratified bootstrap method.

The results support equivalence of the 2 products for  $AUC_{0-5}$ ,  $AUC_{0-3}$ , and  $AUC_{0-2}$ , but not for  $AUC_{0-1}$ .

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