In-Vitro Dissolution Profile Comparison: Statistics and Analysis, Model Dependent Approach

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Purpose. To develop and propose a 'model dependent' approach for the *in-vitro* dissolution profiles comparison.

Methods. Diltiazem hydrochloride tablet dissolution profiles were compared using a statistical approach based on a mathematical model. A similarity region (SR) was defined based on the intra- and inter-lot parameter variances of the final production size standard lots. Statistical distances between the test and reference lot parameter means were computed and normalized. A 90% confidence region (CR) was developed around the statistical distance. The confidence region was compared with the similarity region to assess the similarity or dis-similarity of the test and reference (REF) lot dissolution profiles. Two test lots, one with a 'minor' modification (mm) the other with a 'major' modification (MM), were evaluated.

Results. 'Weibull' was selected as the 'model' function. A comparison of the confidence regions around the statistical distance of 'mm-REF' and 'MM-REF' with the similarity region, suggested that the dissolution profiles of the 'minor' modification lot were similar and that of 'major' modification lot were dis-similar to the reference lot.

Conclusions. A 'model dependent' approach was shown to be useful for the inter-lot *in-vitro* dissolution profiles comparison.

KEY WORDS: dissolution profile comparison; statistics; model dependent analysis.

INTRODUCTION

The dissolution of immediate release formulations is invariably conducted using a suitable USP dissolution method and apparatus (1). Towards the approval, the dissolution method and specification '*Q*' are set by the FDA. This is done by considering the physico-chemical properties of the drug, discrimination with respect to different manufacturing/formulation variables and to assure adequate and complete release in a reasonable time frame. The specifications going into the firm's manufacturing and controls section are usually a single or multiple point '*Q*' estimates with a one sided window. Though this 'point estimate' approach is suitable for drug products with rapid dissolution, it may not be adequate for the products with either low solubility actives or modified release characteristics. In these situations, some times, the drug products with inherently different dissolution profiles, may comply with the point estimate '*Q*'. This in turn may inadvertently lead to the declaration of similar dissolutions.

Some approaches have been suggested (2,3) for the assessment of difference between the two dissolution profiles. These approaches however, appear to be mathematical than statistical. They propose point estimate measures which may not account for the within/between lot variances and covariances. Moore and Flanner (4) have proposed an index criterion for dissolution profile comparison. Its application is however limited to the data with small within-lot variation. Tsong and coworkers (5) have proposed a statistical measure of the difference between the two dissolution profiles and decision making rule. Their approach is limited in that the data comparisons have to be made using identical sampling schemes. We hereby propose a 'model dependent' mathematical, statistical approach which accounts for the within and between-lot variance and covariance.

METHODS

The 'Model Dependent' approach is recommended for the 'dissolution data rich' scenario, hereby defined as consisting of at least four or more dissolution data points. The approach requires a model specification, characterized by a suitable mathematical function, to describe the dissolution data. Once the mathematical function is selected, the dissolution profiles are evaluated in terms of the model parameters. The approach may be worked out through the following steps:

1. Define a suitable mathematical function to describe the dissolution data of the standardized production size lots or batches.
2. Fit the individual unit dissolution data from different standardized production size batches and assess the inter- and intra-batch variabilities for the model parameters.
3. Define an appropriate similarity region or criterion based on the intra- and inter-batch variances of parameters.
4. Fit the dissolution data from the '*N*' individual units of the reference (pre-change) and test (post-change) batches using the mathematical function used in step 1 and estimate the model parameters.
5. Calculate a statistical distance between the mean parameters of the test and the reference batches.
6. Compute a 90% confidence region around the statistical distance.
7. Compare the confidence region with the pre-defined similarity region to declare either similarity or dis-similarity of the profiles.

The method is described by the example of diltiazem HCl tablet dissolution. Since the approach is primarily targeted for the analysis of scale-up and post approval changes, two test scenarios, involving (a) a minor modification and (b) a major modification lots are discussed. Diltiazem hydrochloride is a cardio-active calcium channel blocker prescribed for its negative inotropic effect. Currently, the USP monograph recommends the dissolution of immediate release diltiazem HCl tablet, in 900 ml water at pH 7.0 and 37°C, using apparatus II (paddle) at 100 rpm rotation speed.

The mean dissolution data and profiles of the three production size diltiazem lots are given in Table I and Figure 1 respectively. The first step of the model dependent analysis involved selection of a suitable mathematical function to describe the dissolution data. After plotting the data with respect to various mathematical expressions given in Table II, such as (i) Probit,

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Table I. Diltiazem HCL Mean Dissolution Data for Different Production Size Lots Using USP Dissolution Method (Apparatus II, 900 ml water, Q1 = NMT 60% in 30', Q2 = NLT 80% in 180')

Time (min)	Ref, Std 1	Std 2	Std 3	Weibull Fit	'mm'	'MM'
0	0.0	0	0	0	0	0
10	—	—	16.58	14.20	—	—
15	—	27.16	—	21.65	—	—
20	—	—	29.33	28.78	—	—
30	40.34	43.56	40.00	41.75	37.95	28.38
45	—	56.40	—	57.71	—	—
60	67.15	67.96	65.33	69.80	62.13	50.88
90	87.00	86.33	—	85.14	80.86	73.01
120	—	96.67	96.67	92.95	—	—
150	—	97.72	—	96.75	—	—
180	97.73	98.58	102.83	98.54	95.91	100.64
240	—	—	103.58	99.72	—	—

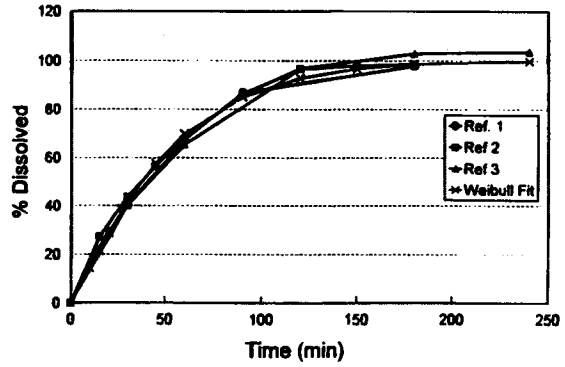

Fig. 1. Diltiazem HCl (120 mg tablet), mean (N = 12) dissolution profiles of the three standard batches with the Weibull function fit.

(ii) Logistic, (iii) Weibull, (iv) Quadratic, and (v) Exponential, a preliminary selection of Probit, Logistic and Weibull function fits was made based on the parsimony of parameters with tight residual mean square errors (6). The three functions were then re-fitted to the average mean dissolution data curve to select the best fitting function using non-linear regression. The selection was made considering robustness of fit based on the standard goodness of fit criteria such as the least residual mean square error, Akaike Information Criteria (7) (which accounted for the number of parameters and sample points) and a tight coefficient of variation for the parameters (<3%) to minimize

Table II. Selected Mathematical Functions for the Percent Dissolved 'X' with Respect to Time 't' and Model Parameters 'α' and 'β'

Function	Form
1. Probit	$X/100 = \Phi(\alpha + \beta*(\log(t))$ where Φ = Standard normal distribution.
2. Logistic	$X/100 = e^{[\alpha+\beta*\log(t)]}/\{1 + e^{[\alpha+\beta*\log(t)]}\}$
3. Weibull	$X/100 = 1 - e^{-\alpha*(t)**\beta}$
4. Quadratic	$X/100 = \alpha + \beta1*(t - \bar{t}) + \beta2*(t - \bar{t})^2$ where $\bar{t}$ = average of all sampled time values.
5. Exponential	$X/100 = 1 - e^{-\alpha*(t)}$

the information loss due to the use of parameter estimates as true estimates. Based on these, Weibull function, which was found to fit and describe the data best, was the ultimate choice for the model. Weibull function is described as follows:

$$X_{(t)} = X_{(\text{inf.})} * [1 - e^{**}(-\alpha*(t^{**}\beta))]$$

where,

$X_{(t)}$ = percent dissolved with respect to time t,
$X_{(\text{inf.})}$ = percent dissolved at infinite time i.e. 100%.
α = scale factor corresponding to the apparent rate constant.
β = shape factor

For convenience, the linearized form of Weibull function,

$$\ln(-\ln(1 - X_{(t)}/X_{(\text{inf.})})) = \ln(\alpha) + \beta \ln(t)$$

was fitted to the individual unit dissolution data, to generate parameters. The next step was to define a similarity region (SR) or criterion for the profile comparison. This was done based on the intra- and inter-lot variances of the model function parameters. To assess the variances, Weibull function was fitted to the dissolution data of 12 individual units of the above mentioned three lots. This generated a set of α, β parameters corresponding to the dissolution profile of each individual unit. After logarithmic transformation of the parameters, the intra- and inter-lot variances were pooled as follows:

$$\text{Pooled SD} = \text{Sqrt}[\{(\text{Var}_1 + \text{Var}_2 + \text{Var}_3)/3\} + \text{Var}(\bar{x})]$$

where, Var_1, Var_2, Var_3 are the intra-lot variances of the ln parameters, $\text{Var}(\bar{x})$ is the inter-lot variance of the ln-parameter means. The estimates of α, β were assumed to be true values. Var_1, Var_2, Var_3 and $\text{Var}(\bar{x})$ were calculated without or by neglecting the estimation error of the parameter estimates. Under the condition of total similarity, ln(α)'s and ln(β)'s would be identical and the mean parameter difference would be zero. By assuming the normal distributions of the ln-parameters within each batch and their batch means, the univariate similarity regions, as shown in Figure 2, were constructed. The boxes

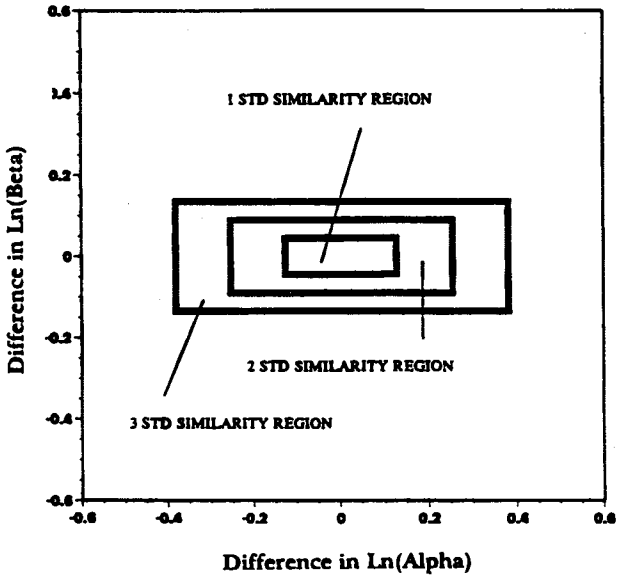

Fig. 2. Pooled SD based Similarity Regions.

corresponding to either one, two and three standard deviations, (corresponding to approximately 68%, 95% and 99% cases) were placed, with respect to zero on the x, y axes. Ideally, if one has adequate number of standard batches, an elliptical multivariate similarity region with correlated ln(α) and ln(β) should be constructed. The construction of a multivariate similarity region using the same data from 3 standard batches is discussed in the Appendix.

RESULTS

The test or post-change batch was envisioned as a minor modification 'mm' (say, a different site) and a major modification 'MM' (say, inactives are substantially different in quality and quantity). It's dissolution was compared with the reference 'REF' or final production (pre-change) batch. The mean diltiazem dissolution profiles (N = 12 tablets, 120 mg strength) of the 'REF', 'mm' and 'MM' batches are given in Figure 3. For the dissolution profile comparisons, individual unit dissolution data from 12 tablets of the two test ('mm' and 'MM') and one reference ('REF') batch were fitted using linearized Weibull function. The resultant sets of α and β parameters corresponding to each unit dissolution, were log-transformed. While comparing the means of 'mm' and 'MM' lots with the 'REF', due to ln- transformation, only the ratio or difference needed to be analyzed. The mean distances in ln(α)'s and ln(β)'s were calculated by using the following statistic often referred to as the 'Mahalanobis' distance[8] (M distance).

$$D^2 = [(X_T - X_R)' S^{-1}_{\text{pooled}} (X_T - X_R)]$$

where,

D^2 = squared 'Mahalanobis' distance,
X_T, X_R = vectors corresponding to the sample means of test and reference lot ln(α)'s and ln(β)'s
S_{pooled} = pooled sample variance/covariance matrix, equal to $(S_{\text{test}} + S_{\text{ref}})/2$, where S_{test} and S_{ref} are the variance/covariance matrix of ln(α) and ln(β) in the test and reference batch respectively.

The squared 'Mahalanobis' distance accounted for both the variances as well as covariances of the parameter differences. The untransformed and ln-transformed parameters, 'Mahalanobis' distance and the subsequent statistics corresponding to 'mm-REF' and 'MM-REF' difference are given in Table III.

In the next step, the 'M-distance' values were normalized using a scaling factor. The normalized statistic, known as Hotelling[8] T^2 was used so that the resultant value was distributed with respect to a statistical 'F' distribution, under the hypothesis $X_T = X_R$. The scaling factor K' and scaled 'M' distance are described as follows

Scaling factor:

$$K' = \{(N_1 + N_2 - P - 1)/[(N_1 + N_2 - 2)P]\} * [(N_1 N_2)/(N_1 + N_2 - P)]$$

where,

N_1, N_2 = Number of units in the lot,
P = Number of parameters

Scaled 'M' distance, $T^2 = K' * D^2$.

Under the assumption of multivariate normal distribution, a 90% confidence region of the true mean difference was computed for the resultant 'Y' value to satisfy the following condition:

$$CR = \{K'[(Y - (X_T - X_R))' S^{-1}_{\text{pooled}} (Y - (X_T - X_R))] <= F_{P,N1+N2-P-1,.90}\}$$

All 'Y' values satisfying the equality of the condition generated the boundary of a 90% confidence region. Since Weibull function had two parameters (α and β), for a 90% confidence region, only an elliptical or circular shape could result. The variances of the differences between ln(α)'s and ln(β)'s being non-identical, in this case, it resulted in ellipses (and not circles) corresponding to 'mm-REF' and 'MM-REF'. The ellipses were then plotted against the similarity regions, Figure 4. The ellipse corresponding to 'mm-REF' was within the 3SD similarity region box. The ellipse corresponding to 'MM-REF' was however outside the 3SD box.

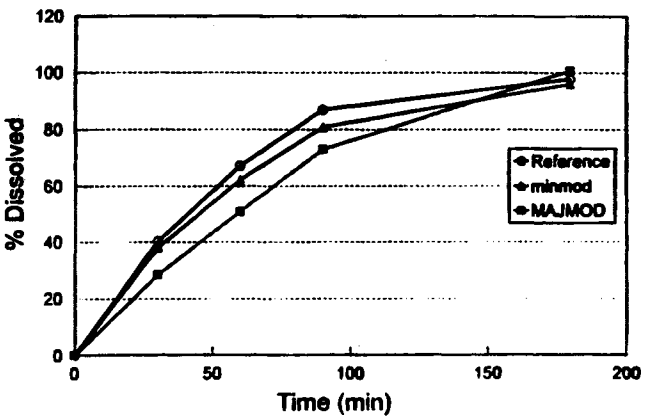

Fig. 3. Diltiazem HCl (120 mg tablet), mean (N = 12) dissolution profiles of the 'Reference', 'minor modification' and 'Major Modification' lots.

DISCUSSION

A meaningful and validated dissolution profile may be important from the perspective of a scale-up and post-approval change of the production lot, identifying important manufacturing variables, defining and evaluating a possible *in-vitro*, *in-vivo* association/correlation and assessing the possibility of bioequivalency study waivers. Since the USP point estimate approach is not adequate for the comparison of dissolution profiles, (which may govern the *in-vivo* input rate and pharmacokinetics), we believe that the above described mathematical and statistical 'Model Dependent' approach is a better tool. Using the ellipses given in Figure 4, the location of true differences between ln(α)'s and ln(β)'s can be estimated with a 90% confidence. The center of the ellipse represents the observed difference between the ln parameter means of the two lots. The confidence region boundaries can be projected to the difference in ln(α) and ln(β) axes to get 90% individual confidence inter-

Table III. Weibull Parameters and the Subsequent Statistics

Table #	Alpha	Beta	Alpha (m)	Beta (m)	Alpha (M)	Beta (M)
1	1.10235	1.2715	1.10642	1.25147	0.67211	1.5971
2	1.02016	1.18755	0.97898	0.98404	0.76722	1.55379
3	1.06385	1.09491	0.96281	1.02701	0.71737	1.53345
4	1.03306	1.08672	1.04511	1.0851	0.87582	1.44743
5	1.53169	1.122	1.01917	1.00332	0.94031	1.34448
6	1.57243	1.09528	0.94143	1.12372	0.8308	1.40506
7	1.18134	1.21115	1.02958	1.06341	0.86558	1.41646
8	1.03462	1.02715	1.0683	1.00525	0.83319	1.4591
9	0.99122	1.12385	0.94917	1.13529	0.75571	1.48513
10	1.06824	1.24445	0.92251	1.15279	0.89086	1.40105
11	1.16791	1.1605	1.04464	1.01963	0.84242	1.42974
12	1.35562	1.09806	1.05833	1.16537	0.77161	1.52781
	Ln Alpha	Ln Beta	Ln Alpha (m)	Ln Beta (m)	Ln Alpha (M)	Ln Beta (M)
1	0.09744	0.24019	0.10113	0.22432	−0.39733	0.46819
2	0.01996	0.17189	−0.02125	−0.01609	−0.26498	0.4407
3	0.06189	0.09067	−0.0379	0.02665	−0.33216	0.42752
4	0.03252	0.08317	0.04413	0.08167	−0.13259	0.36979
5	0.42637	0.11511	0.01899	0.00331	−0.06154	0.29601
6	0.45262	0.09101	−0.06036	0.11665	−0.18537	0.34008
7	0.16665	0.19157	0.02915	0.06148	−0.14436	0.34816
8	0.03404	0.02679	0.06607	0.00524	−0.18249	0.37782
9	−0.00882	0.11676	−0.05216	0.12689	−0.28009	0.39551
10	0.06601	0.21869	−0.08066	0.14218	−0.11556	0.33722
11	0.15522	0.14885	0.04367	0.01944	−0.17148	0.3575
12	0.30426	0.09354	0.05669	0.15304	−0.25927	0.42384
Mean	0.1506813	0.1323537	− 0.0089583	0.0787319	−0.2106028	0.3818601
Standard Deviation	0.1592641	0.0628729	0.0577349	0.0745374	0.0974589	0.050406
Comparison Lot	Mean Ln(Alpha) Difference	Mean Ln(Beta) Difference	'M' Distance	Scaled 'M' Distance	Ln(Alpha) Diff., 90% C.I.	Ln(Beta) Diff., 90% C.I.
'mm-REF'	−0.14172	−0.05362	1.463	12.835	(−0.255; −0.028)	(−0.119; 0.011)
'MM-REF'	−0.36128	0.2495064	4.508	121.948	(−0.486; −0.236)	(0.195; 0.304)

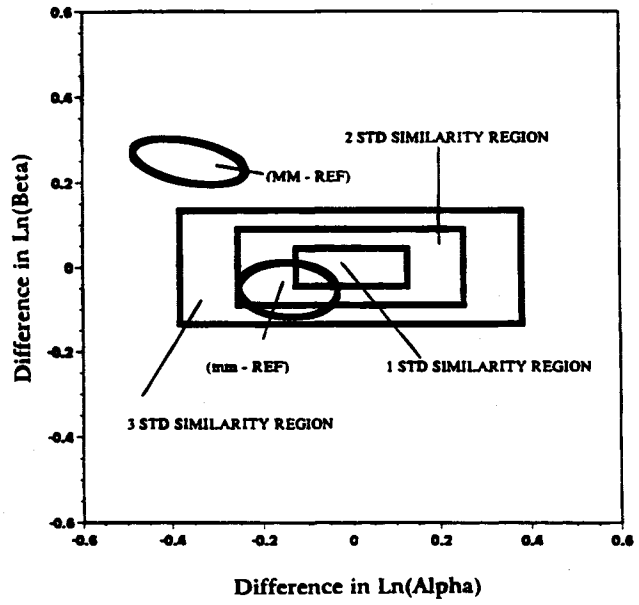

Fig. 4. Ninety percent Confidence Regions (CR) of the mean difference in the Ln Weibull parameters compared to the univariate Similarity Regions (SR).

vals. The approach invariably results in the reduction of dimensions. In this case, the dimensions are reduced from four (sample points) to two (parameters). The method accounts for the variances and covariances of the data. In addition, different sample time point schemes can be accommodated for the test and reference dissolution lots. This is in contrast to model independent approach[5] which requires identical sampling time points. In case of Weibull function, the parameters provide in-vitro meaning to the terms relating to the dissolution kinetics, 'α' representing the apparent rate constant of dissolution K_d or the scale factor and 'β' representing the shape factor. The approach however requires many meaningfully spaced dissolution data points. For the correct profile similarity assessment, the data points should not be clustered near zero or 100% dissolution. Also, if used with only a few sample points, a model misspecification may result.

The logarithmic conversion is used to minimize the skewness of the parameter distribution. In the above example, we used univariate standard deviation similarity region boxes for the comparison of multivariate ellipses. The Appendix gives comparison using multivariate elliptical similarity regions using the same data sets. If one has the dissolution data from many final production size standard batches, we believe that a multivariate similarity region should be constructed and used. The approach is primarily targeted for the SUPAC (scale-up and

post-approval changes) immediate release products. Eventually, it may be extrapolated to scenarios such as bio-waivers, *in-vitro in-vivo* correlations and for the dissolution of extended release formulations. At present, some issues are unresolved such as (i) how many standard deviations (2 or 3) should be used for a similarity criterion, (ii) what to do if the ellipse is only marginally out of the similarity region and (iii) whether this approach may encourage more variable production lots in the first place.

CONCLUSIONS

In summary, the following conclusions could be drawn:

1. A mathematical and statistical model dependent approach which uses a suitable mathematical function such as Weibull, may be utilized to compare dissolution profiles of the test (production size post approval change) and reference (production size final) batches of the immediate release products.
2. The approach accounts for the variances and covariances of the dissolution data sets.
3. The method is flexible in that the dissolution data points for the test and reference lots may be collected using different sampling schemes.

APPENDIX

The dissolution data of the standard batches consist of the inter-batch and intra-batch covariances. To construct a similarity region, the two may be combined by using the following mixed effect model:

$$Y = \mu + A + E$$

where,

Y = matrix of dissolution measurement with respect to batch, unit and time
μ = vector of overall mean dissolution
A = vector of deviation of batch mean from the overall mean
E = vector of deviation of individual tablet from the batch mean

Y may be represented as a $(I*N)*P$ matrix of dissolution values, where I are the total number of batches, N is the number of tablets within each batch and P is the number of parameters corresponding to the dissolution model. The Y values may be represented such that the first 'N' rows of the matrix reflect the dissolution values of the first batch, the second 'N' rows of Y are of the second batch, ...

Let y_{ij} represent the dissolution values of the j-th tablet in the i-th batch, then

$$y_{ij} = \mu + A_i + E_{ij}$$

and

$$y_{ij} - \mu = A_i + E_{ij}$$

Where, μ is the vector of the overall mean dissolution values, A_i is a random vector of the mean deviation of the I-th batch from the overall dissolution mean and E_{ij} is a random vector of the within batch deviation of the tablet dissolution from the batch mean. Both A_i and E_{ij} are independent random vectors. A_i is distributed with a multivariate normal distribution MVN(0, Σ_1), with a mean of 0 and between batch $P \times P$ covariance matrix of mean dissolutions Σ_1. E_{ij} is distributed with a multivariate normal distribution, MVN(0, Σ_2), with a mean of 0 and within batch $P \times P$ covariance matrix of tablet dissolutions Σ_2. Under the exactly identical conditions, the difference between the mean ln(β)'s and ln(α)'s of the two batches would be zero, therefore the similarity region (SR) may be defined as the 99% confidence region of the batch mean differences around zero and can be stated as all $\Delta\mu$'s satisfying the following inequality:

$$SR = K'(\Delta\mu)'(\Sigma_1 + (\Sigma_2/\sqrt{N}))^{-1}(\Delta\mu) \leq F_{P,2N-P-1,.99}$$

where, $\Delta\mu$ is the P vector of mean dissolution differences between two batches, $F_{P,2N-P-1,.99}$ is the 99% value of the F-distribution with degrees of freedom P and $2N$-P-1 and K' is the scaling factor as defined previously. Figure 5 gives the elliptical similarity region contrasted with the 'mm-REF' and 'MM-REF' confidence region ellipses.

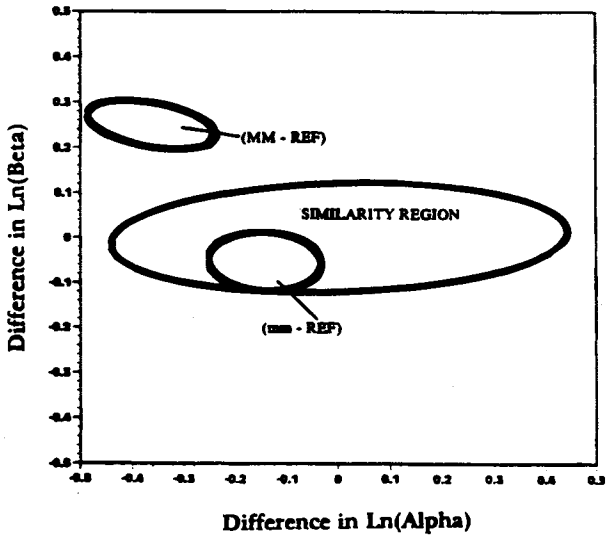

Fig. 5. Ninety percent Confidence Regions (CR) of the mean difference in the Ln Weibull parameters compared to the multivariate Similarity Region (SR).

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