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International Journal of Pharmaceutics 313 (2006) 5–13
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Statistical evaluation of physiological variability of rifampicin in fixed dose combinations

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Received 15 November 2005; accepted 29 December 2005
Available online 6 March 2006

Abstract

Tuberculosis is one of the microbial diseases having a long history of its occurrence and yet to be eradicated from the world. Due to the development of bacterial resistance, treatment has changed from monotherapy to combotherapy to fixed dose combinations (FDCs). Rifampicin has been found one of the most important anti-tubercular drugs, however variable bioavailability of rifampicin in some FDCs as well as separate formulations has been reported in the literature, and led to the development of WHO model protocol for evaluation of FDCs for bioequivalence trials. In present investigation, role of physiological variability in rifampicin bioequivalence was studied. Influence of subject's body weight, inter/intra-individual variability of elimination rate and impact of outliers on the decision of bioequivalence were investigated. Normalization of pharmacokinetic measures for bioequivalence (AUC and C_{max}) were carried out as per body weights and elimination rate constants of subjects, then different statistical tests like *two-way* ANOVA, *hauschke* analysis, normal and log-transformed confidence interval were applied to check for the change in bioequivalence decision. It was found that normalization as per body weights did not play a significant role in the outcome of bioequivalence endpoint. Similarly, elimination rate variability and outliers have been found insignificant regarding final outcome of bioequivalence study. Hence, it has been concluded that physiological variability did not play a significant role in bioequivalence of rifampicin in FDCs.

Keywords: Rifampicin; Variable bioavailability; Physiological factors

1. Introduction

Tuberculosis (TB) has been one of the leading causes of death among the infectious diseases and treatment of drug-resistant tuberculosis is an emerging issue for much of the world. World Health Organization (WHO) and International Union Against Tuberculosis and Lung Disease (IUATLD) propose the use of FDC tablets for the treatment of TB. Recommendation of FDC tablets to replace the single-drug tablets has been justified and evolved as a new tool to deliver the short course chemotherapy (SCC) in a standardized, simpler and potentially more reliable way. Further, FDCs of WHO suggested strengths are designed to provide adequate dosage of all the constituent drugs for a large range of body-weights by simply altering the number of tablets to be ingested per day (Blomberg and Fourie, 2003). Some of the FDCs currently being marketed are found to be substandard as far as their rifampicin bioavailability is concerned; hence, WHO and IUATLD advocate the bioequivalence of only rifampicin because of the variable bioavailability reported with this drug (Agrawal et al., 2001). Bioequivalence is the most important quality control tool as a surrogate for the therapeutic efficacy.

The rate and extent measures become surrogate indicators of therapeutic outcome to assess the drug product performance. The maximum plasma concentration (C_{max}) and the time of its occurrence (T_{max}) are thought to be reasonable measures for rate of absorption (Welage et al., 2001). The determination of the area under the concentration–time curves (AUCs) is the method most commonly used by regulatory agencies to assess the extent of drug absorption after single-dose administration of oral products (Chen et al., 2001).

Bioequivalence studies are often carried out using a two period crossover design. Average bioequivalence is concluded,

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doi:10.1016/j.ijpharm.2005.12.049

if the 90% confidence interval for the mean relative bioavailability falls within the prespecified limits, usually 80–125%, based upon the analysis of the log transformed AUC and C_{max} data (Welage et al., 2001). Bioequivalence studies are generally carried out in healthy volunteers, however, differences in the results are observed (Panchagnula et al., 1999). In many cases marked differences in the product bioavailability were observed, for which several factors could be responsible. Probable main sources of variability described in BIO-international '92 (Blume et al., 1995) are as follows:

- Variability in formulation.
- Intrinsic variability of the active substances (e.g. poorly soluble or poorly absorbed substances, high presystemic clearance, variable systemic clearance, etc.).
- Day to day variability of the subjects.
- Presence of outliers.

One of the assumption for the assessment of bioequivalence in a crossover design is that drug clearance in each subject on the two study days remains the same and any observed differences in AUC and/or C_{max} between the two drug products are due to differences in bioavailability. This was found to be questionable for highly variable drugs (FDA, 1997). Based on the intra-subject/intra-formulation variability, the method for widening of bioequivalence limits, handling of outliers and use of non-parametric statistical methods in bioequivalence studies were suggested. Assessment of variability due to formulation was proposed to be an integral part of bioequivalence studies of highly variable drug products (Blume et al., 1995).

Although rifampicin is reported to be absorbed completely after oral administration, several studies have shown considerable inter-individual differences in bioavailability. Significant correlation was found between average AUC values and body weight of the volunteer. Apart from dose to body weight ratio, inter-individual variation in rifampicin bioavailability was explained by different rates of drug metabolism (Pahkla et al., 1999). Moreover, rifampicin anti-TB activity is found to be dose dependent (Panchagnula et al., 1999). According to the fundamental equation for rate of input, there is a relation between AUC, dose and clearance of the drug. Change in the dose of the drug will cause change in AUC and hence the bioavailability endpoint. AUC and clearance are independent factors while K_{el} is dependent factor, which depends on the subject's physiological condition. Variability in K_{el} can be observed in case of drugs (e.g. rifampicin) following saturation kinetics (Ritschel and Kearns, 1999).

In a bioavailability/bioequivalence study, a commonly encountered problem is that the data set may contain some extreme or outlying values/subjects, which causes product failure or subject-by-formulation interaction (Wang and Chow, 2003). Because bioequivalence studies are usually carried out as crossover studies, the most important type of subject outlier is the within-subject outlier, where one subject or a few subjects differ notably from rest of the subjects. Hence, the objective of this investigation was to clearly understand the implication of physiological variability on the out-come bioequivalence decision.

2. Materials and methods

Data of the different bioequivalence studies of rifampicin in FDCs (Table 1) was collected from the National Institute of Pharmaceutical Education and Research (NIPER) bioavailability center for the statistical evaluation of physiological variability to understand the implication on bioequivalence decision.

2.1. Normalization of the pharmacokinetic measures

In case of bioequivalence studies, dose given to different volunteers is the same for every individual; hence pharmacokinetic measures like AUC and C_{max} were adjusted for dose normalization according to individual body weight. Inter- and intra-individual variability of the elimination rate for the same drug was observed in the bioequivalence study hence correction for the observed terminal elimination rate constant data were carried out as mentioned below (Ritschel and Kearns, 1999):

$$\text{AUC corrected for dose normalized to body weight} = \text{AUC}/(\text{dose}/\text{body weight})$$

AUC normalized to body weight and elimination rate constant was calculated as follows:

$$\text{Normalized AUC} = \text{AUC}/(\text{dose}/(\text{body weight} \times K_{el}))$$

2.2. Outlier detection

Dixon's test was applied for the detection of the extreme values in the data obtained from different bioequivalence studies

Table 1
Bioequivalence studies conducted at NIPER (FDCs vs. separate formulations) undertaken for statistical evaluation

No.	Fixed dose combinations	No. of volunteers used	Sampling period (h)	Strength (mg)			
				R	H	Z	E
1	4 Drugs RHZE	13	36	150	75	400	275
II	4 Drugs RHZE	14	24	150	75	400	275
III	4 Drugs RHZE	13	24	150	75	400	275
IV	4 Drugs RHZE	14	24	225	150	750	400
V	4 Drugs RHZE	22	24	150	75	400	275
VI	3 Drugs RHZ	19	24	150	75	400	–

R: rifampicin; H: isoniazid; Z: pyrazinamide; E: ethambutol.

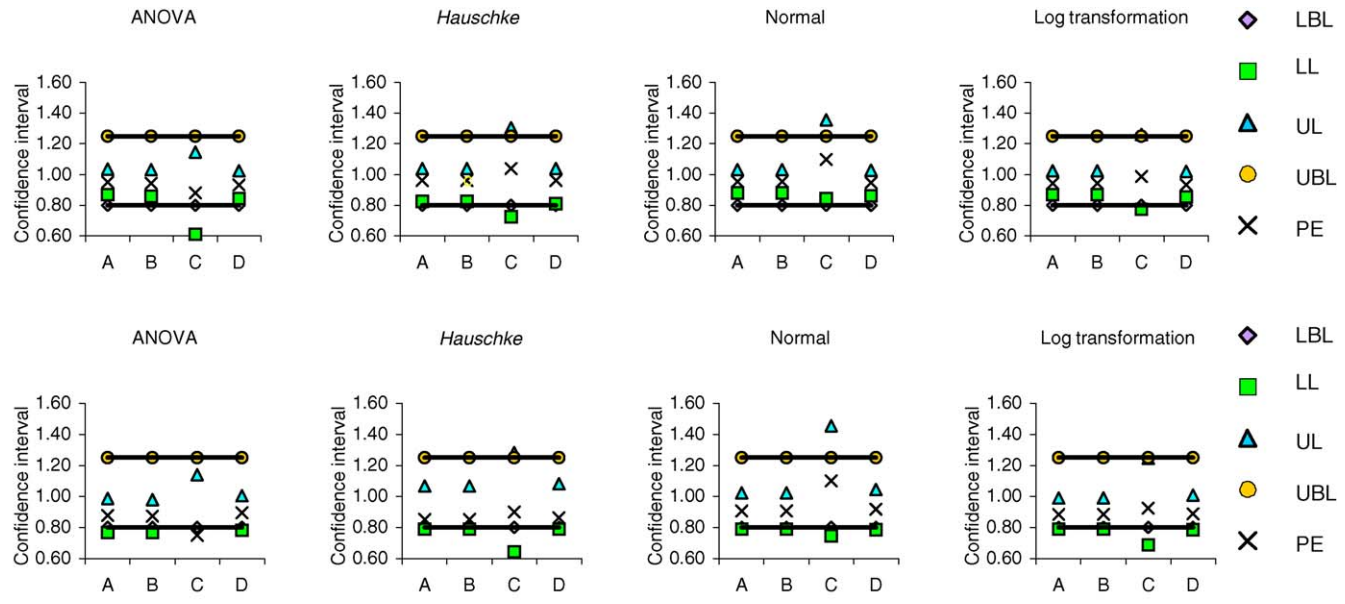

Fig. 1. Statistical limits observed for AUC (upper panel) and C_{max} (lower panel) values obtained from different statistical tests for study I.

(Bolton, 1990). After calculating the ratio as described in the test, calculated value was compared with tabulated value according to sample size and decision for the presence or the absence of the outliers were made. In case of the presence of outlier, that value was omitted as an outlier and remaining data was tested again for the outliers using the same procedure.

2.3. Bioequivalence estimation

After carrying out the normalization of the pharmacokinetic measures and outlier removal from the data, AUC and C_{max} were evaluated by normal confidence interval, log transformed confidence interval, parametric (*two-way* ANOVA) and non-

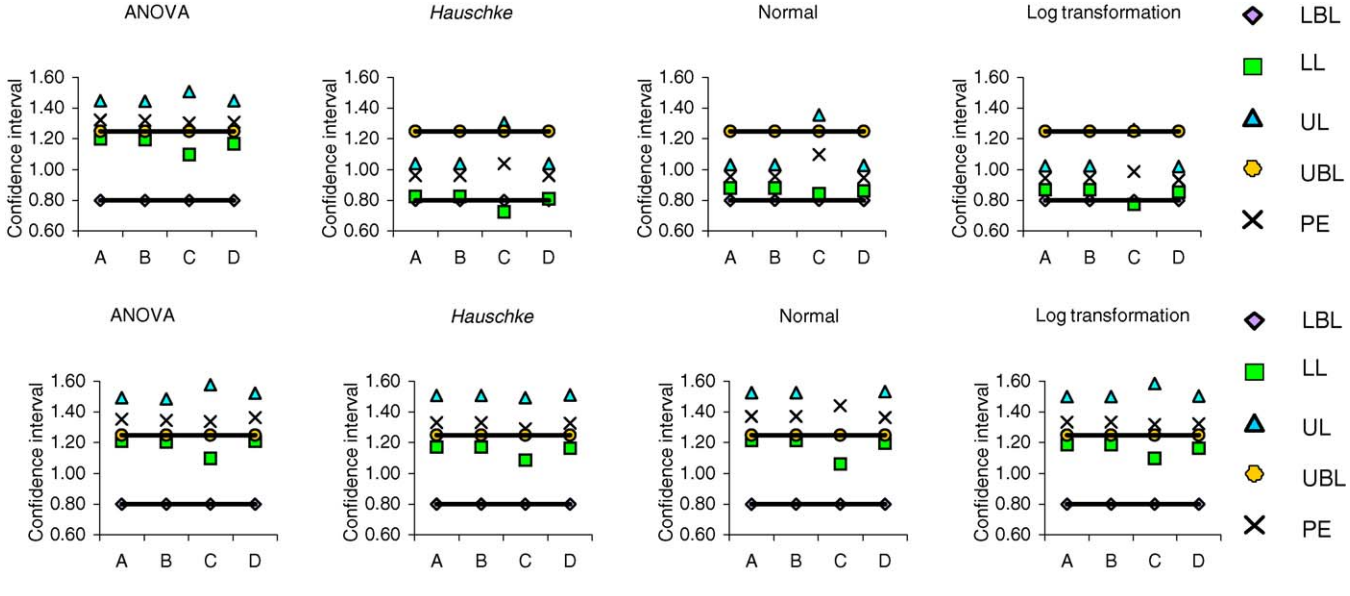

Fig. 2. Statistical limits observed for AUC (upper panel) and C_{max} (lower panel) values obtained from different statistical tests for study II.

parametric (*Hauschke* analysis) tests at 90% confidence interval (Hauschke et al., 1990). *Hauschke* analysis is the only statistical test for the bioequivalence which takes into consideration all the possible sources of variation and is strongly recommended method of statistical evaluation of bioequivalence by WHO and IUATLD (Anonymous, 1999).

3. Results and discussion

3.1. Dose normalization as per body weight

Dose normalization of both AUC and C_{max} values were carried out as per individual's body weights. These normalized

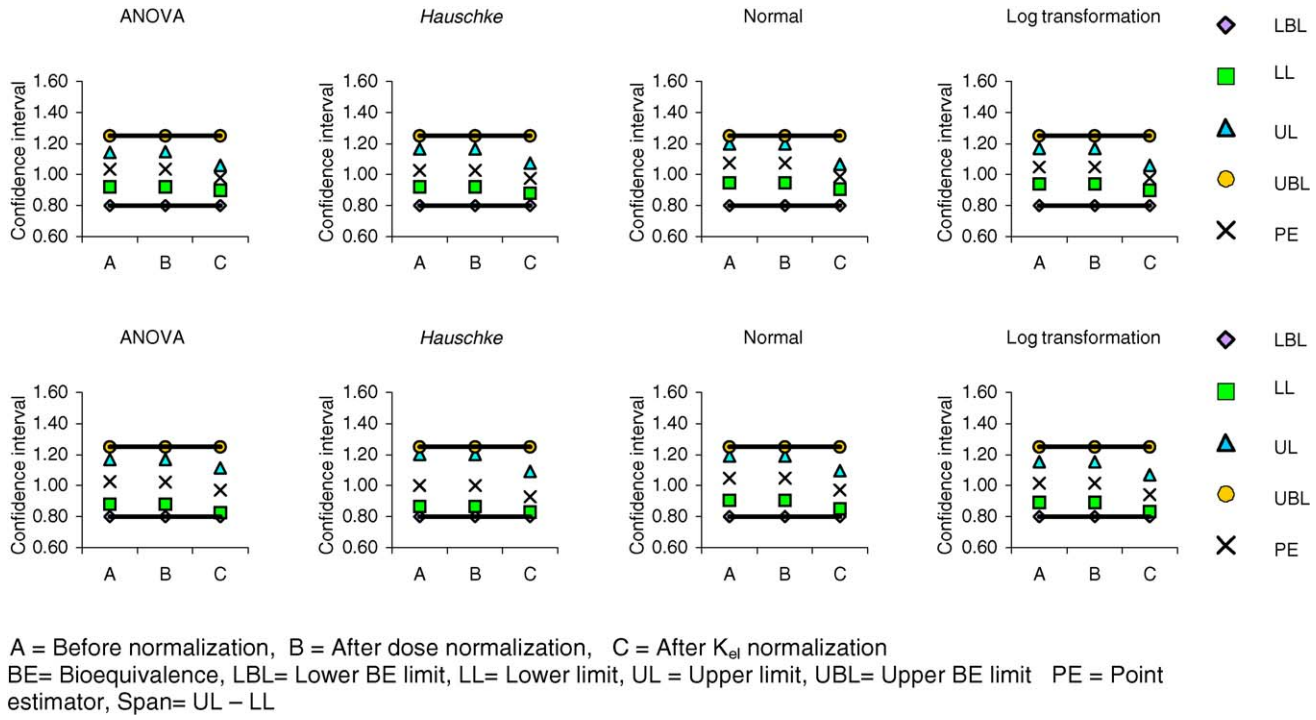

Fig. 3. Statistical limits observed for AUC (upper panel) and C_{max} (lower panel) values obtained from different statistical tests for study III.

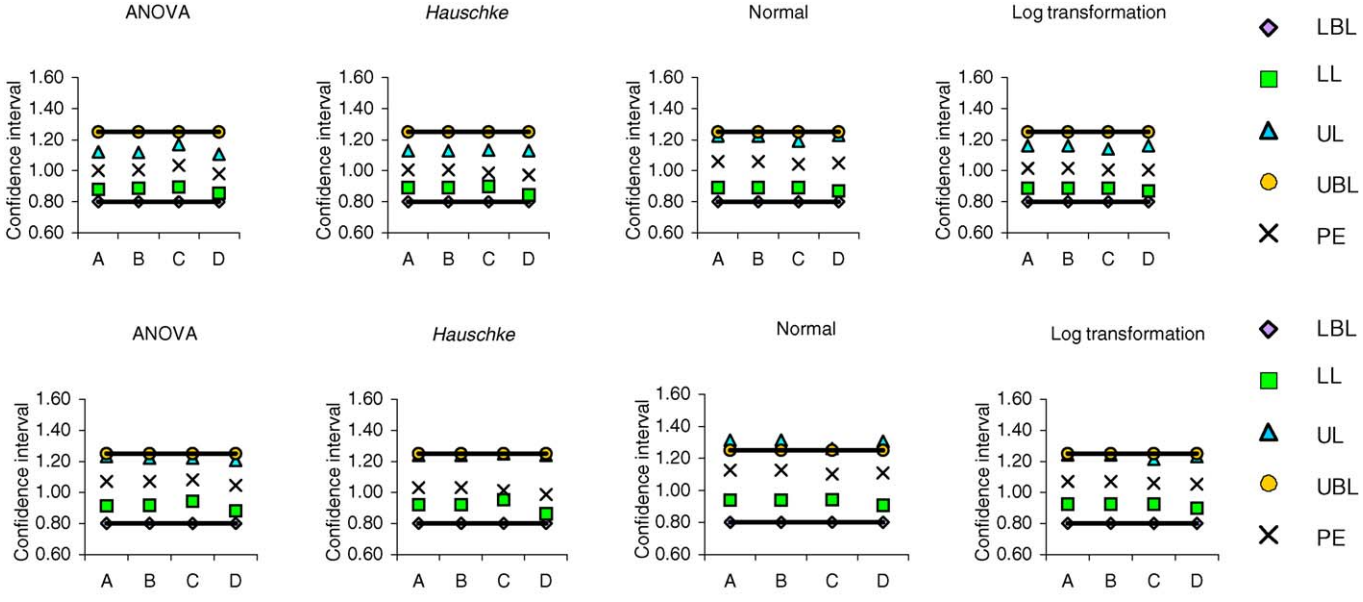

Fig. 4. Statistical limits observed for AUC (upper panel) and C_{max} (lower panel) values obtained from different statistical tests for study IV.

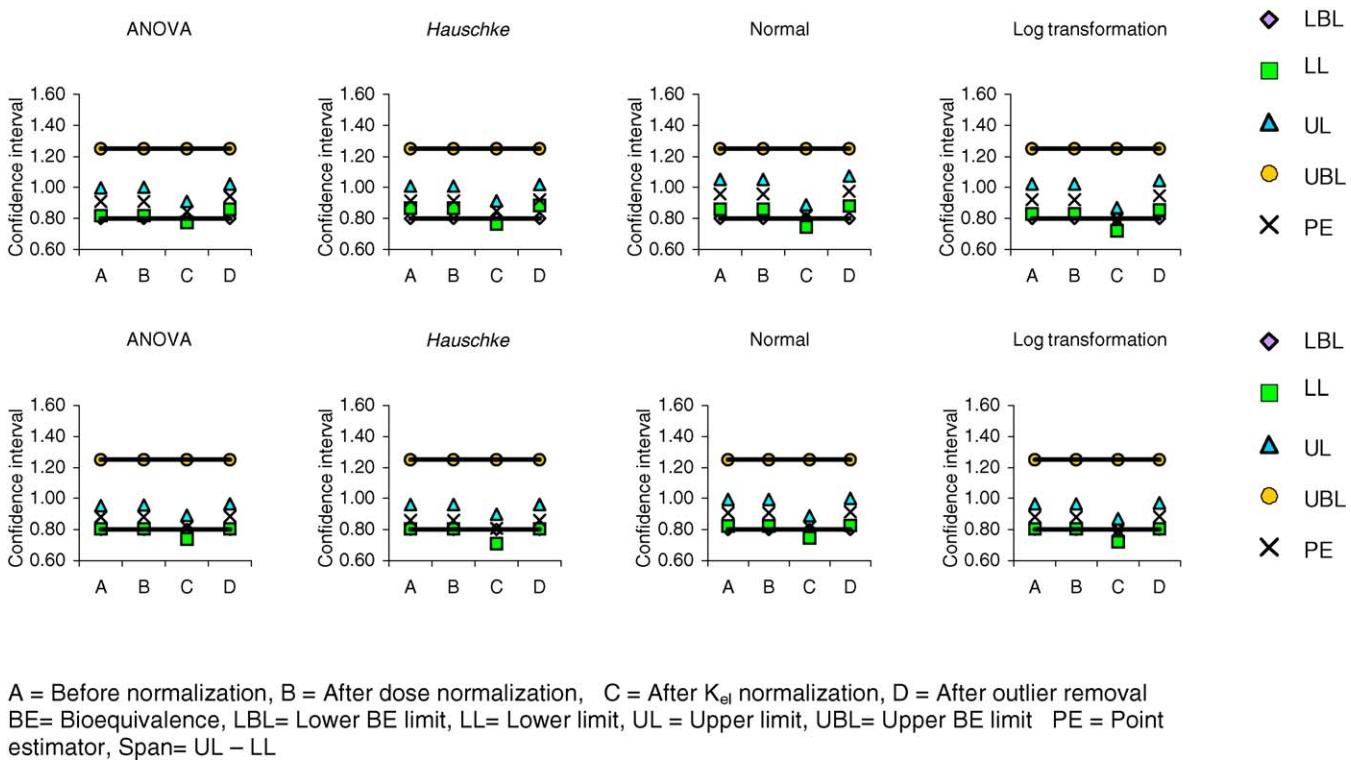

Fig. 5. Statistical limits observed for AUC (upper panel) and C_{max} (lower panel) values obtained from different statistical tests for study V.

AUC and C_{max} values were then evaluated by different tests like parametric *two-way* ANOVA, *hauschke* analysis, normal and log-transformed confidence interval (Panchagnula et al., 2000). After dose normalization to the individual body weights % CV of AUC values were changed (data not shown). As evident from Fig. 1, in study I dose normalization of AUC had shown no significant change in the bioequivalence limits and ultimate decision of bioequivalence remains the same. Similarly dose normalization of C_{max} values also had no significant effect on the bioequivalence endpoints (Fig. 1).

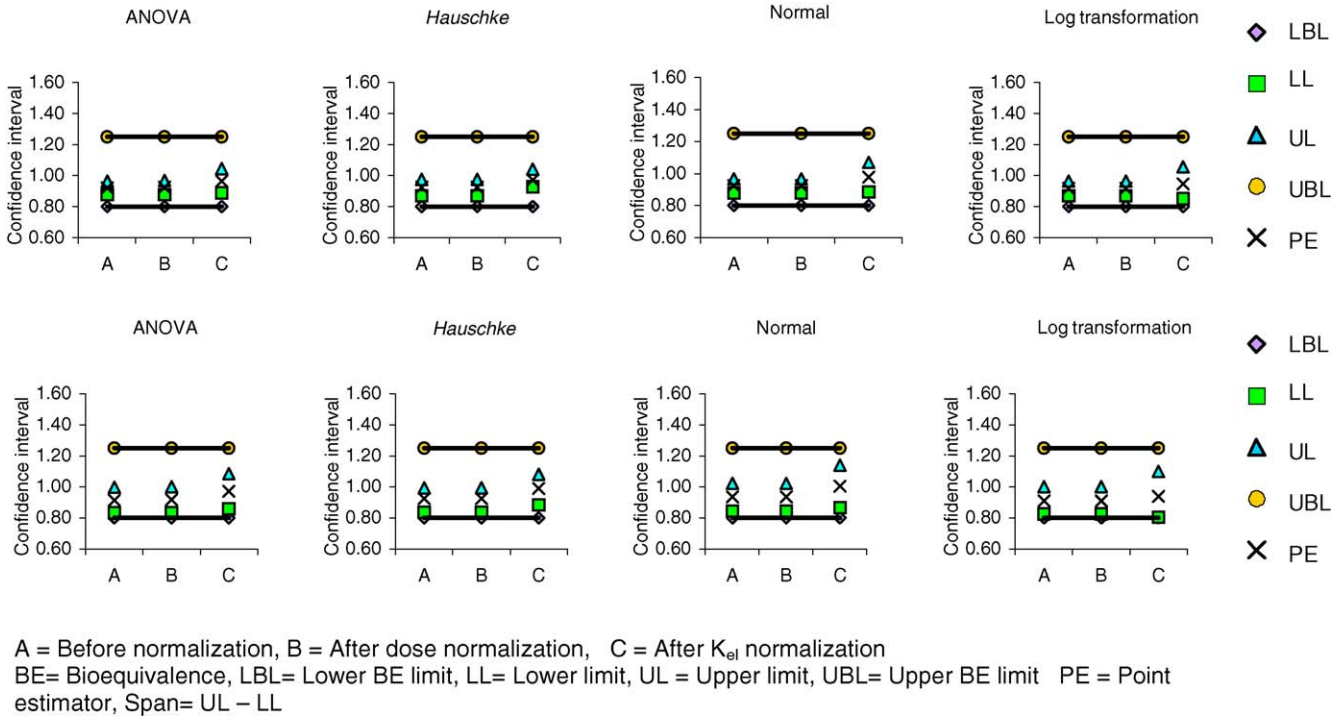

Fig. 6. Statistical limits observed for AUC (upper panel) and C_{max} (lower panel) values obtained from different statistical tests for study VI.

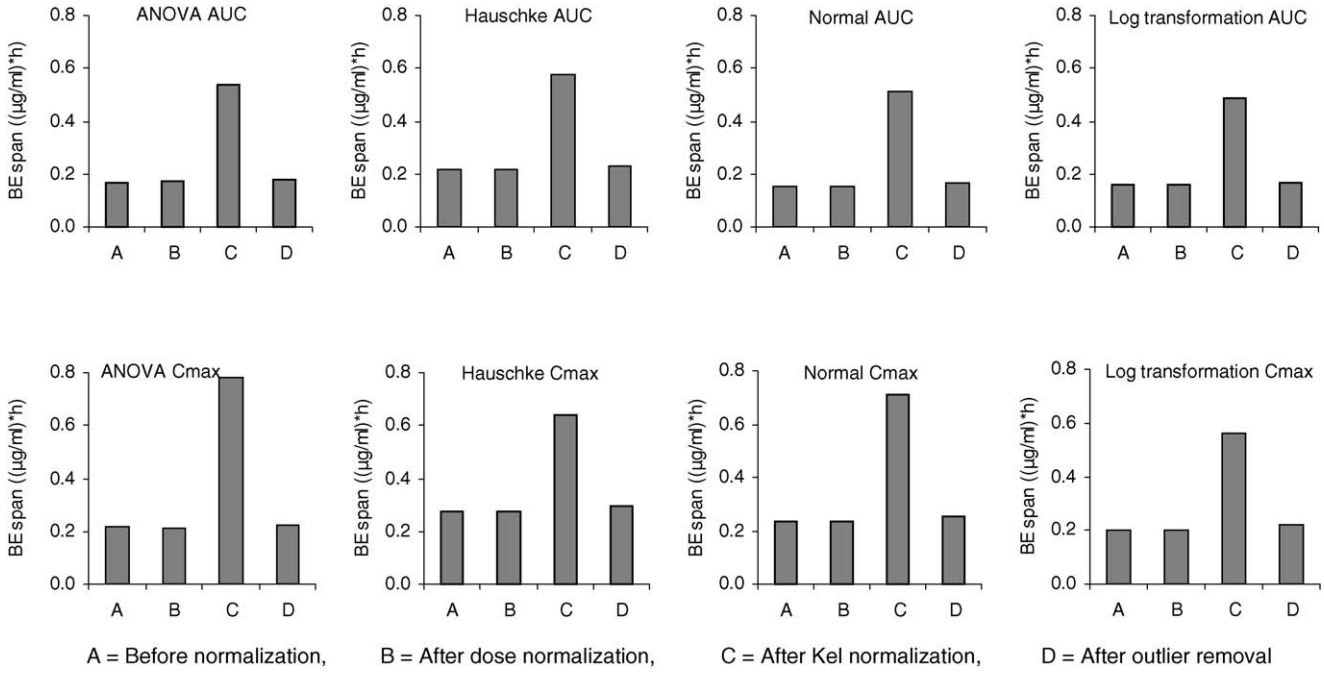

Fig. 7. Change in bioequivalence span observed for AUC and C_{max} values obtained from different tests for study I.

Similar trend was observed in all the studies (studies II–VI) and dose normalization of AUC and C_{max} values had shown no significant change in the ultimate conclusion regarding bioequivalence. Data from various studies are shown in Figs. 2–6.

Dose normalization in both combined and separate formulations was to the same extent and in case of bioequivalence estimation test/reference ratio is taken into account in *hauschke* analysis, normal and log-transformed confidence interval calculations and hence it shows no difference in the bioequivalence endpoint. However, in case of *two-way* ANOVA, difference between test and reference is also taken into consideration hence change in the bioequivalence confidence limits was observed, but it was found to be insignificant. Therefore, span of the bioequivalence (difference between upper and lower limit) do not show significant change.

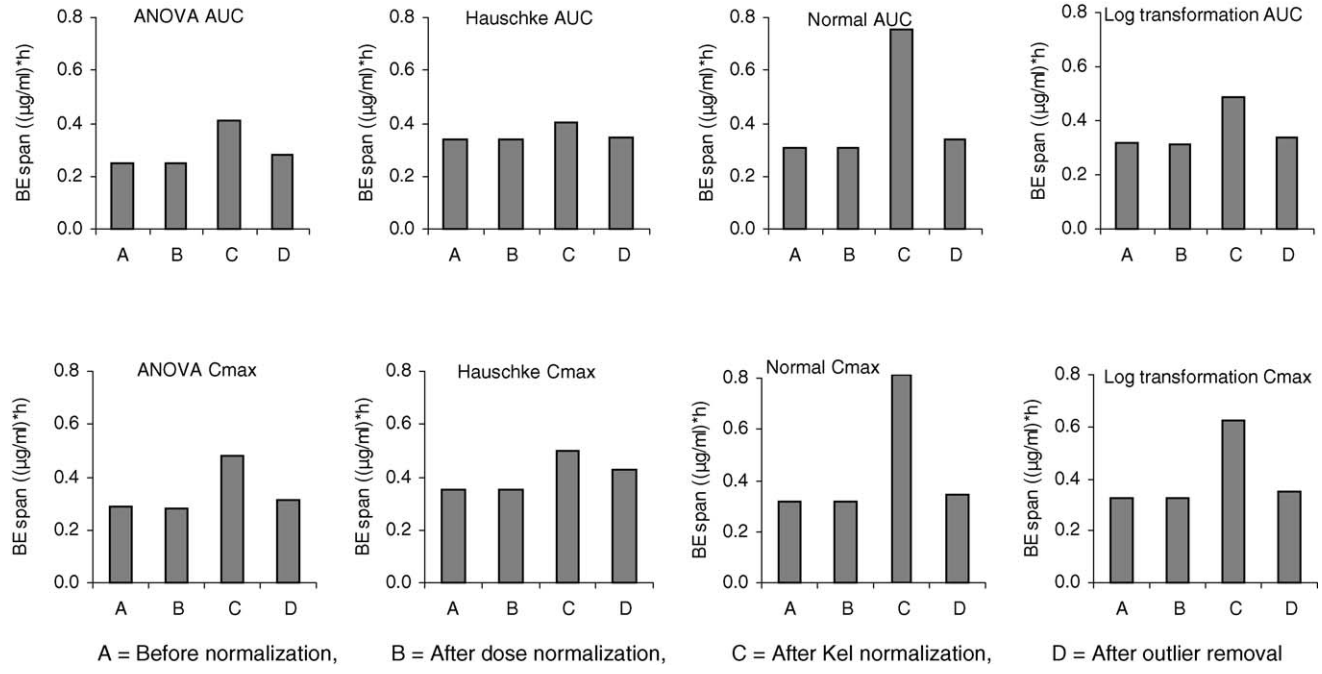

Fig. 8. Change in bioequivalence span observed for AUC and C_{max} values obtained from different tests for study II.

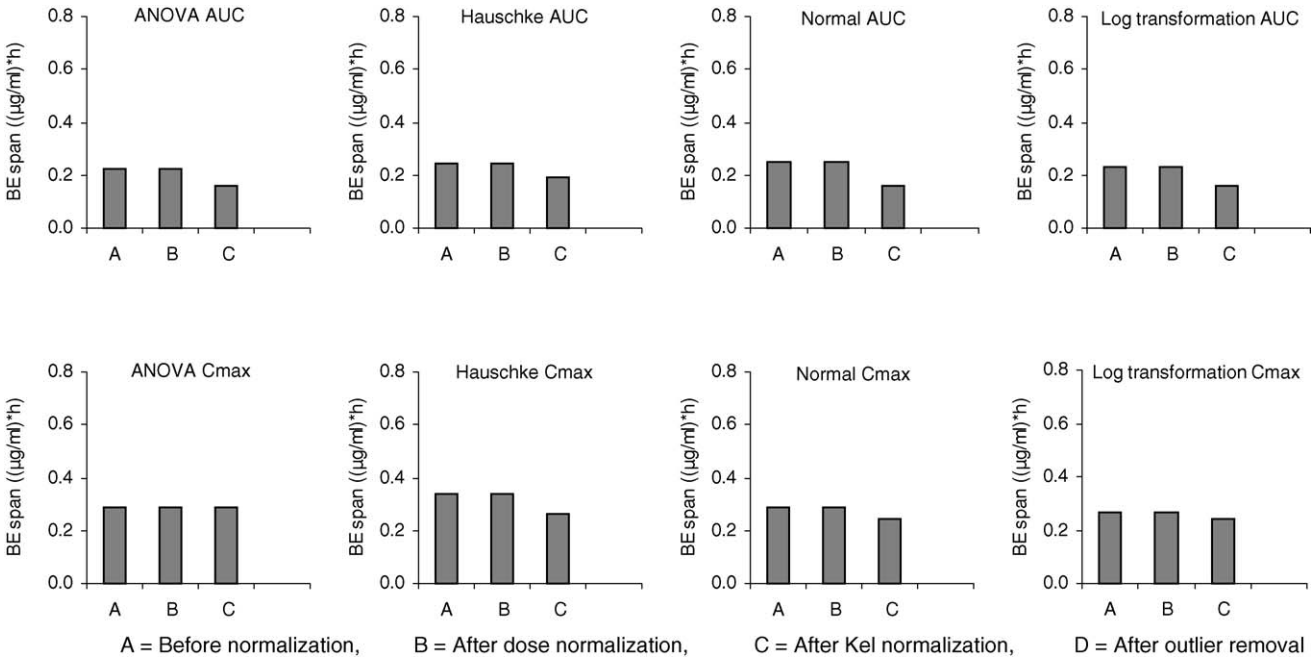

Fig. 9. Change in bioequivalence span observed for AUC and C_{max} values obtained from different tests for study III.

3.2. Normalization according to body weight and elimination rate constants

Normalization of AUC and C_{max} was carried out both according to individual's body weight and elimination rate constant and then subjected to tests like *two-way* ANOVA, *hauschke* analysis, normal and log-transformed confidence interval. As shown in Figs. 1–6, after normalization of AUC to body weight and elimination rate constant, change in the bioequivalence limits was observed.

It is clear from Fig. 7, span of the bioequivalence also remained unchanged in study I, after dose normalization of both AUC and C_{max}. Similar trend was also observed in all the studies. In case of study I widening of the bioequivalence span was observed after normalization of dose and K_{el} (Fig. 7). Similar observations were made in studies II, IV and VI (Figs. 8, 10 and 12). Similar trend was observed with normalized C_{max} values.

In case of studies III and V, downward shift of the bioequivalence limit was observed after both AUC and C_{max} normalization

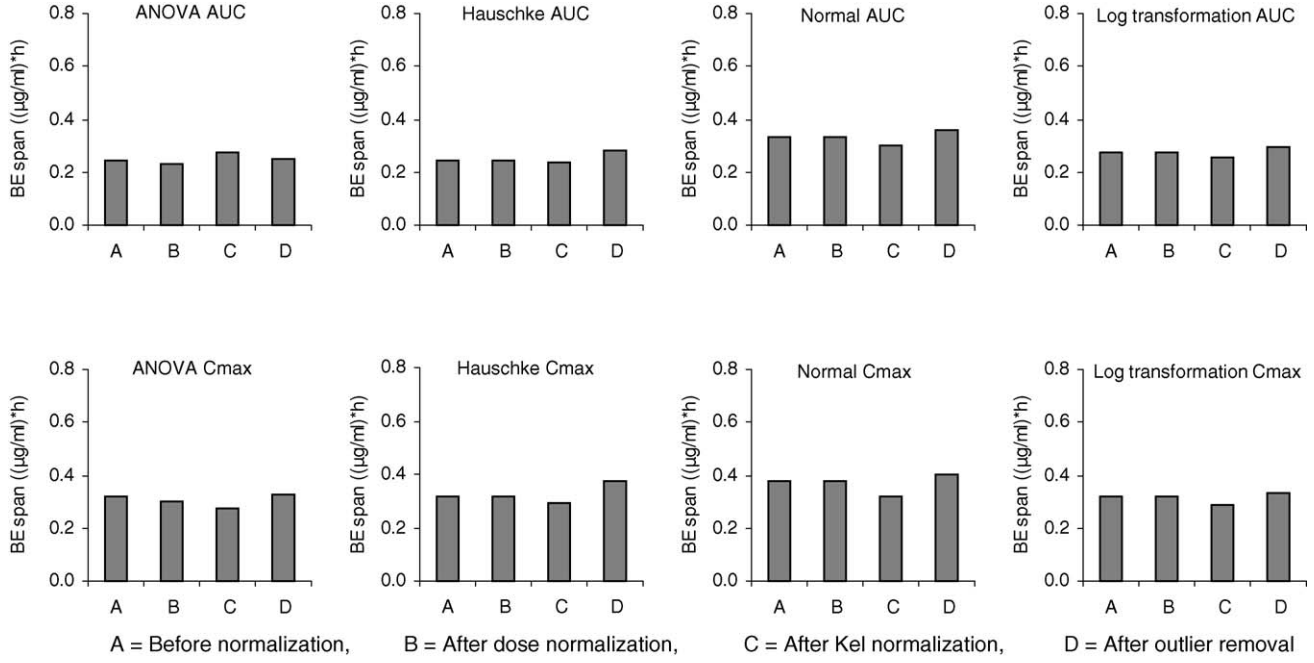

Fig. 10. Change in bioequivalence span observed for AUC and C_{max} values obtained from different tests for study IV.

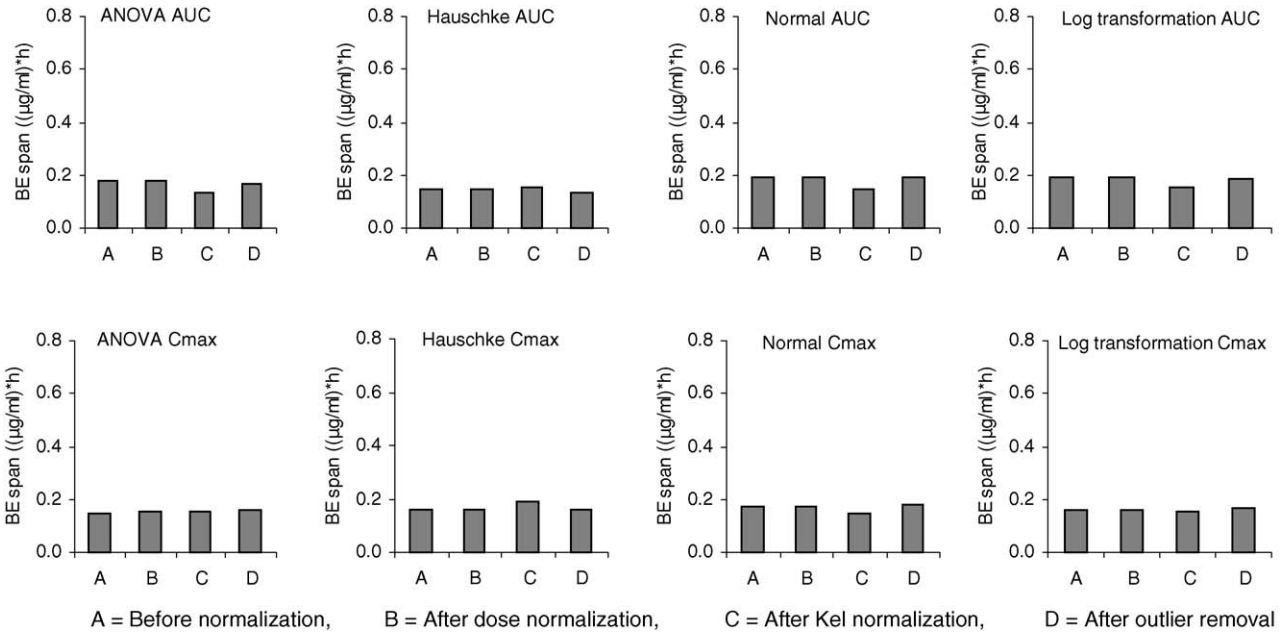

Fig. 11. Change in bioequivalence span observed for AUC and C_{max} values obtained from different tests for study V.

to both body weight and K_{el} (Figs. 3 and 5). However, in studies IV and VI, upward shift of the bioequivalence limits was observed (Figs. 4 and 6). In spite of this shift there is no change in bioequivalence end point.

After normalization of AUC, span of the bioequivalence was increased in studies I, II and VI (Figs. 7, 8 and 12) while in case of studies III, IV and V (Figs. 9–11) it was found to be decreased After normalization of C_{max}, span of bioequivalence was increased in studies I, II and VI and span was decreased in case of study IV. In studies II and V, no significant change was observed in bioequivalence span. In studies involving failed formulations, bioequivalence limits were widened, while in all other cases, either upward or downward shift in the bioequivalence limits was observed. There was no change observed in decision of bioequivalence after normalization of both AUC and C_{max} as per individual body weights and elimination rate constants.

3.3. Outlier detection and outlier removal

Dixon's test for the outlier detection was carried out for all the studies. After detection of the outlier, that data was removed and then remaining data was again checked for outlier. In studies III and VI, no outlier was detected both for the AUC and C_{max} values (Figs. 3 and 6). While in other studies I, II, IV and V, outlier was detected and after outlier removal data was evaluated by *two-*

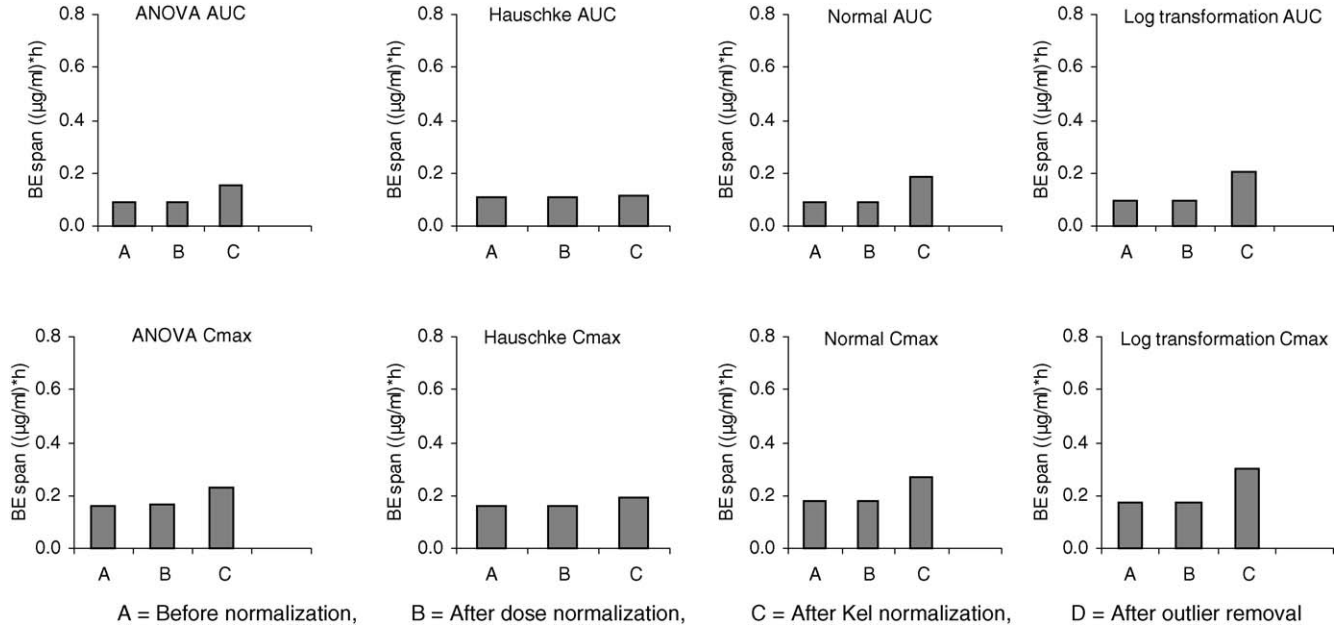

Fig. 12. Change in bioequivalence span observed for AUC and C_{max} values obtained from different tests for study VI.

way ANOVA, *hauschke* analysis, normal and log-transformed confidence interval tests (Figs. 1, 2, 4 and 5).

In studies I and II, AUC values after outlier removal had shown no significant difference in the bioequivalence limits. In study IV, after outlier removal AUC values had shown downward shift in bioequivalence limits (Fig. 4) while in study V, upward shift was observed in bioequivalence limits (Fig. 5). No change in the bioequivalence endpoint was observed in all the studies after outlier removal from both AUC and C_{max} values. Span of the bioequivalence also had shown insignificant change in all the studies after outlier removal from the AUC and C_{max} data (Figs. 7–12).

4. Conclusions

Dose adjustment as per body weights of the individuals did not play a significant role for decision of the bioavailability/bioequivalence. Inter/intra-individual variability in elimination rate showed a change in bioequivalence limits, however it did not play any significant role on the bioavailability end point. The effect of outliers on the assessment of bioequivalence has been found insignificant during the present investigation. Overall, study indicates that physiological variability does not play any role in variable bioavailability of the rifampicin observed in FDCs. Therefore, the variability seen in rifampicin bioavailability is mainly due to formulation factors, rate of dissolution and not due to methodology adopted in the protocol. These results further substantiate the validity of WHO model protocol for evaluation of bioequivalence of rifampicin containing FDCs.

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