

## Effect of Replicate Design on Drug Variability and Bioequivalence in Humans

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### Abstract

The purpose of this study is to investigate the effect of using replicate design on the intra/inter subject variability and bioequivalence of drugs in healthy volunteers. Model drugs used for analysis were amoxicillin/clavulanic acid combination. 24 healthy subjects participated in this study using 4-phase replicate cross over design. Individual disposition kinetic parameters of areas under plasma concentrations ( $AUC_{0-t}$ ) and maximum concentration ( $C_{max}$ ) were calculated by non-compartmental analysis using Kinetica program V 4.2 using all phases. The 90 % confidence intervals for log-transformed  $AUC_{0-t}$  and  $C_{max}$  were calculated for phases I & II; then for phases I, II and III; and for phases I, II, III and IV respectively. The intra and inter-subject variability values did not show a trend to decrease by the increase in phases included in analysis in both drugs and for both parameters. In addition, the 90 % confidence intervals for log-transformed  $AUC_{0-t}$  and  $C_{max}$  passed the 80-125 % limit range in both drugs for all phase combinations, even though  $C_{max}$  variability was shown high for clavulanic acid. However, individual bioequivalence was shown for AUC and not shown for  $C_{max}$  of both drugs. These results suggest not using replicate design as an approach to show the high inter/intra subject variability of highly variable drugs and hence justify wider acceptance limits of 75-133 % as recommended by the draft EMEA guideline. Literature information about drug high variability should be adequate to justify using wider acceptance limits of 75-133%.

**Keywords:** Replicate design; Acceptance limits; Bioequivalence; EMEA

### Introduction

Studies to measure bioavailability and/or establish bioequivalence of a product are important elements in support of the different drug applications and their supplements (Draft Guideline on the Investigation of Bioequivalence, 2008). Of special interest are bioequivalence studies of highly variable drugs. Hence, it was recommended by the new draft EMEA guideline that 90% confidence intervals for log-transformed areas under curve for the maximum plasma concentration ( $C_{max}$ ), to also fall between 80-125%. Wider acceptance limits of 75-133 can be justified by using replicate design to prove that drug is highly variable (Draft Guideline on the Investigation of Bioequivalence, 2008).

Statistical analysis for pharmacokinetic measures, such as area

under the curve, using the standard average bioequivalence involves the calculation of a 90% confidence interval for the ratio of the averages (population geometric means) of the measures for the test and reference products. However, the average bioequivalence method does not assess a subject-by-formulation interaction variance, that is, the variation in the average test and reference difference among individuals. In contrast, the individual bioequivalence (IBE) approach assesses within-subject variability for the test and reference products, as well as the subject-by-formulation interaction (Guidance for Industry, 2001).

The purpose of this study is to investigate the effect of using replicate design on the bioequivalence of high (variability > 30%) and low (variability < 30%) variable drugs. Model drugs used amoxicillin/clavulanic acid combination.

### Materials and Methods

#### Drugs

Drug formulations were amoxicillin/clavulanic acid combination.

#### Subjects and study design

24 healthy adult male volunteers participated in the two formulations, two sequence (TRTR, RTTR), four period cross-over single oral dose study. Sample size for each study was calculated based on reported intra-subject variability of pharmacokinetics primary parameters, considering  $\alpha = 0.05$ , the bioequivalence range (0.8-1.25) and to obtain a statistical power greater than 80%. All subjects had mean age, mean body weight and mean height. The volunteers were instructed to abstain from taking any drug including over-the counter (OTC) for 2 weeks prior to and during the study period. Studies were performed according to the revised Declaration of Helsinki for bio-medical research involving human subjects and the rules of Good Clinical Practices. Also, study protocols were approved by Institutional Review Board (IRB) of IPRC (Idkaidek, 2004).

#### Experimental and assay procedure

In each study, following a ten-hour overnight fast, single oral dose of each drug was administered followed by 240-ml water

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Drug – Parameter	Phases	Intra , Inter % CV	Point Estimate	90% Lower Limit	90% Upper Limit	BE Conclusion
Amoxicillin- AUC <sub>t</sub>	I & II	5.8 , 18.7	97	94	101	Pass
Amoxicillin- C <sub>max</sub>	I & II	19.1, 27.9	89	81	99	Pass
Amoxicillin- AUC <sub>t</sub>	I, II & III	9.8, 19.3	95	89	101	Pass
Amoxicillin- C <sub>max</sub>	I, II & III	22.2, 29.8	95	86	105	Pass
Amoxicillin- AUC <sub>t</sub>	I, II, III & IV	9.3, 19.2	96	92	100	Pass
Amoxicillin- C <sub>max</sub>	I, II, III & IV	21.2, 28.8	92	84	99	Pass
Clavulanic a- AUC <sub>t</sub>	I & II	16.3, 28.9	92	82	102	Pass
Clavulanic a- C <sub>max</sub>	I & II	19.1, 31.6	94	84	106	Pass
Clavulanic a- AUC <sub>t</sub>	I, II & III	22.1, 29.4	101	92	110	Pass
Clavulanic a- C <sub>max</sub>	I, II & III	24.4, 31.8	100	88	114	Pass
Clavulanic a- AUC <sub>t</sub>	I, II, III & IV	19.9, 27.9	96	89	104	Pass
Clavulanic a- C <sub>max</sub>	I, II, III & IV	22.7, 30.6	97	88	108	Pass
Amoxicillin- AUC <sub>t</sub>	IBE*	H <sub>η1</sub> = - 0.081 (Pass)				
Amoxicillin- C <sub>max</sub>	IBE*	H <sub>η1</sub> = 0.019 (Fail)				
Clavulanic a- AUC <sub>t</sub>	IBE*	H <sub>η1</sub> = - 0.0234 (Pass)				
Clavulanic a- C <sub>max</sub>	IBE*	H <sub>η1</sub> = 0.0517 (Fail)				

\*IBE: Individual Bioequivalence analysis results, from reference 2.

**Table 1:** Average Bioequivalence Analysis Results for Test / Reference Ratios after Log Transformation for AUC<sub>0-t</sub> and C<sub>max</sub> Parameters.

in each study. Blood samples were collected up to 10 hour after dosing. Samples were stored at -20 °C until parent drugs are analyzed by validated and sensitive hplc method (Idkaidek, 2004).

### Data analysis

Areas under plasma concentrations (AUC<sub>0-t</sub>), maximum concentration (C<sub>max</sub>), time to reach maximum were calculated by non-compartmental analysis for all subjects. Confidence intervals, point estimates and parameter variability analysis for log-transformed AUC<sub>0-t</sub> and C<sub>max</sub> were also calculated for phases I & II; then for phases I, II and III; and for phases I, II, III and IV using Excel and Kinetica® software (Kinetica V 4.2, 2007).

### Results and Discussion

Average bioequivalence analysis and IBE results were summarized in Table 1. As shown in table 1, the 90 % confidence intervals for log-transformed AUC<sub>0-t</sub> and C<sub>max</sub> were calculated for phases I & II; then for phases I, II and III; and then phases I, II, III and IV respectively. The intra and inter-subject variability values did not show a trend to decrease by the increase in phases included in analysis in both drugs and for both parameters. In addition, the 90 % confidence intervals for log-transformed AUC<sub>0-t</sub> and C<sub>max</sub> passed the 80-125 % limit range in both drugs for all phase combinations, even though C<sub>max</sub> variability was shown high for clavulanic acid. Thus use of replicate design did not add advantages in terms of decreasing inter/intra subject variability and also for bioequivalence limits.

However, individual bioequivalence was shown for AUC and not shown for C<sub>max</sub> of both high and low variable drugs (Idkaidek, 2004). This rendered the IBE approach and the replicate design unsuitable for showing formulation differences. Instead, scaled average bioequivalence of 2X2 cross over design would be more appropriate (WHO Guideline, 2005; Midha, 2005). Actually, US FDA guideline recommends using average cross over design, as a default design for bioequivalence studies (Guidance for Industry, 2003).

### Conclusion

These results suggest not using replicate design as an approach to show the high inter/intra subject variability of highly variable drugs and hence justify wider acceptance limits of 75-133 % as recommended by the draft EMEA guideline. Literature information about drug high variability should be adequate to justify using wider acceptance limits of 75-133%.

### References

1. Draft Guideline on the Investigation of Bioequivalence (2008) Doc. Ref. CPMP/EWP/QWP/1401/98 Rev. 1. London. » [CrossRef](#) » [PubMed](#) » [Google Scholar](#)
2. Guidance for Industry (2001) Statistical Approaches to Establishing Bioequivalence. Population and Individual Bioequivalence Working Group of the Biopharmaceutics Coordinating Committee in the Office of Pharmaceutical Science, Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration (FDA). » [CrossRef](#) » [PubMed](#) » [Google Scholar](#)
3. Guidance for Industry (2003) BA and BE Studies for orally administered drug products – General Considerations. Center for Drug Evaluation and Research (CDER), Food and Drug Administration (US FDA). » [CrossRef](#) » [PubMed](#) » [Google Scholar](#)

4. Idkaidek N, Alghazawi A, Najib N (2004) Bioequivalence assessment of Amoxicillin/Clavulanic acid 250/125 mg combination tabs in Healthy Human Volunteers: Use of replicate design. *Biopharmaceutics and Drug Disposition* 25: 367-372. » [CrossRef](#) » [PubMed](#) » [Google Scholar](#)
5. Kinetica V 4.2, (2007) Innaphase Corp., France. » [CrossRef](#) » [PubMed](#) » [Google Scholar](#)
6. Midha K, Rawson M, Hubbard J (2005) The bioequivalence of highly variable drugs and drug products. *Int J Clin Pharmacol Ther* 43: 485-498. » [CrossRef](#) » [PubMed](#) » [Google Scholar](#)
7. WHO Guideline (2005) Multisource (GENERIC) Pharmaceutical Products: Guidelines on Registration Requirements to Establish Interchangeability. Quality Assurance & Safety: Medicines (QSM), Department of Medicines Policy and Standards (PSM), World Health Organization. » [CrossRef](#) » [PubMed](#) » [Google Scholar](#)