RESEARCH ARTICLE

# Assessment of polymorphic metabolite data in bioavailability/bioequivalence studies – considerations and challenges

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Bioavailability (BA)/ bioequivalence (BE) studies are the cornerstone for the approval of generic drugs. While BA/BE assessment involving the pharmacokinetic data of the parent compound has been routinely performed, the introduction of the assessment of metabolite(s) data, alone or in addition to parent compound, has also emerged. In this context, the assessment of BA/BE of metabolite(s) may pose additional complexities and challenges, if the metabolic pathway is under the influence of a polymorphic enzyme. This communication provides brief perspectives on the challenges and study design considerations for the assessment of polymorphic metabolite in BA/BE studies.

***Key words:*** *Bioavailability, bioequivalence, metabolite, polymorphic, CytochromeP450*

## INTRODUCTION

The subject matter of bioavailability (BA)/bioequivalence (BE) is a topic that has evolved over time, including design-related considerations.[1-7] BA/BE studies are typically conducted to confirm whether or not the rate and extent of absorption of the parent drug is identical when delivered in a prototype formulation (test) relative to the reference formulation. In most cases, the pharmacokinetic evaluation is performed for the parent drug using peak concentration (Cmax) and area under the plasma/serum concentration versus time data (AUC). However, in recent times, the metabolite pharmacokinetic data, in addition to the parent data, are being considered in the assessment of BE of the two formulations. This communication focuses on some general perspectives for BA/BE study design considerations if the metabolite formation is under the control of polymorphic enzyme.

## MAJOR ISSUE

By nature, the biotransformation of compound(s) that is/ are under the control of polymorphic cytochrome P450 (CYP) isozymes (notably, CYP2D6, CYP2C9 or CYP2C19) is tricky – whereas subjects who express little or no enzyme tend to exhibit higher parent levels, the subjects who have over expression of these enzyme(s) tend to display higher proportion of metabolite(s). Therefore, in a given population pool, higher than normal variability needs to be expected relating to both parent and metabolite (s) pharmacokinetic data of polymorphic drug substrates. The work of Tomalik-Scharte *et al*. (2009) has captured the significant role of polymorphic play due to defective alleles in the drug metabolizing enzymes inclusive of scores of enzymes like *N*-acteyltransferase, thiopurine *S*-methyl transferase, uridine diphosphate glucuronosyltransferase, etc., responsible for Phase II metabolism and disposition of xenobiotics.[8] The importance of CYP2D6 genetic polymorphism has been recently highlighted for the clinical therapy with anti-depressants – this article covers areas such as drug regimen selection, dose alterations, drug switches, and drug withdrawals for the effective management of patients with chronic depression.[9] Overall, although the scope of the present communication is restricted to BA/BE study design considerations, the impact of genetic polymorphism can influence the safety, efficacy and drug-drug interaction (DDI) potential of scores of xenobiotics.[10-12]

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As a consequence of the variability in the data set, a larger pool of subjects may be needed to establish BE. Additionally, sample size is an important consideration for DDI study using BE assessment because one may conclude either a potential interaction when there was none and vice versa.

## BIOEQUIVALENCE STUDY DESIGN CONSIDERATIONS

Several options may be formulated, as enumerated in Figure 1. Each of the options would provide a different strategic posture, risks and challenges for execution.

a) An interesting option is to consider a study population that is homogeneous in nature (i.e., pre-screen the phenotype of subjects and include only the extensive metabolizers for the study). Such a strategy would be supportive for a BE study of two drug products, where only product characteristics are expected to be evaluated. However, the drawback of such an approach would be lack of data obtained in the poor metabolizers of the drug and the lingering question is whether the drug formulation would behave differently in the poor metabolizer phenotypes. Although it may be equally possible to only enrol subjects who are poor metabolizers to provide homogenous baseline, however, such a strategy is not recommended because the enrolment of subjects belonging to poor metabolizer phenotype may take time and is non-productive.

b) Another option is to consider the known distribution of the phenotypes in the population at large. Following this, randomly pre-screen the subjects and enrol them *a priori* to include certain percentage of the various phenotypes, comprising extensive, intermediate and poor metabolizer phenotypes. While this would be an ideal study setting to ensure due considerations were given to reflect the population phenotype distribution, the challenges of such an approach would be: i) related to enrolment of the required number of pre-specified phenotypes which may lead to study delays; ii) inflexibility for subject replacement(s); and iii) need to conduct the study in multicenters, adding another layer of complexity.

c) Another design option, typically followed, is simply to enrol the subjects without regard to the phenotypes – here one would determine the sample size (assumption of certain variability factoring various phenotypes) and enrol subjects at random until the desired sample size is reached. The distribution of phenotypes in the study will only become apparent when the pharmacokinetic data becomes available.

While in options (a) and (b) stated above, the certainty of study population is known *a priori*, it may not be the case if one considers the design option (c). In study design option (c) which tends to be most commonly applied, one major drawback is obviously related to the potential inadvertent

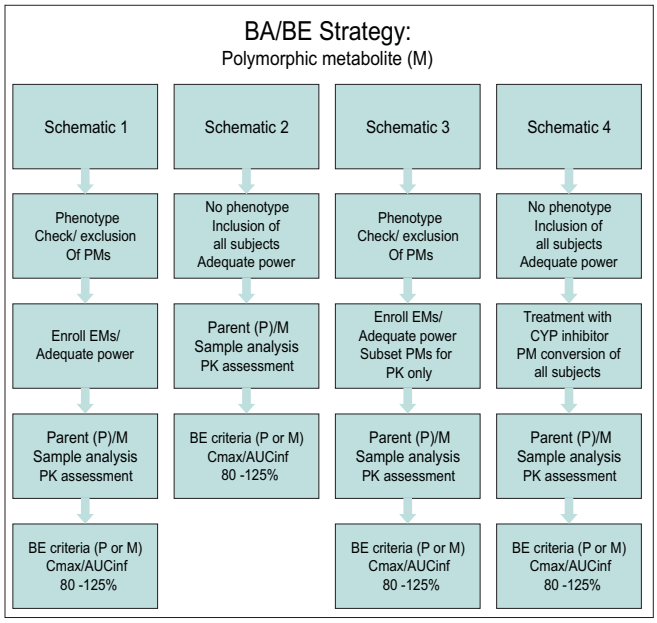


**Figure 1:** Schematic representation of various study design considerations for bioequivalence for drugs that exhibit polymorphic metabolism

over-enrolment of a specific phenotype which may contribute for a higher variability than what was known from the previous pharmacokinetic data of the compound. Such an occurrence could lead to either error in data interpretation and/or inconclusive outcome of the study. In both study designs (b) and (c), an important consideration is to ensure the replacing subjects in the study represent the same phenotype of the original subject(s) who is/are not able to participate in the study. Such an underlying principle will ensure that the study power/sample size is not altered. Another interesting study design consideration [schematic 4 in Figure 1] relates to the conversion of all subjects to the same phenotype by the introduction of another agent that can inhibit for a transient period the polymorphic CYP enzyme (for instance, CYP2D6 can be inhibited by a small dose of quinidine). While one may consider this type of study design to be akin to DDI study, nevertheless, it will address the variability associated with the formation of polymorphic metabolite by converting the entire population into a homogenous group. In this design, all subjects will be temporarily converted into poor metabolizer phenotypes, and therefore the parent drug measurements should not pose a challenge. However, it is important to point out that due to the inhibition of polymorphic CYP enzyme, the formation of the metabolite may be completely hindered or limited such that low levels of the metabolite may be floating in the systemic circulation. This would necessitate the development of a sensitive bioanalytical assay for the measurement of low levels of the metabolite. Some drawbacks of this design stem from the fact that another agent is being introduced for the inhibition of polymorphic CYP enzyme, and therefore absorption/BA of the agent may introduce variability. Additionally, it may be possible that

the extent of inhibition may vary amongst subjects. Perhaps to avoid the issue of absorption/BA and, to some extent, degree of inhibition due to incomplete dissolution, one could consider dosing of the desired CYP inhibitor in the form of a solution.

## CASE STUDY – ILLUSTRATION USING CLINICAL DATA

Figure 2 provides illustration on the challenges likely to be posed if polymorphic metabolism is involved in the conversion of parent compound, while Figure 3 depicts similar challenges for the disposition of the primary metabolite which is under the influence of polymorphic metabolism.

The context to the issues of polymorphic metabolite and CYP inhibitor is provided by a recently reported DDI study of rupatadine with azithromycin.[13] One important distinction that needs to be made from the case study is that it deals with the polymorphic metabolism of the primary metabolite of rupatadine, as illustrated by schematic 2 in Figure 1. Solans *et al*. (2008) have designed and published an elegant, properly controlled and well-executed DDI study using BE criteria between rupatadine and azithromycin in healthy subjects.[13] The study was performed under steady-state conditions reflecting the ideal clinical scenario.[13] The measurements of two active metabolites, desloratadine and 3-hydroxyloratadine, also ensured the appropriateness for a DDI clinical study.[13]

A cursory look at the pharmacokinetic data from this study[1] suggested that there may be an involvement of a genetic polymorphism in the disposition of rupatadine such that there were distinct phenotypes on the basis of metabolite(s) to parent drug exposure ratios.[13] While literature citations that implicated rupatadine as a polymorphic substrate were not readily found, interestingly a recent review article on rupatadine that included clinical pharmacology and metabolism attributes claimed that it was unlikely that rupatadine would be a substrate for polymorphic CYP isozymes.[14] However, Solans *et al*. (2008) provided a hint that there was a genetic variability in the pharmacokinetic parameters in earlier studies for desloratadine[15,16] and pointed out that the formation of 3-hydroxyloratadine metabolite from desloratadine was under the control of genetic polymorphism.[13,15,16] Interestingly, the CYP enzyme responsible for the formation of 3-hydroxyloratadine has not been identified.

Based on the above facts, this study, in particular,[1] assumed a greater significance because rupatadine is metabolized predominantly by CYP3A4, while one of rupatadine's key metabolites (desloratadine) is subjected to metabolism by polymorphic CYP enzyme(s) and azithromycin is a classical mechanism based CYP3A4 inhibitor. Since it is a rarity to see publications of this complexity,[1] using this particular DDI

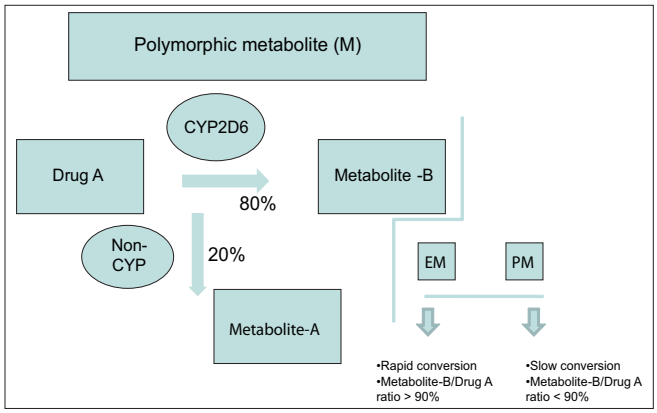


**Figure 2:** Schematic to represent formation of a polymorphic metabolite from the parent compound

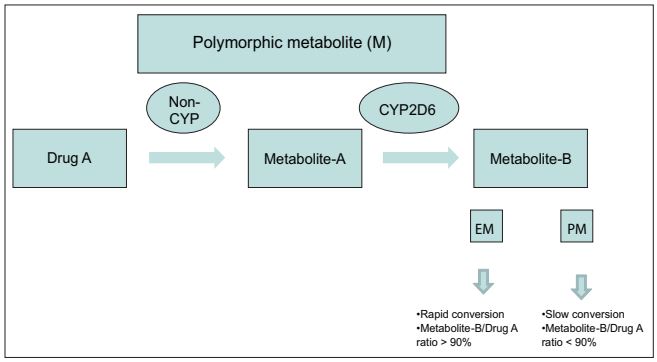


**Figure 3:** Schematic to represent formation of a polymorphic metabolite from the primary metabolite of the parent compound

study as a reference, the following thoughts are provided for introspection: 1) Despite having more than the expected number of slow metabolizers of desloratadine, the data reported in the study provided convincing evidence of BE, and therefore lack of DDI potential between rupatadine and azithromycin.[13] However, closer scrutiny of the data suggested that point estimates for both parent and the polymorphic metabolite were above 1.00 and the upper limit of 90% CI was close to 1.25, suggesting supra bioavailability of both rupatadine and desloratadine when co-administered with azithromycin; 2) In the event bioinequivalence was observed for both rupatadine and/or desloratidine, would it suggest a true DDI phenomenon or would it reflect the inherent variability with the data set, especially with the polymorphic metabolite further confounding the data? The case study also points out to the recognition of a specific metabolite that may be affected by polymorphic metabolism once it is formed and the disposition of metabolism in certain patient population may be difficult to predict and therefore could lead to increased variability in the pharmacokinetics.

In summary, the data from the presented case study[13] are a learning lesson that the issue of genetic polymorphism may arise from the secondary/subsequent metabolism of the key primary metabolite(s) but not necessarily the parent

compound. Additionally, the existence of such genetic polymorphism may go unnoticed for considerable time due to lack of appropriate study population as evidenced by lack of literature citations on this aspect of rupatadine's metabolism until recently.[14,17] While more publications of these types of DDI studies would provide a strong base to appreciate the complexities involved in the polymorphic metabolism of primary metabolite(s), it is prudent to adopt a study design that provides the right balance in achieving the study objectives if existence of polymorphic metabolism is suspected in the disposition of primary metabolite without risking a study failure to unsuspected "polymorphic" metabolism.

## DISCUSSION

BA/BE studies are the cornerstone for the introduction of generic small molecule drugs and the criteria for submission of such clinical pharmacokinetic data of test and reference formulations have been clearly stated. While the standard BE criteria have been strictly followed in the submission package for the approval of generic drugs, such BE criteria are also involved in making decisions in formulation switches during drug development as well as in the introduction of drugs to market when the Phase 3 formulation used in pivotal registration studies was different from the intended marketed product.

While generally BA/BE strategy is applicable for most of the drugs using the parent compound itself, sometimes one may have to consider the use of metabolite along with the parent compound, and in other instances the use of metabolite may have to be considered for assessing BA/BE between the test and reference formulations. However, there is no strict regulatory guidance on the use of metabolite(s) in lieu of the parent compound and the appropriateness for the use of metabolite data in lieu of the parent data may have to be justified by the sponsor. In this context, the involvement of polymorphic metabolism as illustrated in this communication may add further complexity for the BA/BE assessment if one is considering the use of metabolite(s) data in lieu of the parent data to compare the reference to test formulations. The communication is intended to think creatively in designing clinical studies for BA/BE assessment in such instances where a polymorphic metabolite is confirmed and/or there is a suspicion that the pathway of metabolism of parent/metabolite to subsequent metabolite(s) is governed by a polymorphic enzyme. The conventional wisdom dictates the use of the pharmacokinetic data (rate and extent of absorption) of the parent compound as the key surrogate for BA/BE assessment which is generally applicable for many compounds. However, in the event the pharmacological activity or pharmacodynamic responses are largely governed by the active metabolite(s), it may be prudent to include such metabolite data in the BA/BE consideration. Since metabolism of compounds, if involving multiple pathways and polymorphism, may lead to more variability in the pharmacokinetic data of both the parent and the generated metabolite, it may be prudent to consider various study design considerations as suggested in this communication. In the event the sponsor chooses a study design which is not typically used, it may be prudent to get a regulatory buy-in to ensure that data generated and interpreted for BA/BE assessment would be considered as a suitable alternative for demonstrating the BE of the two formulations.

## CONCLUSIONS

The BA/BE criteria of using the pharmacokinetic data of the parent compound have stood the test of time and are universally accepted. However, the use of metabolite(s) in addition to parent or instead of parent compound in BA/BE assessment has been a more recent phenomenon. In this context, the presence of polymorphic metabolite may pose additional challenges and complexities. The communication provides a platform for some out of box thinking and concepts to address the challenges/complexities that are involved with the generation of polymorphic metabolites.

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