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Questions & Answers: positions on specific questions addressed to the Pharmacokinetics Working Party (PKWP)

Background

In the context of assessment procedures, the input of the Pharmacokinetics Working Party (PKWP), (previously the Therapeutic Subgroup on Pharmacokinetics of the Efficacy Working Party (EWP-PK subgroup)), may be sought by the CHMP or, following CHMP's agreement, by other Committees, Working parties or the CMD(h). The objective is to address specific questions in relation to pharmacokinetic evaluations and particularly the requirements and assessment of bioequivalence studies. The positions, which are subsequently elaborated by the PKWP in response to such questions, are then forwarded to the enquiring party for consideration in their assessment.

It is understood that such positions will be reflected in procedure-related assessment reports, if applicable. In some cases however, these positions might also be of more general interest as they interpret a very specific aspect that would not necessarily be covered by a guideline. This paper summarises these positions which have been identified as being within this scope. In addition, general clarifications related to guidelines authored by the PKWP are subject to specific positions in this paper.

It should be noted that these positions are based on the current scientific knowledge as well as regulatory precedents. They should be read in conjunction with the applicable guidelines on bioequivalence in their current version. If the questions have initially been raised in the context of specific assessment procedures, details of these procedures have been redacted for reasons of confidentiality.

This compilation will be updated with new positions as soon as they become available. Likewise, if a position is being considered outdated, e.g. due to new evolutions in the scientific knowledge including revisions to the applicable guidelines, positions will be removed from this document. Positions previously prepared by the EWP-PK subgroup are endorsed by the current PKWP unless removed from this document.

The positions in this document are addressing very specific aspects. They should not be quoted as product-specific advice on a particular matter as this may require reflection of specific data available for this product. By no means should these positions be understood as being legally enforceable.

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Note: Although previous versions of certain guidelines may be cited in some cases, the requirements referred to remain valid and in line with current guideline recommendations. All relevant current guidelines can be found in the scientific guidelines section of the EMA website under clinical pharmacology and pharmacokinetics.

The following positions have been deleted in the latest update because respective contents have been implemented in new/revised guidance documents:

Position	**Date of deletion**	**Reasoning**
Requirements for food interaction studies for modified release formulations	July 2015	Covered by the revised Guideline on the pharmacokinetic and clinical evaluation of modified release dosage forms (EMA/CPMP/EWP/280/96 Corr1)
Bioequivalence of gastro-resistant preparations (e.g. omeprazole)	July 2015	Covered by the Guideline on the pharmacokinetic and clinical evaluation of modified release dosage forms (EMA/CPMP/EWP/280/96 Corr1)
Requirements for demonstration of bioequivalence for generics of biphasic modified release formulations for oral use	July 2015	Covered by the Guideline on the pharmacokinetic and clinical evaluation of modified release dosage forms (EMA/CPMP/EWP/280/96 Corr1)
BCS classification of memantine	July 2015	Covered by the memantine product-specific bioequivalence guidance (CHMP/PKWP/EMA/423734/2013)

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1. Bioequivalence studies in children

Date of publication: 22 January 2009

The EWP-PK subgroup was asked to address the following questions: “Treatment of children often requires that new formulations or strengths are developed. If chemical-pharmaceutical data are not considered sufficient to establish bioequivalence should bioequivalence studies be conducted in children or would healthy volunteers suffice?”

The position of the EWP-PK subgroup is as follows:

In vivo bioequivalence is almost always established in healthy volunteers unless the drug carries safety concerns that make this unethical. This model, *in vivo* healthy volunteers, is regarded adequate in most instances to detect significant formulation differences and the results will allow extrapolation to populations in which the drug is approved (the elderly, patients with renal or liver impairment etc.). The same reasoning applies also to children. Hence, in the vast majority of cases BE studies in healthy volunteers are adequate for products intended for use in children.

2. Bioequivalence studies for generic products containing clopidogrel

Date of publication: 25 June 2009 (Rev. 1)

The platelet aggregation inhibitor clopidogrel is pre-systemically hydrolysed to the inactive metabolite clopidogrel carboxylic acid. The plasma levels of the unchanged drug are up to 2000 fold lower than those of the carboxylic acid metabolite. Another metabolite, clopidogrel thiol, formed by a parallel pathway, is the pharmacologically active form of clopidogrel and is generated in the intestine and liver primarily by the CYP2C19 enzyme isoform. Due to its chemical instability and low circulating levels, its detection in plasma is problematic. Clopidogrel thiol irreversibly binds to the P2Y12 receptors of ADP on the platelet membranes in portal and systemic circulation, leading to the inhibition of platelet aggregation.

During the evaluation of the Marketing Authorisation applications for generic product of clopidogrel, the following questions were addressed by the CHMP to the EWP-PK subgroup and the EWP-CVS subgroup group[1], respectively:

1. Which substance should be studied in bioequivalence studies: the parent compound clopidogrel or the metabolite(s) of clopidogrel?

The *Guideline on the investigation of bioequivalence* (CPMP/EWP/QWP/1401/98 Rev 1) states "*Also for inactive prodrugs, demonstration of bioequivalence for parent compound is recommended. The active metabolite does not need to be measured."*

At the time of approval of the reference product Plavix, no reliable and validated methodology for the determination of the pharmacokinetics of the parent prodrug clopidogrel or of the active metabolite clopidogrel thiol was available. Thus, at the time, the pharmacokinetic profile of clopidogrel was established based on the pharmacokinetics of clopidogrel carboxylic acid, which is the non-active metabolite. In the meantime, the pharmacokinetic profile characterisation of clopidogrel has improved by development of a sensitive analytical technique (e.g. LC-MS-MS) enabling for a suitable investigation of the parent prodrug, clopidogrel. A more accurate picture of the PK profile of clopidogrel can be obtained.

Position of the EWP-PK subgroup:

The demonstration of bioequivalence between the reference and the generic compound should be based on the parent prodrug, clopidogrel.

2. Is demonstration of bioequivalence under fed conditions necessary in addition to the demonstration under fasting conditions?

At the time the innovative drug-product was developed, no data regarding the effect of food on the bioavailability of clopidogrel parent compound were available. More recently, the investigation of food intake influence on the bioavailability of clopidogrel has been investigated. The results obtained by Nirogi *et al.* (Nirogi, RV *et al.*, Arzneimittelforschung 2006; 56(11); 735-9: *Effect of food on bioavailability of a single oral dose of clopidogrel in healthy male subjects*) indicate that in the fed state the bioavailability of a single oral dose of clopidogrel increases dramatically (500 - 600 %) but the systemic exposure to the major but inactive carboxylic acid metabolite increases only by approximately 10-20 %. The current Summary of Product Characteristics (SPC) for the originator states that clopidogrel should be given as a single daily dose of 75 mg with or without food.

[1] EWP Therapeutic Subgroup on Cardiovascular Issues

Position of the EWP-PK subgroup:

The Guideline on the investigation of bioequivalence (CPMP/EWP/QWP/1401/98 Rev 1) states *"In general, a bioequivalence study should be conducted under fasting conditions as this is considered to be the most sensitive condition to detect a potential difference between formulations. For products where the SmPC recommends intake of the reference medicinal product on an empty stomach or irrespective of food intake, the bioequivalence study should hence be conducted under fasting conditions."*

The food effect on the bioavailability (BA) of the unchanged clopidogrel - not recognised in the SPC - was not investigated by the innovator before approval of the originator product since a sensitive analytical method was not available at the time of approval. However, a publication by Nirogi et al. (2006) suggested a significant food effect with a high-fat meal. Similar results have been observed in applications for generic medicinal products. The food effect might be due to a protection from acidic hydrolysis in the stomach in a fasting state, since the BA is enhanced under fed conditions. The EWP-PK subgroup reviewed the solubility properties of clopidogrel salts and these indicate that when administration of clopidogrel occurs under fasting conditions, the dissolution in the gastric media with a subsequent hydrolysis and formation of the inactive carboxy-acid metabolite is maximal. As a consequence, the extent of unchanged drug that still is available for absorption (at the intestine level) is reduced. Conversely, the dissolution of clopidogrel is limited in the gastric media under fed conditions, the acidic hydrolysis in the stomach is reduced and the BA of clopidogrel is improved.

The EWP-PK subgroup acknowledges that as a consequence, the solubility of salts might be important. However, all clopidogrel salts have high solubility at low pH and the risk for acidic hydrolysis may therefore be similar. The food effect could consequently be expected to be similar to the reference product for different salts. Hence, the EWP-PK subgroup considered that there was currently an insufficient scientific rationale to justify a deviation from the revised bioequivalence guideline and bioequivalence should be demonstrated under fasting conditions irrespective of the salt.

Should further information on the food effect of clopidogrel become available, the SPC would be amended accordingly.

3. Bioanalytical methods: Should there be any special requirements to ensure that the risk of back-conversion of the major metabolite to clopidogrel could be excluded?

Within several centralised clopidogrel applications, the CHMP raised concerns about the possible back-conversion of the major metabolite of clopidogrel (clopidogrel carboxylic acid) to clopidogrel during the bio-analytical analysis of the samples. Considering that plasma levels of clopidogrel carboxylic acid observed in patients or healthy volunteers treated with clopidogrel are much higher than that of the parent drug, a minimum back-conversion of the metabolite could potentially lead to a huge over-estimation of clopidogrel plasma levels and would bias the outcome of bioequivalence study.

Position of the EWP-PK subgroup:

The EWP-PK subgroup confirmed that back-conversion could potentially occur when methanol is used as (part of) extraction solvent, reconstitution solvent, chromatography mobile phase or for the preparation of calibrators, quality control (QC) solutions and internal standards during bioanalysis. Therefore, testing for the back-conversion of clopidogrel carboxylic acid metabolite should be part of the validation process of analytical methods used for the measurement of clopidogrel plasma levels.

It should be demonstrated that there is no back-conversion of the major metabolite to the parent drug clopidogrel under all conditions for sample handling (including extraction procedures) and storage.

4. Could the acceptance criteria for C_{max} be widened?

According to the *Guideline on the investigation of bioequivalence* (CPMP/EWP/QWP/1401/98 Rev 1) widening of the acceptance criteria for C_{max} is possible for highly variable drug products provided that a wider difference in C_{max} is considered clinically irrelevant based on a sound clinical justification. The revised bioequivalence guideline provides detailed advice on how the acceptance criteria can be widened for highly variable drug products with a bioequivalence study of replicate design and using the scaled-average-bioequivalence approach. However, a prerequisite for widening the acceptance criteria is that a wider difference in C_{max} is considered clinically irrelevant. This issue was assessed by the EWP-CVS subgroup.

Position of the EWP-CVS subgroup:

The EWP-CVS subgroup evaluated the request from widening the 90% confidence interval for C_{max} from the efficacy and safety perspectives. The EWP-CVS subgroup considered what would be the degree of the impact of the possible variations in the C_{max} following the 75 mg dose, since some data suggest the existence of a plateau response in the inhibition of platelets aggregation. However, it is currently not entirely clear what would be the influence of variable clopidogrel concentrations on pharmacodynamics. It is important to note that clopidogrel is approved and recommended for use in acute clinical conditions, for which a high loading dose is advised in order to attain a fast antiplatelet action. Whether in these situations a lower C_{max} might be of clinical relevance is unknown, but cannot be completely excluded.

In conclusion, it is not definitely proven that widening C_{max} acceptance range for clopidogrel is devoid of clinically relevant implications, both in terms of safety and efficacy, for all situations where the drug is used in clinical practice. Under these circumstances, the widening of 90% confidence intervals for C_{max} is not recommended.

3. Acceptance criteria for bioequivalence studies for losartan

Date of publication: 22 July 2010 (Rev. 2)

The EWP-PK subgroup was asked to address the following question: Which analyte, parent and/or metabolite, should be used for the decision of bioequivalence in the case of losartan, and which acceptance criteria should be applied.

Position of the EWP-PK subgroup:

Losartan is not a pro-drug. It is an angiotensin II antagonist at the AT1-subtype receptor. In humans, losartan competitively binds to the AT1 receptor, while the metabolite E3174 binds non-competitively.

The active metabolite E3174 is not directly formed from losartan, but from an intermediate product, metabolite E3179. Alternatively, the E3179 intermediate can also be hydroxylated to an inactive metabolite. It has been estimated that about 14% of the orally administered losartan dose is converted into E3174. In addition, 5 other minor metabolites exists that exhibit activity but much less than parent.

AUC of the active metabolite is 4 – 8 fold higher than parent, as it is cleared about 10-fold slower than parent.

Plasma free fractions of parent are 1.3% and that of the active metabolite 0.2%. Losartan and its metabolite E3174 shows linear pharmacokinetics.

It has been shown in vitro that the IC50 for binding to the AII receptor in smooth muscle cells is 10-fold more potent for the metabolite than parent and that the in vitro AII concentration dependent contractile response in rabbit aorta is 33-fold higher for the metabolite. In vivo, in normotensive and renal hypertensive rats, the active metabolite has been shown to be 15 – 20-fold more potent compared to the parent.

Based on in vivo studies in rat, in which the potency was 15 – 20-fold higher for the metabolite, and assuming a more or less comparable protein binding as that observed for human plasma (literature indicated for losartan a binding >99% in rat plasma), the metabolite activity is about 76 – 100-fold higher than the parent compound.

Hence, based on total exposure (AUC), the metabolite accounts for the majority of the activity. However, losartan and the active metabolite have different plasma-concentration time course, with considerably higher losartan plasma concentrations during the first hours after administration. Considering the plasma concentration time course, difference in activity and protein binding, losartan may account for a large part of the activity during the first hour after the first drug administration, and at losartan tmax, which occur after about one hour, contribution to activity may be almost equal for losartan and the metabolite. Thereafter, the metabolite's contribution to activity is much larger.

Moreover, as the active metabolite E3174 is formed via an intermediate product and not direct from the parent, the pharmacokinetic data for metabolite E3174 may not reflect the rate of absorption of parent.

Therefore, bioequivalence for losartan should be proven based upon parent data. Regarding what acceptance criteria to apply, the submitted documents do not allow any conclusion to be drawn on this and consequently a conservative approach using 90% CI of 80 – 125% for AUC and Cmax applies.

4. Bioequivalence assessment of generics for tacrolimus

Date of publication: 22 July 2010 (Rev. 2)

In relation to the bioequivalence guideline, which has been drafted by the EWP-PK subgroup, a question was raised regarding the assessment of bioequivalence for tacrolimus generic products. There were different views whether the normal (80-125%) or a tightened (90-111%) acceptance range for the 90% CIs, for both AUC and Cmax, should be applied.

The decision on the bioequivalence criteria requires the clinical judgement whether tacrolimus is considered a narrow therapeutic index drug (NTID). Therefore, the response to this question has been prepared by the Efficacy Working Party (EWP) taking the EWP-PK's general position on bioequivalence criteria for NTIDs into account.

The position of the EWP is as follows:

The decision on whether a particular active substance may be considered to be a narrow therapeutic index drug (NTID) and whether narrowing of the bioequivalence acceptance limits should apply needs to be based on clinical considerations of the dose- or concentration-response relationships for both efficacy and safety.

The following key issues are identified for tacrolimus:

- Tacrolimus is a drug that requires individual dose titration to achieve a satisfactory balance between maximizing efficacy and minimizing serious dose related toxicity. Plasma level monitoring is routinely employed to facilitate dose titration.
- Recommended Therapeutic Drug Monitoring schemes often set desirable levels close to the upper or lower limit of the therapeutic window (5 ng/ml or 20 ng/ml), for example the use of "minimisation protocols" using low levels during maintenance phase. It is well established from clinical experience with the drug that even small changes of dose can lead to crossing the upper or lower limits of the therapeutic window
- In the case of kidney and heart transplantation, there is only a twofold difference in the upper and lower limit of the proposed therapeutic range (whole blood levels from 10 to 20 ng/mL). This is comparable to the therapeutic range for "classical" NTIDs such as digoxin.
- The consequences of over-dosing and of under-dosing (including morbidity/mortality associated with graft rejection) are of major clinical importance and can substantially affect clinical outcome.

For the above reasons the EWP considers that tacrolimus is a drug with a narrow therapeutic index.

In a number of EU countries generic prescribing is the norm and pharmacies may dispense either the branded product or a generic. Where multiple generics are available patients may be switched from one generic to another when renewing their prescription. Changes of formulation in this situation would not normally be accompanied by re-titration. The usual frequency of whole blood drug level measurements in clinical practice (typically once per month during maintenance phase) is not sufficiently frequent to ensure avoidance of over or under dosing as a result of a patient switching to a different formulation in the event of generic substitution of tacrolimus. Therefore, in order to ensure

the safety and efficacy of generic tacrolimus products it is necessary to apply tighter bioequivalence acceptance criteria than the conventional 80-125%.

The EWP discussion also covered whether the narrowing of the bioequivalence acceptance criteria to [90-111%] can be limited to AUC and will not be needed for C_{max}. For tacrolimus, this is supported by the following PK and PK/PD characteristics. Total drug exposure (AUC) is considered to be the key parameter of importance for dose titration of tacrolimus; in comparison peak whole blood levels do not seem to be critical for either safety or efficacy. As tacrolimus has a long elimination half-life C_{min} trough levels can be used as a surrogate for AUC in clinical practice. Given the long terminal half-life, tacrolimus accumulates during repeated dosing. Due to this accumulation, a potential difference between formulations in C_{max} after single dosing can be expected to be less at steady state, if AUC is the same for the two formulations. Therefore, the normal acceptance criteria for C_{max} [80-125%] can be used in single dose bioequivalence studies for tacrolimus.

Conclusion: The EWP recommends that the bioequivalence acceptance criteria for tacrolimus should be [90-111%] for AUC and [80-125%] for C_{max}.

5. Requirements for demonstration of bioequivalence for ciclosporine generics

Date of publication: 22 July 2010 (Rev. 2)

The reference product Neoral soft gelatine capsule concerns a specific formulation of ciclosporin which undergoes microemulsification process at administration (in the presence of water). For Neoral, the SmPC indicates a 33% decrease in Cmax and a 13% decrease in AUC, in case the product is taken with a high fat meal.

As indicated in the guideline on bioequivalence (CPMP/EWP/QWP/1401/98 Rev 1.), for products with specific formulation characteristics, like Neoral, bioequivalence studies performed under both fasted and fed conditions are required unless the product must be taken only in the fasted state or only in the fed state. Neoral may be taken with or without food, and in clinical practice, ciclosporin is often recommended to be taken in a standardised way in relation to food. Hence, a generic ciclosporin product must be bioequivalent with the originator product both in fasting and in fed state.

As EWP has defined ciclosporin to be a NTID, for which both AUC and Cmax are important for safety and efficacy, a narrowed (90.00-111.11%) acceptance range should be applied for both AUC and Cmax, under fasting as well as under fed conditions, in line with the guideline on bioequivalence (CPMP/EWP/QWP/1401/98 Rev 1.).

Although a generic product with a reduced food effect could be considered an improvement, this would not be considered acceptable for a ‘generic application’, but could be considered for a “hybrid” application, article 10(3) with additional data to support an application under this legal basis.

6. Requirements for demonstration of bioequivalence for mycophenolate mofetil generics

Date of publication: 26 January 2011 (Rev. 3)

The CMDh requested from the PKWP a position concerning interpretation of the revised Guideline on the Investigation of Bioequivalence with respect to the bioequivalence data for inactive pro-drugs in relation to both parent drug and metabolite in the context of demonstration of bioequivalence for mycophenolate mofetil.

The questions relate to the circumstances under which it is acceptable to base bioequivalence decision solely on metabolite data if a pro-drug plasma level is measurable. The revised guideline states: "*Also for inactive pro-drugs, demonstration of bioequivalence for parent compound is recommended"*.

1) If the exact meaning of the word "*recommended"* in the context of mycophenolate mofetil (MMF), depends on:

    - either the feasibility of the technical detection limits, in which the concentrations of the inactive prodrug are approximately 12000- to 6000-fold lower, for AUC and C_{max}, respectively, than that of the active metabolite mycophenolic acid;
    - or should specific PK-parameters be taken into account, low exposure of the parent resulting in a short Tmax, which makes it not relevant to measure the parent drug.

Position of the PKWP:

The bioequivalence guideline states "*for inactive prodrugs, demonstration of bioequivalence for parent compound is recommended*". The guideline further clarifies: *"However, some pro-drugs may have low plasma concentrations and be quickly eliminated resulting in difficulties in demonstrating bioequivalence for parent compound. In this situation it is acceptable to demonstrate bioequivalence for the main active metabolite without measurement of parent compound."* Hence, although the guideline recommends the use of parent compound also for inactive pro-drugs, exceptions are possible. The acceptability of use of main active metabolite instead of parent compound will be determined based both on the feasibility of measuring parent compound and on the pharmacokinetic characteristics for parent compound and active metabolite. For pro-drugs with a very large difference in exposure between parent and active metabolite and where the pro-drug is quickly eliminated, it is expected that there can be difficulties in demonstrating bioequivalence for parent compound and demonstration of bioequivalence based on active metabolite alone can be accepted.

For mycophenolate mofetil (MPM) specifically, the parent compound undergoes extensive presystemic metabolism to the active metabolite MPA. Moreover, MPM half-life is very short (0.60 to 1.20 h as reported) resulting in approximately 12000- and 6000-fold lower AUC and C_{max} respectively, for parent compound compared to metabolite. MPM has a tmax of 0.5 h and a t1/2 of less than 1 h, which limits the characterisation of the early plasma concentrations. As a consequence reliable estimation of Cmax will be difficult. "*In this situation it is acceptable to demonstrate bioequivalence for the main active metabolite without measurement of parent compound"* as stated in the bioequivalence guideline.

2) Is it acceptable NOT to follow this recommendation and use ONLY metabolite data to demonstrate bioequivalence between two products of the same pro-drug mycophenolate mofetil, even when current analytical assays allow measuring the parent with acceptable sensitivity?

Position of the PKWP:

A recommendation leaves room for an exceptional decision on a case by case basis. In this case it is clear that the parent compound is inactive and completely converted into the active metabolite yielding a 12000 fold difference in AUC. Due to this, demonstration of bioequivalence between two products of the same pro-drug can be based on metabolite data only. The argument that current analytical assays allow measuring the parent with acceptable sensitivity cannot be readily taken considering the short tmax and t1/2 of the parent compound which will limit a reliable estimation of Cmax of the parent compound.

7. Recommendations on determination of absolute and relative bioavailability

Date of publication: 26 January 2011 (Rev. 3)

Absolute bioavailability

Information on absolute bioavailability is important in the overall evaluation of the pharmacokinetics of the drug substance. For some new chemical entities information on absolute bioavailability facilitates the evaluation of the mass balance study, and enables conclusions regarding the contribution of different elimination routes to drug clearance. This information is important when determining the need for studies in subjects with renal and hepatic impairment as well as the need for drug-drug interaction studies at biliary excretion level. The information is also useful when predicting the consequences of pre-systemic drug-drug interactions, both at absorption and metabolism level. Therefore, for new active substances intended for systemic action, the absolute bioavailability should, if possible, be determined by comparing the bioavailability of the intended pharmaceutical form for an extra-vascular route of administration with an intravenous administration. For substances with non-linear pharmacokinetics, consideration should be given to the dose(s) used for evaluation of absolute bioavailability. Furthermore, data on absolute bioavailability is valuable in the evaluation of BCS based biowaivers (see Guideline on the investigation of bioequivalence, CPMP/EWP/QWP/1401/98 Rev. 1).

Relative bioavailability

It is recommended to obtain information on the relative bioavailability of different dosage forms (or formulations) used during drug development. By definition relative bioavailability is the comparison of different dosage forms (or different formulations thereof) administered by the same or a different non-intravenous route (e.g. tablets vs. oral solution).

Regarding formulation changes during drug development, unless BCS based biowaiver is applicable bioequivalence studies are needed if there has been a change between the formulation used in phase III and the final marketing formulation which may affect rate or extent of absorption. Relative bioavailability studies (or comparative bioavailability studies) are recommended between different formulations used during phase I, II and III. There is no requirement for demonstration of bioequivalence between phase II and phase III formulations. It is assumed that any difference in rate or extent of absorption between these formulations is taken into account in the design of the phase III studies. The clinical relevance of any differences in exposure between formulations used in phase I, II and III studies should be discussed in applications for NCEs in Module 2.5 and 2.7.1 and taken into account in the assessment of pharmacokinetic data in Module 2.7.2.

Recommendations for suprabioavailable products

A suprabioavailable product displays appreciably larger extent of absorption than an approved reference medicinal product.

If suprabioavailability is found, the development of a lower dosage strength should be considered. In this case, the biopharmaceutical development should be reported and a final comparative bioavailability study comparing the reformulated new product with the approved reference medicinal product should be submitted. The potential for a difference in food effect on the rate and/or extent of absorption or a difference in absorption interactions between the reformulated new product and the approved reference product should be discussed and when relevant evaluated in vivo.

In the case where a lower dosage strength has not been developed the dosage recommendations for the suprabioavailable product will have to be supported by clinical studies.

8. Clarification on the recommended statistical method for the analysis of a bioequivalence study

Date of publication: 26 January 2011 (Rev. 3)

1. Introduction

The following text on the general analysis of bioequivalence studies is included in the guidance document. The bold text is the main sentence of interest for this discussion.

> **4.1.8 Evaluation**
>
> **Statistical analysis**
>
> The assessment of bioequivalence is based upon 90% confidence intervals for the ratio of the population geometric means (test/reference) for the parameters under consideration. This method is equivalent to two one-sided tests with the null hypothesis of bioinequivalence at the 5% significance level.
>
> **The pharmacokinetic parameters under consideration should be analysed using ANOVA.** The data should be transformed prior to analysis using a logarithmic transformation. A confidence interval for the difference between formulations on the log-transformed scale is obtained from the ANOVA model. This confidence interval is then back-transformed to obtain the desired confidence interval for the ratio on the original scale. A non-parametric analysis is not acceptable.
>
> The precise model to be used for the analysis should be pre-specified in the protocol. The statistical analysis should take into account sources of variation that can be reasonably assumed to have an effect on the response variable. The terms to be used in the ANOVA model are usually sequence, subject within sequence, period and formulation. **Fixed effects, rather than random effects, should be used for all terms.**

Following the publication of revised version of the Guideline on the Investigation of Bioequivalence (CPMP/QWP/EWP/1401/98 Rev.1) this paragraph raised several questions from interested parties. The reason for this interest was twofold. Firstly, the new guideline gives more emphasis to replicate design trials and evaluation of such trials is a more complex task compared to a conventional two-period two sequence crossover trial. Secondly, the current standard for the analysis of replicate design trials is a likelihood-based linear mixed model with random subject effects.

The question of whether to use fixed or random effects is not important for the standard two period, two sequence (2×2) crossover trial. In section 4.1.8 of the guideline it is stated that “subjects in a crossover trial who do not provide evaluable data for both of the test and reference products should not be included.” Provided this is followed the confidence intervals for the formulation effect will be the same regardless of whether fixed or random effects are used.

Therefore all that remains to be discussed is the analysis method for replicate designs. In section 2 three models for analysing data from replicate bioequivalence trials are considered. To illustrate these approaches, in section 3 data from a four-period unbalanced study (see data set I) and data from a three-period balanced study (data set II) were analysed using different statistical models and computer programs.

2. Studied methods

2.1 Approach compatible with CHMP guideline (Method A)

The approach envisaged when the current guideline was written was to simply use the same analysis method for replicate designs as is used for 2×2 trials.

```
proc glm data=replicate;
class formulation subject period sequence;
model logDATA= sequence subject (sequence) period formulation;
estimate "test-ref" formulation -1+1;
test h=sequence e=subject(sequence);
lsmeans formulation / adjust=t pdiff=control("R") CL alpha=0.10;
run;
```

For this model there is only one variance term estimated, $\sigma^2_{w,}$ the within subject variability.

2.2. Slight modification to approach compatible with CHMP guideline (Method B)

The same model as specified above could be used in PROC MIXED and subject specified as a random effect.

```
proc mixed data=replicate;
class formulation subject period sequence;
model logDATA= sequence period formulation;
random subject(sequence);
estimate "test-ref" formulation -1 1 / CL alpha=0.10;
run;
```

This means there are two variance terms estimated σ^2_w and σ^2_b, as a distribution is also fitted to the between subject variability. If subject is a fixed effect (as in the previous model) each subject is treated as being selected in some way rather than being sampled from a random distribution and a subject effect is estimated individually for each patient as is done for the period effect.

This model will give the same results as Method A if all subjects included in the analysis provide data for all treatment periods.

2.3. Method C

The FDA Guidance for Industry document “Statistical approaches to establishing bioequivalence” specifies the code to be used for the analysis of replicate designs using PROC MIXED.

```
proc mixed data=replicate;
classes sequence subject period formulation;
model logDATA= sequence period formulation / ddfm=satterth;
random formulation/type=FA0(2) sub=subject G;
repeated/grp=formulation sub=subject;
estimate 'test-ref' formulation -1 1/ CL alpha=0.10;
run;
```

This model allows a different subject effect for each formulation (i.e. a subject by formulation interaction), and therefore has 5 variance terms (within subject for reference, within subject for test, between subject for test, between subject for reference, covariance for between subject test and reference – the last three are combined to give the subject ×formulation interaction variance component.)

This model will provide the same point estimate as methods A and B if all subjects provide data for all treatment periods. However it will generally give wider confidence intervals than those produced by methods A and B.

3. Results

3.1. Data set I

The following data reflect a four period crossover study where subjects receive both test and reference twice, with some subjects providing data for only a subset of the treatment periods. Results obtained with methods A, B and C are shown in the following table.

	Point estimate	**90% confidence interval**
Method A (guideline recommended)	115.66	107.11, 124.89
Method B (random effects)	115.73	107.17, 124.97
Method C (random effects with interaction)	115.66	107.10, 124.89

Within subject CV% (from method C) – reference 47.3%, test 35.3%

The results are generally very similar although missing treatment periods for some subjects causes the results to be different for all three approaches.

3.2. Data set II

Data of a three period crossover study where all subjects receive reference twice and test once were analysed using Methods A, B and C.

The results are given in the Table below

	Point estimate	**90% confidence interval**
Method A (guideline recommended)	102.26	97.32, 107.46
Method B (random effects)	102.26	97.32, 107.46
Method C (random effects with interaction)	102.26	97.05, 107.76

Within subject CV% (from method C) – reference 11.5%

As there are no subjects with missing treatment periods the results from methods A and B are identical, and the point estimate is the same for all three approaches. Method C gives wider intervals.

3.3. Alternative computer programs

SAS (version 9.1, SAS Institute Inc., NC) was used in the previous computations. Results obtained by alternative, validated statistical programs are also acceptable except spreadsheets because outputs of spreadsheets are not suitable for secondary assessment.

3.4. Estimating the within subject variability

The guideline introduces the possibility of widening the acceptance limits for C_{max} if the within-subject variability for the reference product is greater than 30%. This is calculated using:

$$CV(\%) = 100\sqrt{e^{s^2_{WR}} - 1}$$

The widening is on a smooth function, i.e. the permitted widening increases as the variability increases (to a maximum of 50%). It is not an all or nothing criteria with 30% being a critical point.

An advantage of Method C is that it directly calculates s^2_{wr} However, sometimes the algorithm fails to converge. For that reason the preferred way to get an unbiased estimate of σ^2_{wr} is using the data from the reference product only.

The following code removes all the test data from the data-set and then fits a model where the residual variance corresponds to the within subject variance for the test product.

```
data var;
set replicate;
if formulation='R';
run;

proc glm data=var;
class subject period sequence;
model logDATA= sequence subject (sequence) period;
run;
```

Results obtained with the different methods for Data Set I and II are summarised in the table below.

Reference within subject CV%

	Model A/B	Model C
Data set I	47.0%	47.3%
Data set II	11.2%	11.5%

The data shows that the variability estimates given by the two approaches are very similar for these examples. There is no dependence on random effects mixed models to estimate within subject variability for a formulation.

4. Discussion

The Guideline on the Investigation of Bioequivalence (CPMP/QWP/EWP/1401/98 Rev. 1) recommends analysing bioequivalence studies using ANOVA and specifying all factors, including subject, as fixed rather than random.

For a 2×2 crossover trial the confidence intervals for the formulation effect will be the same regardless of whether fixed or random effects are used for subject.

For replicate designs the results from the two approaches will differ if there are subjects included in the analysis who do not provide data for all treatment periods. Either approach is considered scientifically acceptable, but for regulatory consistency it is considered desirable to see the same type of analysis across all applications.

For multi-period studies other, more complex statistical models are possible. One of the possibilities is to include a subject by formulation interaction term. Analysis of data currently available shows that the subject by formulation interaction is negligible and therefore models without the interaction effect adequately control the type I error. Thus the same statistical models can be used regardless of the design.

5. Conclusion

The Guideline on the Investigation of Bioequivalence (CPMP/QWP/EWP/1401/98 Rev. 1) recommends analysing bioequivalence studies using ANOVA and specifying all factors, including subjects, as fixed

rather than random. The analysis presented above show that this approach (Method A) is feasible even for unbalanced replicate design studies. The advantage of this approach is that it is straightforward and that it appears to be software and software option independent. A simple linear mixed model, which assumes identical within-subject variability (Method B), may be acceptable as long as results obtained with the two methods do not lead to different regulatory decisions. However, in borderline cases and when there are many included subjects who only provide data for a subset of the treatment periods, additional analysis using method A might be required.

For highly-variable drugs it is recommended to estimate the within subject variance using data from the reference formulation only.

ANNEX

Data set I

SUBJECT	DATA	FORMULATION	PERIOD	SEQUENCE	logDATA
1	2285.96	R	1	BABA	7.734541
1	1955.82	T	2	BABA	7.578565
1	1345.94	R	3	BABA	7.204848
1	2856.24	T	4	BABA	7.957261
2	3151.72	T	1	ABAB	8.055704
2	2589.3	R	2	ABAB	7.859143
2	2992.94	T	3	ABAB	8.004011
2	2413.4	R	4	ABAB	7.788792
3	3264.74	T	1	ABAB	8.090935
3	3257.92	R	2	ABAB	8.088844
3	3100.54	T	3	ABAB	8.039332
3	3094.16	R	4	ABAB	8.037272
4	1206.36	T	1	ABAB	7.095363
4	1306.56	R	2	ABAB	7.175153
4	1583.12	T	3	ABAB	7.367153
4	1349.44	R	4	ABAB	7.207445
5	3880.9	R	1	BABA	8.263822
5	7322.88	T	2	BABA	8.898759
5	4429.66	R	3	BABA	8.396078
5	3322.88	T	4	BABA	8.108587
6	978.08	R	1	BABA	6.885591
6	1211.04	T	2	BABA	7.099235
6	973.88	R	3	BABA	6.881288
6	1150.8	T	4	BABA	7.048213
7	2924.06	T	1	ABAB	7.980728
7	2289.98	R	2	ABAB	7.736298
7	2494.28	T	3	ABAB	7.821755
7	3239.14	R	4	ABAB	8.083063
8	2425.46	R	1	BABA	7.793776
8	3705.74	T	2	BABA	8.217638
8	1891.06	R	3	BABA	7.544893
8	8979.12	T	4	BABA	9.102657
9	3825.02	R	1	BABA	8.249319
9	5315.04	T	2	BABA	8.578296
9	5813.16	R	3	BABA	8.667880
9	11475.9	T	4	BABA	9.348004
10	4112.26	R	1	BABA	8.321728
10	3822.86	T	2	BABA	8.248754
10	2459.82	R	3	BABA	7.807843

10	4616.76	T	4	BABA	8.437448
11	3170.3	T	1	ABAB	8.061581
11	2267.1	R	2	ABAB	7.726257
11	1703.32	R	4	ABAB	7.440335
12	2997.18	T	1	ABAB	8.005427
12	2954.78	R	2	ABAB	7.991179
12	5252.66	T	3	ABAB	8.566490
12	3744.54	R	4	ABAB	8.228054
13	2055.7	T	1	ABAB	7.628372
13	983.3	R	2	ABAB	6.890914
13	1771.3	T	3	ABAB	7.479469
13	3293.18	R	4	ABAB	8.099609
14	1590.62	R	1	BABA	7.371879
14	1141.54	T	2	BABA	7.040134
14	1238.34	R	3	BABA	7.121527
14	1285.8	T	4	BABA	7.159136
15	1470.5	T	1	ABAB	7.293358
15	1122.84	R	2	ABAB	7.023616
15	1592.18	T	3	ABAB	7.372859
15	1753.16	R	4	ABAB	7.469175
16	1886.14	R	1	BABA	7.542288
16	2077.28	T	2	BABA	7.638815
16	2197.62	R	3	BABA	7.695130
16	2194.64	T	4	BABA	7.693773
17	629.16	T	1	ABAB	6.444386
17	498.34	R	2	ABAB	6.211283
17	551.74	T	3	ABAB	6.313077
17	382.18	R	4	ABAB	5.945892
18	464.96	R	1	BABA	6.141951
18	2949.84	T	2	BABA	7.989506
18	1205.58	R	3	BABA	7.094716
18	2145.96	T	4	BABA	7.671342
19	1889.26	R	1	BABA	7.543940
19	5837.14	T	2	BABA	8.671996
19	2375.84	R	3	BABA	7.773106
19	1673.46	T	4	BABA	7.422649
20	793.44	T	1	ABAB	6.676378
20	1169.72	R	2	ABAB	7.064520
20	1072.8	R	4	ABAB	6.978027
21	2085.78	R	1	BABA	7.642898
21	2373.2	T	2	BABA	7.771995
21	1557	R	3	BABA	7.350516
21	2135.28	T	4	BABA	7.666353
22	288.06	R	1	BABA	5.663169
22	309.98	T	2	BABA	5.736508
22	324.18	R	3	BABA	5.781299
22	307.58	T	4	BABA	5.728735
23	524.8	T	1	ABAB	6.263017
23	372.84	R	2	ABAB	5.921149
23	518.92	T	3	ABAB	6.251750
23	604.56	R	4	ABAB	6.404501
24	5866.94	T	1	ABAB	8.677088
24	5547.78	T	3	ABAB	8.621153
24	4386.8	R	4	ABAB	8.386355

25	4008.46	T	1	ABAB	8.296162
25	1898.84	R	2	ABAB	7.548998
25	1565.22	T	3	ABAB	7.355782
25	4875.32	R	4	ABAB	8.491941
26	1197.46	T	1	ABAB	7.087958
26	330.82	R	2	ABAB	5.801574
26	1276.16	T	3	ABAB	7.151611
26	394.82	R	4	ABAB	5.978430
27	13823.18	R	1	BABA	9.534102
27	7618.82	T	2	BABA	8.938377
27	9493.34	R	3	BABA	9.158346
27	8928.44	T	4	BABA	9.096997
28	940.86	R	1	BABA	6.846794
28	1188.7	T	2	BABA	7.080616
28	882.02	R	3	BABA	6.782215
28	1226.38	T	4	BABA	7.111822
29	2175.24	R	1	BABA	7.684894
29	2654.36	T	2	BABA	7.883959
29	3235.26	R	3	BABA	8.081865
29	3033.3	T	4	BABA	8.017406
30	1194.9	T	1	ABAB	7.085818
30	826.66	R	2	ABAB	6.717393
30	610.38	T	3	ABAB	6.414082
30	594.14	R	4	ABAB	6.387115
31	4108.68	R	1	BABA	8.320857
31	7399.52	T	2	BABA	8.909170
31	4461.62	T	4	BABA	8.403267
32	792.22	T	1	ABAB	6.674839
32	999.74	R	2	ABAB	6.907495
32	1179.4	T	3	ABAB	7.072761
32	1678.96	R	4	ABAB	7.425930
33	3925.52	R	1	BABA	8.275254
33	3789.74	T	2	BABA	8.240053
33	3463.82	R	3	BABA	8.150127
33	4576.64	T	4	BABA	8.428720
34	1708.58	R	1	BABA	7.443418
34	2500.84	T	2	BABA	7.824382
34	1263.3	R	3	BABA	7.141483
34	2048.42	T	4	BABA	7.624824
35	943.06	T	1	ABAB	6.849130
35	769.22	R	2	ABAB	6.645377
35	848.8	T	3	ABAB	6.743824
35	1193.88	R	4	ABAB	7.084964
36	2540.42	T	1	ABAB	7.840085
36	2091.18	R	2	ABAB	7.645484
36	2583.66	T	3	ABAB	7.856962
36	1993.98	R	4	ABAB	7.597888
37	851.44	T	1	ABAB	6.746929
37	653.88	R	2	ABAB	6.482924
37	2371.3	T	3	ABAB	7.771194
37	1275.38	R	4	ABAB	7.150999
38	6054.76	R	1	BABA	8.708600
38	7322.18	T	2	BABA	8.898663
38	6746.98	R	3	BABA	8.816850

38	7130.7	T	4	BABA	8.872165
39	5825.64	T	1	ABAB	8.670024
39	6462.82	R	2	ABAB	8.773821
39	7400.48	T	3	ABAB	8.909300
39	6196.84	R	4	ABAB	8.731795
40	1690.42	R	1	BABA	7.432732
40	1292.9	T	2	BABA	7.164643
40	1522.4	R	3	BABA	7.328043
40	1066.58	T	4	BABA	6.972213
41	2783.06	R	1	BABA	7.931306
41	1149.08	T	2	BABA	7.046717
41	877.92	R	3	BABA	6.777555
41	572.42	T	4	BABA	6.349873
42	4759.06	T	1	ABAB	8.467805
42	5831.92	R	2	ABAB	8.671102
42	4154.76	R	4	ABAB	8.332010
43	5399.28	T	1	ABAB	8.594021
43	5425.9	R	2	ABAB	8.598939
43	4344.5	T	3	ABAB	8.376666
43	4507.04	R	4	ABAB	8.413396
44	5611.1	T	1	ABAB	8.632502
44	5444.14	R	2	ABAB	8.602295
44	4805.9	T	3	ABAB	8.477600
44	4960.66	R	4	ABAB	8.509294
45	707.68	R	1	BABA	6.561992
45	3681.66	T	2	BABA	8.211119
45	18454.26	R	3	BABA	9.823051
45	1003.46	T	4	BABA	6.911209
46	2400.64	T	1	ABAB	7.783491
46	1420.6	R	2	ABAB	7.258835
46	1146.68	T	3	ABAB	7.044626
46	5005.72	R	4	ABAB	8.518337
47	483.08	R	1	BABA	6.180182
47	1033.3	T	2	BABA	6.940513
47	644.54	R	3	BABA	6.468537
47	675.3	T	4	BABA	6.515157
48	2157.08	R	1	BABA	7.676511
48	3117.36	T	2	BABA	8.044742
48	2816.14	R	3	BABA	7.943122
48	2850.4	T	4	BABA	7.955215
49	14261.54	T	1	ABAB	9.565322
49	26489.56	R	2	ABAB	10.184506
49	23525.66	T	3	ABAB	10.065847
49	21243.76	R	4	ABAB	9.963818
50	1552.24	T	1	ABAB	7.347454
50	1569.32	R	2	ABAB	7.358398
50	2090	T	3	ABAB	7.644919
50	1479.98	R	4	ABAB	7.299784
51	3834.44	R	1	BABA	8.251779
51	4899.76	T	2	BABA	8.496942
51	3702.9	R	3	BABA	8.216872
51	5677.02	T	4	BABA	8.644182
52	5925.92	R	1	BABA	8.687091
52	967.9	T	2	BABA	6.875129

52	797.02	R	3	BABA	6.680880
52	939.38	T	4	BABA	6.845220
53	3528.48	T	1	ABAB	8.168622
53	2037.36	R	2	ABAB	7.619410
53	3211.68	T	3	ABAB	8.074549
53	2906.74	R	4	ABAB	7.974787
54	937.16	R	1	BABA	6.842854
54	6327.96	T	2	BABA	8.752733
54	1054.92	R	3	BABA	6.961220
54	1766.02	T	4	BABA	7.476484
55	3437.98	T	1	ABAB	8.142639
55	3731.8	R	2	ABAB	8.224646
55	4832.72	T	3	ABAB	8.483165
55	3310.24	R	4	ABAB	8.104776
56	1011.14	T	1	ABAB	6.918834
56	654.02	R	2	ABAB	6.483138
56	858.58	T	3	ABAB	6.755280
56	908.12	R	4	ABAB	6.811377
57	1003.34	R	1	BABA	6.911090
57	4739.94	T	2	BABA	8.463780
57	697.84	R	3	BABA	6.547990
57	2504.52	T	4	BABA	7.825852
58	6496.34	R	1	BABA	8.778994
58	5949.36	T	2	BABA	8.691039
58	6003.38	R	3	BABA	8.700078
58	6373.72	T	4	BABA	8.759939
59	1247.58	R	1	BABA	7.128961
59	1116.88	T	2	BABA	7.018294
59	1166.74	R	3	BABA	7.061969
59	2658.38	T	4	BABA	7.885472
60	33929.62	T	1	ABAB	10.432044
60	24943.44	R	2	ABAB	10.124366
60	19110.22	T	3	ABAB	9.857979
60	12805.18	R	4	ABAB	9.457605
62	2280.5	T	1	ABAB	7.732150
62	1714.48	R	2	ABAB	7.446865
62	4034.28	T	3	ABAB	8.302583
62	3420.76	R	4	ABAB	8.137618
63	3376.72	T	1	ABAB	8.124660
63	2242.8	R	2	ABAB	7.715480
63	1719.54	T	3	ABAB	7.449812
63	2342.32	R	4	ABAB	7.758897
64	912.34	R	1	BABA	6.816013
64	2104.42	T	2	BABA	7.651795
64	2061.04	R	3	BABA	7.630966
64	1496.5	T	4	BABA	7.310884
65	3957.94	R	1	BABA	8.283479
65	5895.6	T	2	BABA	8.681962
65	5859.58	R	3	BABA	8.675833
65	5073.48	T	4	BABA	8.531782
66	1165.7	T	1	ABAB	7.061077
66	1248.62	R	2	ABAB	7.129794
66	1168.68	T	3	ABAB	7.063630
66	1300.42	R	4	ABAB	7.170443

67	1197.4	R	1	BABA	7.087908
67	1119.34	T	2	BABA	7.020495
68	1709.72	R	1	BABA	7.444085
68	2532.4	T	2	BABA	7.836923
68	1581.02	R	3	BABA	7.365825
68	2807.4	T	4	BABA	7.940014
69	2798.84	T	1	ABAB	7.936960
69	2454.1	R	2	ABAB	7.805515
69	5334.84	R	4	ABAB	8.582014
70	4318.42	R	1	BABA	8.370645
70	2182.66	T	2	BABA	7.688300
70	1649.16	R	3	BABA	7.408021
70	1620.32	T	4	BABA	7.390379
71	470.24	T	1	ABAB	6.153243
71	208.04	R	2	ABAB	5.337730
72	2098.3	T	1	ABAB	7.648883
72	1919.76	R	2	ABAB	7.559955
72	2817.76	T	3	ABAB	7.943698
72	2041	R	4	ABAB	7.621195
73	6667.32	T	1	ABAB	8.804973
73	5289.84	R	2	ABAB	8.573543
73	7300.28	T	3	ABAB	8.895668
73	9711.84	R	4	ABAB	9.181101
74	2036.76	R	1	BABA	7.619116
74	1948.04	T	2	BABA	7.574579
74	1539.58	R	3	BABA	7.339265
74	2079.14	T	4	BABA	7.639710
75	767.3	T	1	ABAB	6.642878
75	1046.3	R	2	ABAB	6.953015
75	1390.36	T	3	ABAB	7.237318
75	3019.18	R	4	ABAB	8.012741
76	12097.5	T	1	ABAB	9.400754
76	12694.42	R	2	ABAB	9.448918
76	10999.24	T	3	ABAB	9.305581
76	9406.52	R	4	ABAB	9.149158
77	1115.5	R	1	BABA	7.017058
77	1115.3	T	2	BABA	7.016879
77	1111.78	R	3	BABA	7.013718
77	2352.82	T	4	BABA	7.763370
78	20373.54	R	1	BABA	9.921992
78	13689.6	T	2	BABA	9.524392
78	20585.02	R	3	BABA	9.932319
78	24498.14	T	4	BABA	10.106352

Data Set II

SUBJECT	DATA	FORMULATION	PERIOD	SEQUENCE	logDATA
1	4053.6	R	1	2	8.307361
1	3970.4	T	2	2	8.286622
1	3748.8	R	3	2	8.229191
2	2986.2	R	1	2	8.001757
2	2378.8	T	2	2	7.774351
2	2804.6	R	3	2	7.939016
3	3464.4	R	1	3	8.150295
3	3340.2	R	2	3	8.113786
3	4028.8	T	3	3	8.301224

4	4105	T	1	1	8.319961
4	3191.2	R	2	1	8.068152
4	3803.6	R	3	1	8.243703
5	4767.8	T	1	1	8.469640
5	4542.6	R	2	1	8.421255
5	3940	R	3	1	8.278936
6	3050.8	R	1	3	8.023159
6	3027.2	R	2	3	8.015393
6	2419.6	T	3	3	7.791358
7	2530.2	R	1	2	7.836054
7	3072	T	2	2	8.030084
7	2962.6	R	3	2	7.993823
8	2205	T	1	1	7.698483
8	2041.4	R	2	1	7.621391
8	2018	R	3	1	7.609862
9	4647.6	R	1	2	8.444106
9	4159.6	T	2	2	8.333174
9	3400	R	3	2	8.131531
10	2228.2	T	1	1	7.708949
10	2360.4	R	2	1	7.766586
10	2221.2	R	3	1	7.705803
11	1863.8	R	1	3	7.530373
11	2212.4	R	2	3	7.701833
11	2394.4	T	3	3	7.780888
12	2278.4	R	1	3	7.731229
12	3170.4	R	2	3	8.061613
12	3927.2	T	3	3	8.275682
13	2640.4	R	1	3	7.878686
13	2430.4	R	2	3	7.795811
13	2869.2	T	3	3	7.961789
14	3030.8	R	1	2	8.016582
14	2459.8	T	2	2	7.807835
14	2970.4	R	3	2	7.996452
15	2254.4	R	1	2	7.720639
15	1994.8	T	2	2	7.598299
15	2724.4	R	3	2	7.910003
16	2959.6	T	1	1	7.992809
16	3442	R	2	1	8.143808
16	3342.6	R	3	1	8.114504
17	2396.8	T	1	1	7.781890
17	2659.4	R	2	1	7.885856
17	2172	R	3	1	7.683404
18	2725	R	1	3	7.910224
18	2805.6	R	2	3	7.939373
18	3146.6	T	3	3	8.054078
19	2418.8	R	1	2	7.791027
19	2749.8	T	2	2	7.919283
19	2504	R	3	2	7.825645
20	2662.4	R	1	3	7.886983
20	2929.8	R	2	3	7.982689
20	3037.2	T	3	3	8.018691
21	2869.6	R	1	3	7.961928
21	2666.4	R	2	3	7.888485
21	3069	T	3	3	8.029107

22	2949	T	1	1	7.989221
22	2926.8	R	2	1	7.981665
22	2855.4	R	3	1	7.956967
23	3154.8	T	1	1	8.056680
23	3185.6	R	2	1	8.066396
23	3548.6	R	3	1	8.174308
24	1874.8	R	1	2	7.536257
24	1808.8	T	2	2	7.500419
24	2730.8	R	3	2	7.912350

9. Effect of sorbitol on the pharmacokinetics of highly permeable drug substances

Date of publication: 20 September 2012 (Rev. 5)

The CMDh asked for a view on the extent to which the results reported by Chen et al. (1) regarding the effect of sorbitol on bioavailability of metoprolol, taken together with relevant regulatory experience regarding the influence of sorbitol on the oral bioavailability of drug substances, are applicable to other highly permeable drug substances (BCS class 1 and 2).

There is scarce information in the literature (1-5) regarding the effect of sorbitol on the absorption of BCS class I and II (highly permeable drug substances). The article by Chen et al (1) (showing no effect on metoprolol absorption) and another one by Fassihi (2) (showing no effect on Cmax or AUC but an effect on Tmax of theophylline upon 10 g of sorbitol) are worth mentioning.

In Chen et al's article (1), the effect of sorbitol on the absorption of metoprolol (BCS class I) and ranitidine (BCS class III) has been studied. No significant effect of sorbitol (5 g) on the extent (AUC) and a 23% reduction in rate (Cmax) of absorption of a single dose of metoprolol has been recorded, whereas a significant effect has been observed on both AUC and Cmax (44% and 51% reduction, respectively) when sorbitol (5 g) and ranitidine (BCS class III) were administered concomitantly. From these data, the best estimate of a single dose threshold for the sorbitol effect on drug bioavailability is probably around 1 g, affecting all drug BCS classes but mainly low permeability drug substances.

Therefore there is no straightforward answer to this question until more data is collected to determine the actual threshold by exploring sorbitol doses lower than 1.25 g.

The putative effect of sorbitol on GI physiology affecting drug absorption is generally accepted to derive from its osmotic effect, accelerating intestinal transit and increasing intestinal water content. The first effect suggests a higher impact on the absorption of low permeability drugs. The latter can lower the diffusion driving force due to dilution, affecting all drug BCS classes.

Therefore any correlation of sorbitol absorption effect with solubility or permeability is in principle difficult to establish.

It also needs to be recognized that sorbitol intolerance is largely described in the literature (6, 7). This means that a dose effect relationship cannot be established universally due to individual susceptibility. Even minute amounts of sorbitol can elicit a GI effect in a sub-population.

Consistently with these results, the Bioequivalence Guideline (CPMP/EWP/QWP/1401/98 Rev. 1) states in Appendix II, Oral solutions:

"If the test product is an aqueous oral solution at time of administration and contains an active substance in the same concentration as an approved oral solution, bioequivalence studies may be waived. However if the excipients may affect gastrointestinal transit (e.g. sorbitol, mannitol, etc.), […], a bioequivalence study should be conducted, unless the differences in the amounts of these excipients can be adequately justified by reference to other data. The same requirements for similarity in excipients apply for oral solutions as for Biowaivers (see Appendix III, Section IV.2 Excipients)."

Further recommendations in Appendix III, section IV.2 on excipients state: "*As a general rule, for both BCS-class I and III drug substances […] Excipients that might affect bioavailability* ***should be qualitatively and quantitatively the same*** *in the test product and the reference product*."

Therefore, strict compliance with the Bioequivalence Guideline is recommended to be followed in the development and assessment of generic applications.

Sorbitol intolerance should be taken into consideration in the labeling of sorbitol containing drug products.

References

1. *Chen, M., Straughn, A., Sadrieh, N. et al (2007). "A Modern View of Excipient Effects on Bioequivalence: Case Study of Sorbitol". Pharm. Res. 24, 73-80.*
2. *R. Fassihi, R. Dowse, and S. S. D. Robertson (1991). "Influence of Sorbitol Solution on the Bioavailability of Theophylline. Int. J. Pharm. 72:175-178*
3. *D. A. Adkin, S. S. Davis, R. A. Sparrow, P. D. Huckle, A. J. Philips, and I. R. Wilding (1995). The Effects of Pharmaceutical Excipients on Small Intestinal Transit. Br. J. Clin. Pharmacol. 39:381-387*
4. *D. A. Adkin, S. S. Davis, R. A. Sparrow, P. D. Huckle, and I. R. Wilding (1995). The Effect of Mannitol on the Oral Bioavailability of Cimetidine. J. Pharm. Sci. 84:1405-1409*
5. *S. van Os, M. Relleke and Muniz Piniella (2007) "Lack of Bioequivalence between Generic Risperidone Oral Solution and Originator Risperidone Tablets, Int. J. Clin. Pharmacol., 45: 293-299*
6. *Born P., The clinical impact of carbohydrate malabsorption (2011) Arab J Gastroenterol. Mar;12(1):1-4. Epub 2011 Feb 5.*
7. *Fernández-Bañares F, Esteve M, Viver JM. (2009) Fructose-sorbitol malabsorption. Curr Gastroenterol Rep. Oct;11(5):368-74.*

10. Requirement to perform incurred sample reanalysis

Date of publication: 10 December 2012 (Rev. 6)

The requirement to perform incurred sample reanalysis (ISR) has been introduced with the Guideline on bioanalytical method validation (EMEA/CHMP/EWP/192217/2009). Given that this is a new regulatory requirement with the need for consistency in its introduction, the PKWP was asked to discuss if it is possible to give recommendations on how the absence of ISR should be handled and whether it is possible to identify other factors which could be assessed in the absence of ISR to support the validity of the analytical method.

Introduction

Incurred sample reanalysis (ISR) is applied to assess the reliability of bioanalytical methods used in pre-clinical toxicokinetic studies and for a variety of clinical pharmacology studies including bioavailability, bioequivalence, pharmacokinetic, interaction and comparability studies. The need for incurred sample reanalysis is discussed already since 2006[2] and regulators supported the need for incurred sample reanalysis also considering significant bioanalytical deficiencies observed in studies. Therefore, although incurred sample reanalysis is a requirement introduced in Europe for the first time with the new EMA Guideline on bioanalytical method validation (EMEA/CHMP/EWP/192217/2009), which came into force in February 2012, it should be noted that the scientific need to perform ISR as an element of bioanalytical method validation was already identified much earlier. ISR should therefore be considered as part of the validation of the analytical method during study sample analysis.

Different sources can be identified which might contribute to the failure of ISR. Some sources may be more likely to occur than other depending on the method, active substance, and analyst, however they cannot be excluded. Sources of ISR failure may be:

- Execution, i.e. switched samples, instrument issues, scientist performance of method
- Method, i.e. metabolite interferences, back conversion of metabolites, poor ruggedness, internal standard response
- Samples, i.e. matrix effects, mislabelling, handling

It is recognized that some of these sources are also likely to occur during validation, like switching samples and mislabelling.

ISR failure and thus lack of the reliability of the study outcome can happen in each study and as such it is difficult to generalise it. Especially with pivotal studies it should be ensured that the results are reliable. However it is also understood that ISR is an additional confirmation of results next to a complete validation.

Introduction of ISR as a regulatory requirement

The principles for the implementation of a guideline are outlined in the Procedure for European Union guidelines and related documents within the pharmaceutical legislative framework (EMEA/P/24143/2004 Rev.1). While applicants may, with the agreement of the competent authority concerned, choose to apply a guideline in advance of the date for coming into operation of a guideline,

[2] Viswanathan CT, Bansal S, Booth B et al. Workshop/Conference Report: Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. AAPS J. 9(1), E30–E42 (2007)

competent authorities should await this date before requiring a guideline to be taken into account for assessments. The Guideline on bioanalytical method validation came into force on 1 February 2012 meaning that as of this date this document sets the applicable requirements for the regulatory review of applications.

It is acknowledged in the above-mentioned principles that in some circumstances it may not be possible for applicants to fully comply with new guidelines within this timeframe (e.g. data generated from trials started before the implementation of the new guideline). In such cases, the applicant should consider whether departure from the new guideline could be justified. The applicant's justification will then be considered on a case-by-case basis by the relevant competent regulatory authorities.

In compliance with this framework, the regulatory assessment requires the review of the bioanalytical method validation in any application against the current regulatory standards as set out in the guideline, including the requirement to address incurred sample reanalysis. If an element of the validation is missing, e.g. lack of incurred sample reanalysis, then this would need to be scientifically justified by the applicant. Such justification can be considered in the framework of the above exception that a particular validation has been performed before the bioanalytical guideline came into force, i.e. February 2012. Any justification will need to be reviewed on a case-by-case basis considering the overall validation data, the study results, as well as the reliance of the application on these data.

Considerations regarding a potential justification for the lack of ISR data

The attempt to scientifically justify the lack of ISR is considered only appropriate for the very practical reason that a study was performed before the guideline on bioanalytical method validation came into force.

For the scientific justification of the lack of ISR the applicant should take all the following points into consideration:

- metabolite back conversion:

  The applicant should support that back conversion is not an issue for the drug compound or that the risk of back conversion on the outcome of the study results is low as for instance it is known that the drug compound is (almost) not metabolised. For drug compounds for which it is known that back conversion is an issue, i.e. clopidogrel, atorvastatin, ramipril, lack of ISR is considered not acceptable.

- other ISR data obtained in the same laboratory:

  ISR data obtained for the same analyte from other studies carried out in the same laboratory and with the same analytical method may be used as supportive data to justify the lack of ISR.

- data from repeat analysis:

  In most studies repeat analysis of study samples has to be carried out for different reasons. Repeat analysis can be considered as ISR in certain situations, however due to the nature of the reanalysis (for instance run acceptance criteria failure) those data are considered not reliable. The applicant should report the data of these reanalysis and take into account and discuss the reason for the reanalysis in the justification for supportive data.

  In case of a multi analyte analysis, if the repeat analysis was due to run acceptance criteria failure for one of the analytes, but the other has passed, the results of the analyte(s) which passed can be used to infer ISR, if analysed.

- the obtained pharmacokinetic data in the study:

  The applicant should compare the obtained pharmacokinetic data with data obtained previously or with reported data and should show that these are comparable

- 90% confidence interval:

  As one element of such justification, if applicable, the applicant could also take into consideration the width of the 90% confidence interval and the ratio to possibly justify that a false positive outcome due to ISR problems has a low probability.

The last two bullet points need to be thoroughly discussed specifically for bioequivalence studies.

The applicant should also consider the overall reliance of the application on the data generated with the bioanalytical method in question. For new molecular entities the pivotal basis of the application normally rests on clinical efficacy and safety studies, nevertheless pharmacokinetic studies in such an application provide significant information (e.g. general pharmacokinetic profile, interactions), which is also reflected in the labelling, hence the validity of such data needs to be sufficiently ensured. Abridged applications may exclusively rely on pharmacokinetic data, e.g. bioequivalence studies, making overall validity of these data paramount. Therefore, the validity of the data needs to be considered for the assessment of the application and the specific study considering whether the data are pivotal or supportive.

Conclusion

ISR is considered an element of the validation of the analytical method during study sample analysis. It has been discussed for many years in the scientific community and recently been introduced as regulatory requirement in the European guideline. Like for any deviation from a guideline requirement, the lack of ISR requires a scientific justification by the applicant. Such justification could be considered for validations which have been performed before the new guideline came into force. Its scientific validity will need to be reviewed on a case-by-case basis in the light of the overall validation data, the study outcome, as well as the reliance of the application on these data.

11. Number of subjects in a two-stage bioequivalence study design

Date of publication: 13 February 2013 (Rev. 7)

According to the Guideline on the Investigation of Bioequivalence (CPMP/QWP/EWP/1401/98 Rev.1), it is acceptable to use a two-stage approach when attempting to demonstrate bioequivalence. The question was raised whether there were a minimum number of subjects that should be included in the second stage of such a design.

Discussion

From the perspective of type I error control it is considered that there is no minimal number of subjects to be included in the second stage of a two-stage design, so long as it can be demonstrated that the type I error of the study is controlled. However, the analysis model for analysing the combined data also needs to be considered.

The CHMP guideline on the investigation of bioequivalence (Doc. Ref.: CPMP/EWP/QWP/1401/98 Rev. 1/ Corr) states: “When analysing the combined data from the two stages, a term for stage should be included in the ANOVA model.” In addition, to account for the fact that the periods in the first stage are different from the periods in the second stage, a term for period within stage is required. Therefore, the expected ANOVA model for analysis of the combined data from a two-stage design would have the following terms: stage, sequence, sequence*stage, subject (sequence*stage), period (stage), formulation. To fit this model it is necessary to have in each stage at least one patient in each sequence – so a minimum of two patients in each stage of the study, but more if both happen to be randomised to the same sequence.

A model which also includes a term for a formulation*stage interaction would give equal weight to the two stages, even if the number of subjects in each stage is very different. The results can be very misleading hence such a model is not considered acceptable. Furthermore, this model assumes that the formulation effect is truly different in each stage. If such an assumption were true there is no single formulation effect that can be applied to the general population, and the estimate from the study has no real meaning.

Conclusion

1) The expected analysis for the combined data in a two-stage design is ANOVA with terms for stage, sequence, sequence*stage, subject (sequence*stage), period (stage), formulation.

2) This model can be fitted provided that in each stage, there is at least one subject randomised to each sequence. This does not supersede the requirement for at least 12 subjects overall.

3) A term for a formulation*stage interaction should not be fitted.

12. Bioequivalence studies for generic application of omega 3 fatty acid ethylesters in a soft gelatine capsule

Date of publication: 10 October 2013 (Rev. 8)

The CMDh asked for PKWP's view on a generic application of Omega-3 fatty acid ethylesters in a soft gelatine capsule.

The capsule filling of both the generic and the innovator product comprised 1000 mg of the liquid active substance, (omega 3 fatty acid ethylesters), without any excipients. The active substance fully complied with the Ph Eur Monograph on Omega-3 fatty acid ethylesters (EE) which describes an active substance including an allowed (although not defined) low amount of preservative.

Hence, the gelatin capsules only included the oily, liquid active substance, (omega 3 fatty acid ethylesters). However, the liquid active substance contains a slightly different amount of preservative alpha-tocopherol (as 70% in vegetable oil). Furthermore the composition of the capsule itself was roughly the same as for the innovator product but with a slight difference in the amount of glycerol.

This particular situation is not addressed in the current guideline on the investigation of bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **), i.e. a generic application referring to an oily 'liquid composition' in a soft gelatine capsule. Hence, the CMDh asked for PKWP's view on:

- Whether a biowaiver would be acceptable in this specific type of drug formulation if fast and comparable disintegration of the capsules has been demonstrated over the whole physiological range (pH 1 – 6.8).
- Should a bioequivalence trial be required, what would be the preferred study design (fed or fasted).
- In the case of fasted state conditions, would it be possible to determine bioequivalence between drug products including in the analysis subjects that have presented erratic absorption profiles, for which the extrapolation $AUC_{t\text{-}inf}$ could not be estimated or was >20% in more than 50% of the subjects.

Discussion

Bioequivalence (BE) is a means to detect potential formulation differences between generics and innovators. This implies that formulation differences are expected due to e.g. different excipients (quantitatively and/or qualitatively) and/or different manufacturing processes.

Since the oily content of both capsule products including an allowed amount of preservative is considered the active substance (PhEur monograph), a different formulation effect cannot be assumed. Hence, requesting *in vivo* BE between test and reference could hardly be justified as both capsules would contain the same amount of actives within accepted limits of variability without excipients potentially causing different formulation effects. The possibility of different amounts of impurities is expected to be controlled via the monograph, i.e. this could not be the reason for a BE study as it refers to the active substance rather than the formulation.

Therefore, simple characterisation of capsule quality by comparative disintegration tests is deemed sufficient. It should however be noted that the disintegration of capsule shells cannot be used as a BE tool as such as it has no relation to any *in vivo* parameter, but simply describes capsule quality.

In summary, a biowaiver would be acceptable in this specific type of drug formulation if fast and comparable disintegration of the capsules has been demonstrated over the whole physiological range (pH 1 – 6.8). Since the liquid oily active substance of the capsules filled with omega-3 fatty acid EEs will be directly available for absorption after rupture and disintegration and a different formulation effect cannot be expected from the allowed preservative, *in vivo* BE study could be waived.

Should *in vivo* BE trial be requested, it should be performed under fed conditions for the following reasons:

- Plasma concentrations are markedly higher under fed conditions than those quantified in the fasted state,
- Plasma concentrations in the fasted state are rather low and erratic. Unreasonably low values within the PK profiles render them invalid as they indicate the measurements of physiological processes rather than pharmacokinetics.

The last point was addressed in the paragraph above. However, since this is considered a general question not particularly related to the omega-3 fatty acid ethylesters in a soft gelatine capsule, it is further discussed below.

Subjects for which erratic absorption prevent the calculation of extrapolated AUC and/or for which the residual area is more than 20 % should still be included in the regular calculations and evaluation of AUC_t since this is the most relevant pharmacokinetic parameter to compare extent of absorption (see section 4.1.8 in the guideline on the investigation of bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **)). However, the cited guideline clearly states that when this is true "*in more than 20 % of the observations then the validity of the study may need to be discussed*" (see section 4.1.8 Evaluation; Reasons for exclusion). Hence, only in exceptional cases it could still be possible to accept an extrapolation larger than 20% in a significant number of subjects (>20% of the subject's concentration - time profiles) if it is justified that AUC_t has been calculated reliably and it is representative of the extent of drug absorption from the products under comparison. Of note, this rule and reasoning does not apply if the sampling period is 72h or more and AUC_{0-72h} is used instead of AUC_t.

13. Acceptability of an "additional strengths biowaiver" when bioequivalence to the reference product has been established with a BCS-based biowaiver

Date of publication: 10 October 2013 (Rev. 8)

The Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/ Corr) states that: "*If bioequivalence has been demonstrated at the strength(s) that are most sensitive to detect a potential difference between products, in vivo bioequivalence studies for the other strength(s) can be waived*."

The PKWP was asked to comment on the acceptability of this approach when the bioequivalence of the "reference" strength to the reference product has been investigated using the BCS (Biopharmaceutics Classification System)-based biowaiver approach i.e., without an *in vivo* bioequivalence study.

Discussion

Bioequivalence is in principle demonstrated by means of *in vivo* bioavailability studies. These *in vivo* studies can be waived if the product fulfils the requirements defined in surrogate tests like the BCS biowaiver approach.

This is in accordance with the Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/ Corr) which states in this respect that: "*The BCS (Biopharmaceutics Classification System)-based biowaiver approach is meant to reduce in vivo bioequivalence studies, i.e., it may represent a surrogate for in vivo bioequivalence. In vivo bioequivalence studies may be exempted if an assumption of equivalence in in vivo performance can be justified by satisfactory in vitro data"*.

An additional strength biowaiver is a waiver designed to avoid repeating the same *in vivo* study at the other strength level. Hence, when the Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/ Corr) states that: "*If bioequivalence has been demonstrated at the strength(s) that are most sensitive to detect a potential difference between products, in vivo bioequivalence studies for the other strength(s) can be waived*", this implies that when bioequivalence has been demonstrated *in vivo* for the test product, *in vivo* bioequivalence studies for the other strength can be waived.

Indeed, the reference in the sentence above to the sensitivity to detect differences between test and reference products only makes sense in the case of *in vivo* comparisons. This sensitivity varies depending on the solubility and the pharmacokinetic linearity. In the case of highly soluble drugs, the only drugs for which a BCS biowaiver is acceptable, the sensitivity to detect differences *in vitro* is the same at all strengths. Thus, the reference to higher sensitivity at the highest strength refers to *in vivo* studies. Further, the different sensitivities arising from non-linear pharmacokinetics only apply to *in vivo* studies. Therefore, the intent of this text was to refer to *in vivo* studies as evidence of bioequivalence.

Summary

Biowaiver of additional strength should be applied only when the test product have shown bioequivalence to the reference product by means of an *in vivo* bioequivalence study.

14. Question on a generic application for Quetiapine Lambda 200, 300, 400 mg prolonged release tablets

Date of publication: 10 October 2013 (Rev. 8)

The CMDh consulted the PKWP for their input on a generic application (article 10.1 of Directive 2001/83/EC) for Quetiapine Lambda 200, 300, 400 mg prolonged release tablets. The originator product's strengths were 200 mg, 300 mg, 400 mg, prolonged release tablets.

The clinical development plan for Quetiapine Lambda 200, 300, 400 mg prolonged release tablets consisted of a single-dose study under fasting and fed conditions with 200 mg strength in healthy volunteers and a multiple-dose study with the highest, 400 mg tablet in schizophrenic patients.

The application for the 300 and 400 mg strength was referred to the CMDh. The PKWP input was sought on the following points:

1/ Clinical development plan: the need for single dose bioequivalence studies in all strengths, where single-dose study under fasting and fed conditions with 200 mg strength in healthy volunteers and a multiple-dose study with the highest, 400 mg tablet in schizophrenic patients have shown bioequivalence,

2/ The need for inclusion of early time points in the calculation of f2 values for a prolonged release tablet in *in-vitro* dissolution data supportive of a biowaiver.

Preamble

The PKWP acknowledged the following limitations:

- Single dose studies with doses higher than 200 mg are not feasible in healthy volunteers due to unacceptably severe adverse effects,
- Multiple dose studies with doses equal to or higher than 200 mg are not feasible in healthy volunteers due to unacceptably severe adverse effects,
- Single dose studies in patients are not feasible due to ethical reasons (interruption of treatment).

Hence, the PKWP's feedback was based on the assumption that it was not possible to conduct the study with the 300mg dose.

Discussion

1. Would a multiple dose study in the highest strength be considered sufficient to demonstrate bioequivalence despite differences in the dissolution profiles, in case where a single-dose study can be waived because of safety reasons?

PKWP response:

In the case of Quetiapine Lambda the following statement from the MR NfG (1), applies:

In case of prolonged release single unit formulations with multiple strengths, a single dose study under fasting conditions is required for each strength. Studies at steady state may be conducted with the highest strength only if the same criteria for extrapolating bioequivalence studies are fulfilled as described in the Note for Guidance for immediate release forms (linear pharmacokinetics, same qualitative composition, etc.).

Therefore, the following is required:

1. Waive multiple dose studies for the 200 mg and 300 mg strengths based on conditions applicable to IR forms as per BE GL currently in force (2). All conditions were fulfilled except for dissolution (see below).
2. Waive single dose studies for the 300 mg and 400 mg studies based on exceptional circumstances: single dose studies are not feasible both in healthy volunteers and patients (see above). In this case the same rules for waiving different strengths should apply.

As a consequence, the only outstanding issue was the comparison of dissolution profiles.

Overall the dissolution data raised doubts on the extrapolation of the BE results only from the 400 mg and the 200 mg strengths because the comparison of 200 mg vs. 400 mg at pH 4.5 and 6.8 does not meet the f2 criterion. On the contrary, bioequivalent (BE) results could be extrapolated to the 300 mg strength on the basis of dissolution data since respective comparisons complied with the f2 criterion.

It was then investigated whether the differences in the dissolution data were due to an active substance effect (as a result of lack of sink conditions) or a formulation effect. As for the lack of sink conditions, the results of a comparison of equivalent strengths of the test product (TP) (2X200 mg vs. 1X400 mg) at pH 4.5 and 6.8 suggested that the noncompliant results could be explained by an active substance effect, not by a formulation effect. However, the results of a comparison of the 200 mg strength of the reference product (RP) the 400 mg strength of the RP at pH 4.5 and 6.8 did not suggest an active substance effect.

Given the exceptional circumstances that the single dose studies cannot be conducted in patients and that the studies with doses higher than 200 mg cannot be conducted in healthy volunteers, only a multiple 200 mg dose study in patients could have clarified these findings. . However, this study would not be ethically acceptable since there was direct evidence that the lack of comparability between 200 mg and 400 mg in the TP was due to the solubility of the active substance, whereas the formulation effect was based on an indirect observation that this was not the case for the RP.

Moreover, BE results should prevail over dissolution data and the 200 mg strength of the TP was BE to the 200 mg strength of the RP, inasmuch as the 400 mg strength of the TP was BE to the 400 mg strength of the RP.
Finally, a bracketing approach could be applicable in this situation since studies were available at the extreme of the strength interval (200 and 400 mg).

Overall *in vivo* and *in vitro* evidence provided points to a positive answer to this question: a multiple dose study in the highest strength can be considered sufficient to demonstrate bioequivalence despite differences in the dissolution profiles (which can be explained because the dissolution profiles become similar when tested at the same dose level per vessel), in case where a single-dose study can be waived because of safety reasons, taking also into consideration the demonstrated BE in the single dose study with the 200 mg strength and a bracketing approach between the 200 and 400 mg strengths. This conclusion cannot be generalised and a case by case approach will be needed in similar situations.

2. Is it acceptable and/or needed to include early time points of the dissolution profiles in the calculation of f2 values for a prolonged release tablet? Because f2 values are sensitive to the choice of dissolution time points, what recommendations can be made for prolonged release tablets in order to reliably conclude that the dissolution profiles can be considered similar?

PKWP response:

The design of a study comparing two dissolution profiles should take into account, among other factors, the inclusion of relevant sampling time points. It is perfectly reasonable to use 2 h as a first time point in a dissolution test running over 24 h. In the case at hand at 2 h already a relevant amount (10 to 15 %) of the active has been released. On the other hand, early time points, even in the case of a sustained release dosage form, are important in revealing release differences between the products under comparison, because the mechanism controlling the release of the active substance is present from the start.

Moreover, even though the choice of sampling time points could be questioned, there is no scientific reason to exclude valid data in a calculation.

Therefore the PKWP was of the opinion that in this case the 2 h time point should not be omitted not only because there was no scientific reason to exclude it but because the amount released was considered relevant.

The choice of early time points in a comparative dissolution profile test should be based on the relevance (mainly amount released and release controlling mechanism). On the other hand, the conditions stated in Appendix 1 of the BE GL (2) should be complied with, namely

- A minimum of three time points (zero excluded)
- The time points should be the same for the two formulations
- Twelve individual values for every time point for each formulation
- Not more than one mean value of > 85% dissolved for any of the formulations.
- The relative standard deviation or coefficient of variation of any product should be less than 20% for the first point and less than 10% from second to last time point.

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(1) Note for guidance on modified release oral and transdermal dosage forms: Section II (pharmacokinetic and clinical evaluation) – CPMP/EWP/280/96

(2) Guideline on the investigation of bioequivalence – CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **

15. Ebastine: use of metabolite data to demonstrate bioequivalence between inactive pro-drugs

Date of publication: 10 October 2013 (Rev. 8)

Background

The Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev.1), states that:

Inactive pro-drugs

Also for inactive pro-drugs, demonstration of bioequivalence for parent compound is recommended. The active metabolite does not need to be measured. However, some pro-drugs may have low plasma concentrations and be quickly eliminated resulting in difficulties in demonstrating bioequivalence for parent compound. In this situation it is acceptable to demonstrate bioequivalence for the main active metabolite without measurement of parent compound. In the context of this guideline, a parent compound can be considered to be an inactive pro-drug if it has no or very low contribution to clinical efficacy.

In view of the above, and regarding the use of metabolite data to demonstrate bioequivalence between inactive pro-drugs, the CMDh sought the Pharmacokinetics Working Party's (PKWP) opinion on the following question:

Is it acceptable for a generic application for ebastine to demonstrate bioequivalence based on either the parent ebastine or on the active metabolite carebastine, provided proper justification in the study protocol has been provided, or can only one of these analytes be used?

PKWP response:

In the context of the Guideline on the investigation of bioequivalence (Doc. Ref.: CPMP/QWP/EWP/1401/98 Rev. 1), the parent compound ebastine can be considered to be an inactive pro-drug as it has no or very low contribution to clinical efficacy[1-6].

Although demonstration of bioequivalence for parent compound is recommended for inactive pro-drugs, demonstration of bioequivalence with ebastine would only be possible by inclusion of a very high number of subjects. Indeed, ebastine has very low plasma concentrations, is rapidly and extensively metabolised resulting in highly variable plasma concentrations of the parent compound, resulting in a higher variability in pharmacokinetics than carebastine.

Therefore, bioequivalence studies using carebastine for bioequivalence evaluation would be considered acceptable to detect formulation related differences between a test and a reference.

In summary, in accordance with the Guideline on the investigation of bioequivalence, it would be acceptable to demonstrate bioequivalence based on the pharmacokinetics of the active metabolite carebastine. However, in case an application is submitted solely with data on the parent ebastine, it is also acceptable to demonstrate bioequivalence based on the pharmacokinetics of the parent ebastine. In case both ebastine and carebastine are analysed, the analyte to be used for bioequivalence evaluation should be prospectively defined in the protocol.

1. T. Yamaguchi, T. Hashizume, M. Matsuda, M. Sakashita, T. Fujii, Y. Sekine, M. Nakashima, and T. Uematsu. Pharmacokinetics of the H1-receptor antagonist ebastine and its active metabolite carebastine in healthy subjects. Arzneim.-Forsch. (1994) 44(1):59-64

2. Martinez-Tobed A., Tarrús E., Segura J., Roberts D.J. Pharmacokinetic studies of ebastine in rats, dogs and man. Drugs Today (1992) 28(Suppl. B):57-67

3. Wiseman L.R., Faulds D. Ebastine: a review of its pharmacological properties and clinical efficacy in the treatment of allergic disorders. Drugs (1996) 51(2):260-277

4. Presa I.J. H1-antihistamines: a review. Alergol. Immunol. Clin. (1999) 14(5):300-312

5. Rico S., Antonijoan R.M., Barbanoj M.J. Ebastine in the light of CONGA recommendations for the development of third-generation antihistamines. J. Asthma Allergy (2009) 2:73-92

6. Wood-Baker R., Holgate S.T. Dose-response relationship of the H1-histamine antagonist, ebastine, against histamine and metacholine-induced bronchoconstriction in patients with asthma. Agents and Actions (1990) 30:1-2

16. IQ Consortium Induction Working Group Questions:

Date of publication: 7 October 2014 (Rev. 10)

1. **The EMA Guideline on the Investigation of Drug Interactions states that: "the incubation duration of enzyme induction or down-regulation– *in vitro* studies should generally be 72 hrs. Shorter durations should be well justified." Please provide the rationale for the recommendation of a 72 hrs incubation time? Most Pharmaceutical companies currently use a 48 hrs incubation period with media replenishment every 24 hrs. mRNA responses are very quick (often <24h). Longer incubation periods bear the risk of study outcome limiting cytotoxicity. Please comment on the acceptability of shorter incubation times such as 8 to 12h measuring mRNA when obtaining EC_{50} and Emax? This situation could be most relevant for cytotoxic drugs such as used in Oncology. Would reporter gene data and/or PXR and CAR TR-Fret competitive binding assays be acceptable?**

PKWP Response:

When drafting the guideline limited experience with induction studies measuring mRNA was available. Based on studies measuring enzyme activity, an incubation duration of 3 days appeared suitable. However, in accordance with the guideline, shorter incubation times can be sufficient if well justified that adequate sensitivity is maintained. The sensitivity of the specific study is verified by the response of the positive control inducer (see the DDI guideline for details).

We have no experience with very short incubations (8-12 hrs) and we are not aware of any literature reference evaluating this. If adequate sensitivity cannot be supported it is recommended to investigate induction *in vivo* instead, for example by performing a cocktail study.

If an induction signal for a PXR inducible enzyme is detected and EC_{50} and Emax for your investigational drug can be determined, the RIS correlation method (or possibly the Mechanistic static model) as described in the EU DDI guideline could be used with short incubation periods if sensitivity is ensured during the validation.

Receptor binding assays can be used as supportive data only. If using these assays, the applicant needs to provide data supporting the performance of the method, including sensitivity.

2. **Assessment of down-regulation *in vitro*. Does the Agency have examples demonstrating *in vitro* data being confirmed by *in vivo* findings? Could this be an *in vitro* artefact?**

PKWP Response:

The Agency has experience with down-regulation observed in human hepatocytes confirmed *in vivo*.

3. **Please provide the rationale for the use of 50-fold $Cmax_u$ in the *in vitro* studies. Can this value be adjusted based on Vd estimates and/or liver-blood partitioning, e.g. for a compound with low human Vd where it is unlikely that liver partitioning is 50-fold?**

PKWP Response:

The 50-fold safety margin on $Cmax_u$ is experience based and has been applied for more than a decade in the enzyme inhibition assessment in the EU. The safety margin includes factors such as an at least 10-fold inter-study variability in Ki, the possibility of markedly higher concentrations in the hepatocyte than in plasma and higher portal vein concentration than Cmax in plasma during absorption. The

safety factor used for inhibition is also applied in the induction assessment. However, additional issues add to the uncertainty of the IVIVC for induction, such as the possible metabolic and/or chemical degradation during the incubations (37°C for 24 hours) and the lack of control of transporter expression in the cells. Reducing the safety-factor based on Vd cannot be recommended until there is scientific data to support this.

4. **The mRNA cut-off of 2-fold induction may be stringent given the variability between and within donors. Would the use of modelling approaches be better suitable than fold induction to assess the need for a clinical induction-based DDI studies?**

PKWP Response:

The 2-fold cut-off is used in the basic model. This relates to the first investigation of whether the drug could be an inducer and therefore it is suitable to have a simple approach. For PXR mediated induction the applicant may use alternative methods such as the RIS correlation method and the mechanistic static model as stated in the guideline. At present the use of PBPK is not recommended for this purpose.

5. **Please clarify the scientific rationale for recommending CITCO as the positive control for the *in vitro* assessment for CYP2B6 induction. CITCO has poor properties which results in variable inductive responses between studies. In addition, CITCO is not an approved drug which limits the applicability to put *in vitro* data into clinical context. Is the EMA willing to consider alternative compounds such as Efavirenz which is known to cause CYP2B6 induction-based DDIs in the clinic and is known to be a CAR transactivator?**

PKWP Response:

If the CAR activator also activates PXR to a significant extent, presence of CAR regulatory pathways cannot be verified. CITCO at the proposed concentration <100 nM is the only substance we are aware of that activates CAR exclusively. Efavirenz is a PXR and CAR agonist (Sharma *et al*, Biochem Pharmacol 2013). If confirmed that the PXR activation of efavirenz, or another substance, is negligible as compared to the effect on CAR at a certain concentration, the use of that substance as a positive control for CAR could be supported.

6. **Major advancements have been made with regards to the understanding of regulatory pathways of metabolic enzymes and transporters. What are the expectations with respect to co-regulated enzymes including transporters if a compound induces CYP1A2, CYP2B6 or CYP3A4? Rather than assessing induction of CYP2C in the clinic, can *in vitro* data or a paper argument be used to avoid additional targeted clinical DDI studies knowing that PXR is involved in the regulation of CYP3A4 and CYP2B6?**

PKWP Response:

A mechanistic approach to induction is applied. If induction is observed for one of these enzymes, co-regulated enzymes and transporters will be assumed to be also induced. The effect on these enzymes/transporters should preferably be quantified *in vivo*. Based on present knowledge, lack of CYP2C induction is concluded if the drug does not increase CYP3A4 or CYP2B6 mRNA expression.

Additional comment from the PKWP:

Please note that when the aim of an in vivo induction study is to quantify an induction effect, the duration of the treatment of the inducer should be well thought and justified to the agency based on a conservative enzyme degradation constant (kdeg) and time to reach steady state for the inducer.

(Please see the Drug Interaction guideline). At present, to evaluate the full induction effect on a CYP3A4 substrate, a duration of 10-14 days is recommended for a perpetrator that does not accumulate during multiple-dose conditions.

17. Evaluation of orally inhaled medicinal products

Date of publication: 22 January 2015 (Rev. 11)

1. The extent to which plasma levels reflect bio-availability in the lung

PKWP Response:

In the EU, PK bioequivalence studies are considered an acceptable methodology to compare the lung deposition of two inhalation products containing the same active substance. In cases where the oral bioavailability of swallowed drug is negligible, or in case it is made negligible by active charcoal blockade, the plasma concentration time curve reflects both the extent of and the pattern of deposition within the lungs.

To conclude equivalent efficacy, both the amount of drug reaching the lungs and the deposition pattern of drug particles within the lung needs to be equivalent.

The area under the plasma concentration-time curve (or AUC) reflects the amount of drug that has reached the lungs. As the rate of absorption from the inhaled particles is different at different areas of the lung, the deposition pattern within the lung is mirrored by the shape of the plasma concentration-time curve during the absorption phase, i.e. Cmax and tmax.

In the case where intestinal absorption is not prevented, i.e. in a study without charcoal blockade, and thus absorption is the sum of the absorption via the lungs and intestinal absorption, as for other modes of administration, equivalent systemic safety can be concluded if two products give rise to equivalent systemic exposure (AUC and Cmax).

Pharmacokinetic endpoints may be more discriminative than PD or clinical endpoints, in particular the efficacy endpoints available for inhaled corticosteroids.

Use of active charcoal and truncated AUCs

For some inhaled medicinal products, the contribution of intestinal absorption to systemic exposure is negligible (<5%) and a single dose PK study without charcoal can be used for both efficacy and safety comparisons. Reasons for the negligible contribution include poor intestinal absorption (e.g., chromoglycate, nedocromil), or an extensive first-pass metabolism (e.g., beclomethasone, fluticasone, mometasone, ciclesonide). For drugs with significant oral bioavailability (e.g., budesonide, formoterol, salmeterol), a PK study with active charcoal is necessary to assess efficacy, and a study without charcoal is used to assess safety. The charcoal blockade needs to be validated to demonstrate that oral contribution to total bioavailability is negligible. In case the absorption of the drug in the lung is very quick (e.g., tmax $\leq$ 5 min) and absorption occurs before the contribution of gastrointestinal absorption is significant (e.g., salbutamol/albuterol, salmeterol), AUC0-30 min might be acceptable as a surrogate for efficacy and AUC0-t for safety. Thus, in this case, one study without active charcoal blockade is sufficient.

To be noted, most respiratory medicinal products are now being approved in the EU based on PK studies (e.g., nasal sprays of mometasone in suspension; pMDI in suspension of salbutamol, salmeterol, fluticasone and salmeterol/fluticasone; and DPI of salmeterol/fluticasone).

2. Scaling of acceptance limits (for Cmax and perhaps AUC) to allow for variability in reference product for fine particle dose

PKWP Response:

In bioequivalence studies, scaling or widening of the acceptance limits is only acceptable for Cmax when it is caused by high intra-subject variability despite similar in vitro characteristics. Scaling is not a suitable solution to the variability in the in vitro characteristics, i.e. the fine particle dose (FPD) of different batches of the reference product.

Widening of the acceptance range

Widening of the conventional 20% acceptance range based on high variability is only possible for Cmax according to the CHMP Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/Corr) (up to 69.84 – 143.19%) if a replicate design is conducted.

To support safety, it should be demonstrated that the systemic exposure is not higher for the test product than for the reference product, i.e. the upper limit of the 90% confidence interval should not exceed the upper bioequivalence acceptance limit 125.00.

Between-batch variability of the reference product and intra-batch variability over time

Variability in particle-size distribution between batches of the reference product or within a single batch of a reference product through their storage period can be significant. There may even be situations where it may be difficult to demonstrate PK bioequivalence between batches of the same reference product. Therefore, before the in vivo comparison, several batches of both test and reference products could be tested to identify representative batches (within ±15% of the corresponding median fine particle dose (or APSD)) of test and reference, respectively. In case of fixed combinations this may imply, if pre-specified in the protocol, the use of different batches for each component.

The development of an IVIVC may be useful to correct the results of the PK study to justified parts of the APSD of the typical marketed batch of the reference product and the corresponding typical test product batch according to the proposed specifications. The IVIC could also be used as scientific support of the in vitro specification of the test product.

Another approach that might be acceptable is to show that the side batches (batches in the tails of the distribution) representing the test product specifications are not superior and not inferior to the side batches of the reference product obtained from the market.

18. Clarifications on the "Evaluation of the pharmacokinetics of medicinal products in patients with impaired hepatic function" guideline

Date of publication: 22 January 2015 (Rev. 11)

1. Why does the guideline state in Sections 3.4 and 4.1 that it is the FREE fraction of the drug and metabolites that is to be determined?

PKWP Response:

Sections 3.4 and 4.1 of the present guideline clearly state that in the hepatic impairment study groups, the free fraction should be determined if the substance(s) measured are highly bound to plasma proteins. The protein binding may be reduced in hepatic impairment. If using total concentration, an increase in the therapeutically relevant free concentration can be masked or underestimated as both the protein bound fraction and hepatic function are affected. No recommendation can be based on the total concentration in this situation. It has been noted that applicants have not observed this requirement resulting in submission of inconclusive studies.

2. Why does section 2 of the guideline state that biliary secreted drugs should be studied?

PKWP Response:

In section 2 of the guideline it is stated that biliary secreted drugs should be studied. Biliary secretion as well as hepatic metabolism can be affected by hepatic impairment. Furthermore, in reviewed NCE applications, very marked increases in exposure have been found for drugs subject to extensive hepatic uptake, when given to patients with hepatic impairment due to hepatitis C. In view of these findings it is particularly important to study the effect of hepatic impairment in drugs subject to hepatic uptake.

3. How should the subjects to be included in the HI study be selected?

PKWP Response:

The subjects included in the hepatic impairment study should be representative for the actual class, e.g. if moderate impairment is investigated, the subjects should have Child-Pugh scores covering the range of moderate impairment and being spread over the range.

4. How should hepatic impairment be classified?

PKWP Response:

Presently, the Child-Pugh classification is being proposed as the most widely used to categorise hepatic function. Presenting the pharmacokinetic effect as a function of the biochemical Child-Pugh components (e.g. S-albumin, bilirubin, prothrombin time, etc.) is encouraged in the guideline. Research in this area is on-going.

5. What is the role of physiologically based pharmacokinetics (PBPK) when estimating the effect of hepatic impairment?

PKWP Response:

In Section 3.6, the guideline makes a short statement on the use of PBPK as a tool. Predicting the effects of hepatic impairment by PBPK is an interesting application of PBPK and there is a great deal of ongoing research in this area. However at the present time due to low confidence in the use of PBPK

modelling to predict hepatic impairment, it is considered that there is no need to revise the general information given on PBPK modelling.

19. Suitability of a 3-period replicate design scheme for the demonstration of within-subject variability for Cmax

Date of publication: 25 June 2015 (Rev. 12)

The Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev.1), states that: "*for the acceptance interval to be widened the bioequivalence study must be of a replicate design where it has been demonstrated that the within-subject variability for Cmax of the reference compound in the study is >30%.*"

The question was raised whether it is suitable to use a TRT/RTR replicate design to demonstrate that the Cmax of the reference product is highly variable or is it mandatory to use TRTR/RTRT or TRR/RTR/RRT replicate designs?"

PKWP Response:

To demonstrate that the within subject variability for Cmax of the reference product is greater than 30% a replicate design where the reference product is given more than once is required. If a 3 period design is to be used to justify a widening of the limits for Cmax subjects the most efficient study design would randomise subjects to receive treatments in the following order: RRT, RTR or TRR. This design is the most efficient as all subjects receive the reference product twice and hence an estimate of the within subject variability is based on data from all subjects.

The question raised asks if it is possible to use a design where subjects are randomised to receive treatments in the order of TRT or RTR. This design is not considered optimal as explained above. However, it would provide an estimate of the within subject variability for both test and reference products. As this estimate is only based on half of the subjects in the study the uncertainty associated with it is higher than if a RRT/RTR/TRR design is used and therefore there is a greater chance of incorrectly concluding a reference product is highly variable if such a design is used.

The CHMP bioequivalence guideline requires that at least 12 patients are needed to provide data for a bioequivalence study to be considered valid, and to estimate all the key parameters. Therefore, if a 3-period replicate design, where treatments are given in the order TRT or RTR, is to be used to justify widening of a confidence interval for Cmax then it is considered that at least 12 patients would need to provide data from the RTR arm. This implies a study with at least 24 patients in total would be required if equal number of subjects are allocated to the 2 treatment sequences.