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SCIENTIFIC FACTORS ON MICRODOSING APPROACH IN PHARMACEUTICAL DEVELOPMENT

Shein-Chung Chow[1] and Fuyu Song*[2]

[1]Duke University School of Medicine, Durham, North Carolina, USA.

[2]Peking University Clinical Research Institute, Peking University Health Science Center, Beijing, China.

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***Correspondence for Author**
Fuyu Song
Peking University Clinical Research Institute, Peking University Health Science Center, Beijing, China.

ABSTRACT

In recent years, microdosing approach has received much attention in pharmaceutical research and development. The concept of microdosing approach focuses on safety signal detection, which is primarily for exploratory purpose (FDA, 2006; ICH, 2009).[2,4] Burt (2011)[3] indicated that the use of microdosing approach has great potential in shortening development by making critical go/no-go decision in early pharmaceutical development. In addition, it is believed that microdosing approach will increase the probability of success based on a limited number of subjects available. In practice, however, many scientific factors regarding the design and analysis of microdose studies are commonly encountered which have limited the potential use of microdosing approach in pharmaceutical development. These scientific factors and/or issues include, but are not limited to, (i) distinction between the effects due to microdose and placebo, (ii) microdose versus therapeutic dose, (iii) characterization of dose response curve, (iv) accuracy and reliability of predictive model, and (v) possible false positive and false negative rate. These issues are discussed. In addition, a predictive index is proposed to assess the accuracy and reliability of predictive model established based on data collected from a microdose study.

KEYWORDS: *Placebo effect; False positive; False negative; Predictive index.*

1. INTRODUCTION

A microdose is defined as less than 1/100th of the dose of a test substance calculated (based on animal data) to yield a pharmacologic effect of the test substance with a maximum dose of

≤ 100 micrograms. Due to differences in molecular weights as compared to synthetic drugs, the maximum dose for protein products is ≤30 nanomoles. Microdose studies are often designed not only to evaluate pharmacokinetics or imaging of specific targets but also to induce pharmacologic effects. In practice, the risk of microdose to human subjects is considered very limited, while information adequate to support the initiation of such limited human studies can be derived from limited non-clinical safety studies. In the past decade, a microdose is often compared with those observed at a therapeutic dose. As indicated by Lappin (2010)[1], about 80% of the microdose pharmacokinetics available in the public domain has been shown to scale to those observed at a therapeutic dose, within a two-fold difference. As a result, microdosing is now being extended into drug (pharmacokinetics) development in situations where the concentration of a drug in cell or tissue types is relevant to its efficacy. In addition, the concept of microdosing has been applied to address drug–drug interactions by giving human volunteers a microdose of the candidate drug before and after the administration of a drug known to inhibit or induce certain enzymes (Loppin, 2010).[1]

As indicated in the 2006 FDA guidance on *Exploratory IND*,[2] a typical microdose study involves very limited human exposure and it has no therapeutic or diagnostic intent. A microdose study is considered an exploratory study which focuses on the detection of safety signals. Thus, it is suggested that preclinical and clinical approaches, as well as chemistry, manufacturing, and controls (CMC) information, should be considered when planning exploratory studies in humans, including studies of closely related drugs or therapeutic biological products. The FDA currently accepts the use of extended single-dose toxicity studies in animals to support single-dose studies in humans. For microdose studies, a single mammalian species (both sexes) can be used if justified by *in vitro* metabolism data and by comparative data on in vitro pharmacodynamics effects. The route of exposure in animals should be by the intended clinical route. In these studies, animals should be observed for 14 days post-dosing with an interim necropsy, typically on day 2 and endpoints evaluated should include body weights. Because miscrodose studies involve only single exposures to microgram quantities of test materials and because such exposures are comparable to routine environmental exposures, routine generic toxicology testing is not needed. For similar reasons, safety pharmacology studies are also not recommended.

The concept of microdosing approach in pharmaceutical development is encouraging. As indicated by Burt (2011),[3] the use of human microdosing in pharmaceutical development has

the following benefits that (i) it takes just six months from laboratory bench to completion of clinical studies, (ii) smarter lead candidate selection, (iii) reduces expensive late stage attrition (i.e., kill ineffective compound early and cheap), (iv) substantially reduced preclinical toxicology package compared to phase I, (v) only gram quantities of non-GMP drug (typically 10g) are needed, (vi) any route of administration possible, including intravenous, (vii) absolute oral bioavailability calculation, (viii) drugs can be tested in sensitive populations; renal impaired patients, women of child bearing age and cancer patients, and (ix) reduces use of animals in research. However, there are statistical issues/concerns regarding the validity of the human microdosing approach in pharmaceutical development. These statistical issues include (i) the selection of microdose (e.g., "how to distinguish the effects due to microdose and the placebo effect?" and "how to select the initial dose and the dose range under study?"), (ii) the selection of study endpoint (e.g., "Should a clinical endpoint or a surrogate endpoint or a biomarker be used?" and "Whether the surrogate or biomarker is predictive of clinical endpoint?"), (iii) the determination of sample size (e.g., precision analysis or power analysis), (iv) model selection and validation (e.g., "how to handle inter- and intra-subject variabilities?" and "how to determine the impact of non-linearity for the dose range under study?"). In addition, it is a concern that the extrapolation based on results from microdose to regular dose may not be reliable because the variability associated with the dose in usually proportional to the dose.

The remaining of this article is organized as follows. In the next section regulatory perspectives regarding the use of microdose in drug development are discussed followed by some scientific factors that are commonly encountered when applying microdosing in drug development. In Section 4, a predictive index for comparing the microdose and a therapeutic dose is proposed under certain assumptions. Some concluding remarks are given in the last section of this article.

2. REGULATORY PERSPECTIVES

2.1 ICH Guideline

In 2009, the International Conference on Harmonization (ICH) published a guideline which provides international standards for the nonclinical safety studies recommended to support human clinical trials for pharmaceuticals (ICH, 2009). This guidance further suggest reducing the use of animals other resources for drug development by considering alternative methods for safety evaluation. These methods such as microdosing approach can be used to replace

current standard methods. The ICH M3 (R2)[4] guideline recommends two different microdose approaches, which are briefly described below (see also Table 1).

The first approach would involve not more than a total dose of 100 µg that can be administered as a single dose or divided doses in any subject. This could be useful to investigate target receptor binding or tissue distribution in a PET study. A second use could be to assess PK with or without the use of an isotopically labelled agent. A second microdosing approach is one that involves < 5 administrations of a maximum of 100 µg per administration (a total of 500 µg per subject). This can be useful for applications similar to the first microdosing approach described above, but with less active PET ligands.

In some situations, it could be appropriate to carry out a clinical microdose study using the intravenous (IV) route on a product intended for oral administration and for which an oral nonclinical toxicology package already exists. In this case the IV microdose can be qualified by the existing oral toxicity studies as described in Table 1, Approach 3, where adequate exposure margins have been achieved. It is not recommended to investigate IV local tolerance of the drug substance in this situation because the administered dose is very low (100 µg maximum). If a novel IV vehicle is being employed then local tolerance of the vehicle should be assessed.

2.2 FDA Guidance

Similar to the ICH M3 (R2) guideline,[4] in 2006, FDA published guidance on exploratory IND study[2], which is intended for clinical trials that are conducted early in phase 1 such as microdose studies. From FDA's perspective, a microdose study usually involves very limited human exposure, and most importantly, it has no therapeutic or diagnostic intent. As indicated by the 2006 FDA guidance, microdose studies are often conducted prior to the traditional dose escalation, safety, and tolerance studies that ordinarily initiate a clinical drug development program. The duration of dosing in an exploratory IND study is expected to be limited (e.g., 7 days). This guidance applies to early phase 1 clinical studies of investigational new drug and biological products that assess feasibility for further development of the drug or biological product.

In addition, FDA published a guidance on *PET Drugs – Current Good Manufacturing Practice* (cGMP)[5] which is intended for positron emission tomography (PET) drugs. The guidance addresses resources, procedures, and documentation for all PET drug production

facilities. In some cases, the guidance provides practical examples of methods or procedures that PET drug production facilities can use to comply with the cGMP requirements.

3. SCIENTIFIC FACTORS

3.1 Microdose versus Placebo

As indicated earlier, a microdose is less than 1/100th of the dose of a test substance calculated (based on animal data) to yield a pharmacologic effect of the test substance with a maximum dose of $\leq$ 100 micrograms (μg). As a result, a microdose is close to a placebo (inactive dose). Thus, an interesting question is how to distinguish whether the effect (or response) observed is due to the microdose or a placebo.

A placebo response is a simulated or otherwise medically ineffectual treatment for a disease or other medical condition intended to deceive the recipient. Sometimes patients given a placebo treatment will have a perceived or actual improvement in a medical condition, a phenomenon is usually referred to as the placebo effect. In clinical research, placebo effects are often the subject of scientific research aiming to understand underlying neurobiological mechanisms of action (MOA) in pain relief, immunosuppression, Parkinson's disease and depression. For example, Zubieta et al. (2006)[6] indicated that placebo can have real, measurable effects on physiological changes in the brain detected by brain imaging techniques (see also, Vul et al., 2009).[7]

To ensure the success of microdose studies, it is important to rule out that the observed effect is by chance alone and it is due to placebo effect.

3.2 Selection of Therapeutic Dose

An effective dose is defined as the dose or amount of drug that produces a therapeutic response or desired effect in some fraction of the subjects taking it. Therapeutic dose is an effective dose that is required in order to produce a desirable (or clinically meaningful) effect. The therapeutic range of a drug is defined by the range between the minimum effective dose (MED) and the maximum tolerable dose (MTD). The MED is the lowest dose level of a drug product that provides a clinically significant response in average efficacy, which is also statistically significantly superior to the response provided by the placebo. Similarly, the MTD is the highest possible but still tolerable dose level with respect to a pre-specified clinical limiting toxicity. In general, these limits refer to the average patient population. For instances in which there is a large discrepancy between the MED and MTD, it is stated that

the drug has a large therapeutic window. Conversely, if the range is relatively small, or if the MTD is less than the MED, then the pharmaceutical product will have little to no practical value.

In microdose studies, a microdose is often compared with those observed at a therapeutic dose. The ultimate goal is to determine whether the treatment effect observed at the microdose is predictive of that at the therapeutic dose. In practice, however, the following questions raised. First, how to confirm that the selected therapeutic dose is truly an effective dose? Second, is microdose predictive of the selected therapeutic dose?

3.3 Animal Model versus Human Model

In order to establish a predictive model for human, the following predictive models are necessarily established.

(1) For animal population, it is to determine that microdose is predictive of therapeutic dose.
(2) Animal model is predictive of human model at a given dose. For example, at microdose, animal response is predictive of human response, while at therapeutic dose, animal response is predictive of human response.

Let A_M and A_T are animal responses at microdose and at therapeutic dose, respectively, while H_M and H_T are human responses at microdose and at therapeutic dose, respectively. In this case, suppose that we have (1) $H_T = f_1(A_T)$, (2) $A_T = f_2(A_M)$, and (3) $H_M = f_3(A_M)$ or $A_M = f_3^{-1}(H_M)$. As a result, we have $H_T = f_1 f_2 f_3^{-1}(H_M)$ or $H_T = g(H_M)$, where $g = f_1 f_2 f_3^{-1}$. It should be noted that in practice, f_1, f_2, and f_3 may not be linear.

3.4 Characterization of Dose Response Curve

To determine whether the microdose is predictive of therapeutic dose, we first need to characterize the dose response curve based on several micro doses and perhaps a therapeutic dose or a couple of therapeutic doses. With a limited number of dose levels available, it is difficult, if not impossible, to characterize dose response curve. If there are only two dose levels, i.e., microdose and therapeutic dose, extrapolation may not be reliable especially when the therapeutic dose is much larger than the microdose.

In addition, the dose response curve in a dose range under study could be either linear or non-linear. In this case, the traditional analysis of variance (ANOVA) with specific contrasts

(linear or non-linear) type F-tests may not be appropriate. Alternatively, Cheng et al. (2006)[8] proposed the use of slope approach by considering slopes between dose levels to characterize dose response curve. The slope approach (based on either slopes between dose levels and initial dose or slopes between adjacent dose levels) was found to be effective and can describe the shape of the dose response curve and will be able to determine whether there is a clinically meaningful difference in response between microdose and therapeutic dose.

3.5 Variability Associated With Dose

Heterogeneity in variance between microdose and therapeutic dose is a major concern in establishing a predictive model based on microdose for therapeutic dose. At microdose, the sensitivity associated with the analytical methods then plan an important role for accurately and reliably quantitation of the response of a given microdose, which have an impact on the prediction of therapeutic dose.

In dose response studies, it is assumed that the dose response is a monotonic increasing function of dose. In practice, it also recognized that the dose response the variability associated with the response is often proportional to the dose. Under the monotonic assumption and the fact that variability is proportional to the dose, several statistical methods have been proposed in the literature. These methods include (i) testing for linearity or non-linearity contrasts such as quadratic contrast in the analysis of variance (ANOVA) and (ii) slop approach proposed by Cheng et al.(2006)[8] based on normalized responses (i.e., responses adjusted for doses).

3.6 False Negative and False Positive

In practice, an established predictive model needs to be validated. Commonly used criteria are the false negative rate and/or false positive rate, which have an impact on the accuracy and reliability of the established predictive model. The false negative rate and/or false positive rate depend upon not only the study design but also assay variability and assay sensitivity. Thus, it is suggested that a pilot study be conducted for determining the false negative rate and/or false positive rate before the conduct of a microdose study in humans.

4. PREDICTION OF MICRODOSE

4.1 Predicability Index

Let X and Y be the characteristics at a microdose from animal and human, where X and Y are independent and follow normal distributions with means μ_X and μ_Y and variances σ_X^2

and σ_Y^2, respectively. The concept we use here is that if Y is predictive of X, the probability of that the ratio of the standardized responses $V=(X-\mu_X)/\sigma_X$ and $W=(Y-\mu_Y)/\sigma_Y$ equal to 1 could be close to 1. Similar to the idea of the concept for the consistency of raw materials and/or final products from two different sites (Tse et al., 2006),[9] we propose the following probability as an index to assess the predicability of the two characteristics;

$$p=P(1-\delta<\frac{V}{W}<\frac{1}{1-\delta}), \tag{1}$$

where $0<\delta<1$ is defined as a limit that allows for prediction. Thus p tends to 1 as δ tends to 1. We will refer to p as the predictive index. A small δ implies the requirement of a high degree of consistency at the microdose between animal and human. Under the normality assumption of X and Y, the ratio of two centered normal variables, $U=(X-\mu_X)/(Y-\mu_Y)$, is Cauchy variable with probability density function (see Cedilnik et al., 2004)[10]

$$f_U(u)=\frac{1}{\pi}\cdot\frac{\sigma_X/\sigma_Y}{u^2+(\sigma_X/\sigma_Y)^2}.$$

Thus, (1) can be rewritten as

$$\begin{aligned} p&=P\left((1-\delta)\cdot\frac{\sigma_X}{\sigma_Y}<\frac{V}{W}<\frac{1}{1-\delta}\cdot\frac{\sigma_X}{\sigma_Y}\right)\\ &=\left[0.5+\frac{\tan^{-1}(u)}{\pi}\right]_{u=(1-\delta)\cdot\frac{\sigma_X}{\sigma_Y}}^{u=\frac{1}{1-\delta}\cdot\frac{\sigma_X}{\sigma_Y}} \end{aligned} \tag{2}$$

Therefore, the predictive index p is a function of the parameters $\theta=(\sigma_X^2,\ \sigma_Y^2)$. Suppose that observations $X_i, i=1,\ldots,n_X$ and $Y_i, i=1,\ldots,n_Y$ are collected. Thus, using the invariance principle, the maximum likelihood estimate (MLE) of p can be obtained as

$$\hat{p}=\left[0.5+\frac{\tan^{-1}(u)}{\pi}\right]_{u=(1-\delta)\cdot\frac{\hat{\sigma}_X}{\hat{\sigma}_Y}}^{u=\frac{1}{1-\delta}\cdot\frac{\hat{\sigma}_X}{\hat{\sigma}_Y}}, \tag{3}$$

Where $\hat{\sigma}_X^2=\sum_{i=1}^{n_X}(X_i-\bar{X})/n_X$, $\hat{\sigma}_X^2=\sum_{i=1}^{n_Y}(Y_i-\bar{Y})/n_Y$, and $\bar{X}$ and $\bar{Y}$ are sample mean of X and Y, respectively. In other words, $\hat{p}=h(\hat{\theta})=h(\hat{\sigma}_X^2,\hat{\sigma}_Y^2)$. Similar to Tse et al. (2006)[9] it can be verified that :

$$\frac{\hat{p}-p-B(\hat{p})}{C(\hat{p})} \longrightarrow N(0,1),$$

where $B(\hat{p})$ and $C(\hat{p})$ are the expected value and standard deviation of $\hat{p}$. Under this asymptotic result, a $(1-\alpha)$x100% confidence interval of p can be obtained. Let $LL(\hat{p})$ be lower limit of the confidence interval. Following similar idea of consistency proposed by Tse et al. (2006)[9] we propose the following quality control (QC) criterion for examination of prediction of microdose. If the probability that the lower limit $LL(\hat{p})$ of the constructed $(1-\alpha)$x100% confidence interval of p is greater than or equal to a pre-specified quality control lower limit, say, QC_L, exceeds a pre-specified number β (say β = 80%), then we claim that U and W are consistent or similar. In other words, U and W are consistent or similar if

$$P(QC_L \le LL(\hat{p})) \ge \beta, \qquad (4)$$

where β is a pre-specified constant.

4.2. Sample Size Requirement

In practice, it is necessary to select a sample size to ensure that there is a high probability, say β, of consistency between U and W when in fact U and W are consistent. It is suggested that the sample size is chosen such that there is more than 80% chance that the lower confidence limit of p is greater than or equal to the QC lower limit, i.e. □ = 0.8. In other words, the sample size is determined such that $P\{QC_L \le LL(\hat{p})\} \ge \beta$. This leads to

$$P\{QC_L \le \hat{p} - B(\hat{p}) - z_{\alpha/2}\sqrt{Var(\hat{p})}\} \ge \beta.$$

Thus,

$$P\{QC_L + z_{\alpha/2}\sqrt{Var(\hat{p})} - p \le \hat{p} - p - B(p)\} \ge \beta.$$

This gives

$$P\left\{\frac{QC_L - p}{\sqrt{Var(\hat{p})}} + z_{\alpha/2} \le \frac{\hat{p}-p-B(p)}{\sqrt{Var(\hat{p})}}\right\} \ge \beta.$$

Therefore, the sample size required for achieving a probability higher than β can be obtained by solving the following equation:

$$\frac{QC_L - p}{\sqrt{Var(\hat{p})}} + z_{\alpha/2} \le -z_{1-\beta}. \qquad (5)$$

Assuming that $n_X = n_Y = n$, then the common sample size is given by

$$n \geq \frac{(z_{1-\beta} + z_{\alpha/2})^2}{(p - QC_L)^2} \left\{ \left(\frac{\partial \hat{p}}{\partial \mu_X} \right)^2 V_X + \left(\frac{\partial \hat{p}}{\partial \mu_Y} \right)^2 V_Y + \left(\frac{\partial \hat{p}}{\partial V_X} \right)^2 (2V_X^2) + \left(\frac{\partial \hat{p}}{\partial V_Y} \right)^2 (2V_Y^2) \right\}. \quad (6)$$

The above result suggests that the required sample size will depend on the choices of α, β, V_X, V_Y, $\mu_X - \mu_Y$ and $p - QC_L$

Table 1. Recommended Nonlinear Studies to Support Exploratory Clinical Trials

Clinical:		Nonclinical:		
Dose to be Administered	**Start and Maximum Doses**	**Pharmacology**	**General Toxicity Studies[a]**	**Genotoxicity[b] / Other**
Approach 1: Total dose ≤ 100 µg (no inter-dose interval limitations) AND Total dose ≤ 1/100th NOAEL and ≤1/100th pharmacologically active dose (scaled on mg/kg for i.v. and mg/m^2 for oral)	Maximal and starting doses can be the same but not exceed a total accumulated dose of 100 µg	In vitro target/ receptor profiling should be conducted Appropriate characterization of primary pharmacology (mode of action and/or effects) in a pharmacodynamically relevant model should be available to support human dose selection.	Extended single dose toxicity study (see footnotes c and d) in one species, usually rodent, by intended route of administration with toxicokinetic data, or via the i.v. route. A maximum dose of 1000-fold the clinical dose on a mg/kg basis for i.v. and mg/m^2 for oral administration can be used.	Genotoxicity studies are not recommended, but any studies or structure-activity relationship (SAR) assessments conducted should be included in the clinical trial application. For highly radioactive agents (e.g., PET imaging agents), appropriate PK and dosimetry estimates should be submitted.
Approach 2: Total cumulative dose ≤ 500 µg, maximum of 5 administrations with a washout between doses (6 or more actual or predicted half-lives) AND each dose ≤ 100 µg AND each dose ≤ 1/100th of the NOAEL and ≤ 1/100th of the pharmacologically active dose	Maximal daily and starting doses can be the same, but not exceed 100 µg.	In vitro target/receptor profiling should be conducted Appropriate characterization of primary pharmacology (mode of action and/or effects) in a pharmacodynamically relevant model should be available to support human dose selection.	7-day repeated-dose toxicity study in one species, usually rodent, by intended route of administration with toxicokinetic data, or via the i.v. route. Hematology, clinical chemistry, necropsy, and histopathology data should be included. A maximum dose of 1000-fold the clinical dose on a mg/kg basis for i.v. and mg/m^2 for oral administration can be used.	Genotoxicity studies are not recommended, but any studies or SAR assessments conducted should be included in the clinical trial application. For highly radioactive agents (e.g., PET imaging agents), appropriate PK and dosimetry estimates should be submitted.

Table 1 (Continued). Recommended Nonlinear Studies to Support Exploratory Clinical Trials

Clinical:		Non clinical:		
Dose to be Administered	**Start and Maximum Dose**	**Pharmacology**	**General toxicity studies[a]**	**Genotoxicity[b]**
Approach 4: Dosing up to 14 days into the therapeutic range but not intended to evaluate clinical MTD.	With toxicity in both species, follow appropriate regional guidance for clinical starting dose. If toxicity is not seen in either species (i.e., the NOAELs are the highest dose tested and doses used were not otherwise limited, e.g., not an MFD), or is seen only in one species, the clinical starting dose should be one that gives a predicted clinical AUC value (based on either interspecies PK modelling or mg/m^2 conversion) that is approximately 1/50th of the AUC at the NOAEL from the species yielding the lower exposure. For other considerations on initial dosing in humans, e.g., predicted PD activity, regional guidance should be consulted. Without toxicity in both species, it is recommended that the maximum clinical dose not exceed 1/10th the lower exposure (AUC) in either species at the highest dose tested in the animals. When only one species demonstrates toxicity, the maximum clinical dose should	*In vitro* target/receptor profiling should be conducted. Appropriate characterization of primary pharmacology (mode of action and/or effects) in a pharmacodynamically relevant model should be available to support human dose selection. Core battery of safety pharmacology (see Section 2) using doses similar to those used for the toxicity studies.	2-week repeated-dose toxicity studies in rodent and non-rodent with standard parameters assessed and where dose selection in animals is based on exposure multiples of anticipated clinical AUC at maximum dose.	Ames assay (or an appropriate alternative assay if Ames is inappropriate, for example, for an antibacterial product) and an assay (*in vitro* or *in vivo*) capable of detecting chromosomal damage in a mammalian system.

5. CONCLUDING REMARKS

As indicated by Lappin (2010)[1] the application of microdosing as a tool for early drug selection and development is growing and can be applied to drug-to-drug interaction by examining drug concentrations in tissues and certain cell types. Both ICH guideline and FDA guidance, however, emphasize that microdosing approach is for exploratory purpose and it should focus on safety in early phase of clinical/pharmaceutical development rather than on development for efficacy.

In practice, there are many scientific factors and practical issues, which limit the possible application of microdosing in pharmaceutical research and development, remain unsolved. These scientific factors include, but are limited to, (i) the issue of placebo effect both at microdose and at therapeutic dose, (ii) the prediction of animal model to human model, (iii) the selection of therapeutic dose, (iv) the characterization of dose response curve, (v) accuracy and reliability of extrapolation (prediction) of the microdose to therapeutic dose, and (vi) the assessment of false positive/negative of microdose prediction. These practical issues have limitations in many aspects on drug development for efficacy.

In this article, we propose a predictive index for examining the closeness between the treatment effects at microdose and therapeutic dose under a simple study design. Microdosing design and analysis, however, are still not fully understood. Thus, it is suggested that appropriate statistical methods should be developed under a valid design for a rigorous and more accurate and reliable assessment of the performance of microdosing approach in pharmaceutical development.

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