



## **S3A: Note for guidance on toxicokinetics: The assessment of systemic exposure in toxicity studies.**

### **Q&A: FOCUS ON MICROSAMPLING (Step 2b draft)**

International Council for Harmonisation of Technical Requirements  
for Pharmaceuticals for Human Use



### **S3A toxicokinetics: Q&A FOCUS ON MICROSAMPLING**

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## Preface

**S3A (Toxicokinetics) guideline: implemented in 1994**

- **Recently, analytical method sensitivity (such as that of liquid chromatography / mass spectrometry) has been improved, allowing microsampling techniques (very low volume sampling) to be widely used in toxicokinetic (TK) assessment.**

## Objective of the Q&A

To describe points to consider before incorporating microsampling method in TK studies, acknowledging

- Its benefits (and limitations) for assessment of TK in main study animals.
- Important contribution to 3Rs benefits (Replacement, Reduction and Refinement) by reducing or eliminating the need for TK satellite animals.

## 1. INTRODUCTION - SCOPE

### *What is the definition of microsampling?*

A method to collect a very small amount of blood (typically  $\leq 50 \mu\text{L}$ ) to measure TK parameters of the drug and/or its metabolites.

- **Matrices:** blood and its derived plasma or serum, in liquid or dried form.

Excluding other matrices (e.g. lung lavage and lymph) those are not yet validated and thus are outside the scope of this Q&A.

- **Animal Species:** Rodents and non-rodents.

### *What are the benefits/advantages of microsampling?*

#### **Minimizing volume of blood collection**

- Can minimize pain and distress in animals (improvement of the animal welfare: refinement).
- Can reduce or eliminate the number of required animals in a TK satellite group for rodents (reduction), particularly for mice.
- Can make evaluation of the relationship between safety data and drug exposure in the same animals, when performing on main study group.

## **2. BASIC PRINCIPLE ON APPLICATION OF MICROSAMPLING**

*For what types of pharmaceuticals and for what types of safety studies can we use microsampling?*

#### **Types of pharmaceuticals:**

- **Applicable to majority of pharmaceuticals and biopharmaceuticals.**
  - However, consideration should be given on a case-by-case basis as to whether the sensitivity of the measurement method is appropriate with the small sample volumes available.

*(continued)*

**Types of safety studies:**

- **Can be used in any type of safety study**  
e.g., single-dose or repeated-dose safety studies, juvenile and reproductive studies, and others.

**However, microsampling is not warranted when the Lower Limit Of Quantification (LLOQ) of the bioanalytical method is insufficient for the planned sample volume due to low drug exposure levels (e.g., exposure after topical or inhaled administration).**

***What are the points to consider when applying microsampling to TK studies?***

- **A bioanalytical method should be developed and qualified (or validated for GLP studies, in accordance with regulatory guideline/guidance in each region) to ensure the reliability of analytical results.**
- **Analytical characteristics (e.g., LLOQ, matrix effects and the stability of the analyte(s) in the biological matrix for the entire periods) should be carefully assessed.**

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- **Bridging from conventional to microsampling methods can be done by assuring comparability of the exposure measurement between microsampling and conventional methods in a separate pharmacokinetics (PK) study. This separate PK study for comparison may be omitted on a case-by-case basis and with appropriate scientific justification, for example, when using the same assay conditions in the same matrix to test blood samples drawn from the same site.**

In this comparison exercise, it is advisable to check the variability of measurement to evaluate the method of adding internal or external substances to evaluate the method of sample dilution

*(continued)*

- **Ideally, the same matrix should be used throughout the TK studies and also in clinical studies.**

When different matrices are used, drug concentration relationship among matrices should be defined to evaluate systemic exposure appropriately from each measurement using different matrices, considering various factors such as

- hematological parameters
- plasma protein binding rate
- blood/plasma (or serum) ratio of the drug

**What types of blood collection and what types of pretreatment methods are used for microsampling?**

- **Blood can be collected from the tail vein, etc., using capillary tubes or any appropriate miniaturized collection devices and treated either in a liquid or dried form.**

**<Liquid sample methods>**

- **Isolated plasma or serum can also be used when blood samples are centrifuged after collection.**
- **In some cases, the sample is diluted with the appropriate solvents or blank matrices prior to storage, shipment and subsequent analysis.**

***(continued)***

**<Dried sample methods>**

- **Sample is usually spotted onto cellulose-based or other types of materials and dried.**
- **A fixed diameter sub-punch or the whole spot on the card/device can be extracted and measured/analyzed.**
- **Recent and on-going advancements in microsampling devices have demonstrated the ability to collect precise volumes of blood, such that the entire sample can be used for analysis without additional volumetric measurements.**

**Newly developed techniques could also be considered with adequate validation.**

### 3. EFFECT ON SAFETY EVALUATION

*How to evaluate the effect of blood sampling on the toxicity data and wellbeing of the animal in main study group?*

- When blood sampling is done in the main study animals, it is important to consider the effect of blood collection on the physiological condition of animals.

<Main factors to consider for planning protocol>

- Volume and the number of samples taken in a given period
- Properties of the test drug (e.g. effects on red blood cells)
- Test system (e.g. species, age, body weight, total blood volume)
- Site of collection
- Study duration

**Sampling protocols should be appropriately established.**

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<Main animal data to record for physiological evaluation>

- Body weight
- Food consumption
- Hematological parameters (e.g. red blood cell count, hemoglobin level, hematocrit value, mean corpuscular volume, electrolytes, total proteins)
- Any effect on the blood collection site (e.g. tissue damage, inflammation)

**Evaluation of these parameters compared to matching control animals treated in the same way will be important in establishing whether any suspected effects are related to test drug or to procedures.**



#### 4. ISSUES REGARDING THE BIOANALYTICAL METHOD

*What are important points to consider in bioanalytical method development and validation of treatment of liquid or dried samples?*

- Analytical method validation should be stipulated in the bioanalytical guideline/guidance in each regulatory region.
- In addition, the following points should be considered when analyzing samples derived from microsampling.

<Liquid sample method>

- 1) Confirmation of the sample homogeneity
- 2) Small volume handling issues e.g. freezing/drying effects during the storage
- 3) Potential increase in the LLOQ due to limited sample volume
- 4) Impact of addition of anticoagulants to small containers/ capillaries, resulting in dilution of the sample

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*(continued)*

<Dried sample method>

Method with best recovery and lowest matrix interference on the drugs should be selected.

- If the sub-punch of the dried spot approach is used, the effect of different hematocrit values should be evaluated.
- It is important to confirm the uniformity of the spots.
- Both of these issues can be minimized if an accurate volume of blood is collected on the device and the whole sample is subsequently analyzed.

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***(continued)***

**<Incurred Sample Reanalysis (ISR)>**

- **ISR should be conducted according to each regional guidance/guideline, if described.**
- **When doing ISR, care should be taken to ensure sufficient sample volumes that are retained for ISR.**

## **Conclusion**

- Q&A on microsampling for TK studies was drafted.
- Microsampling can contribute to 3Rs and evaluation of relationship between the safety data and drug exposure in the same animals.
- Liquid/dried microsampling can be applicable to majority of pharmaceuticals and biopharmaceuticals.
- When blood sampling is done on the main study animals, it is important to consider the effect of blood collection on the physiological condition of animals.
- Analytical method validation should be stipulated in the bioanalytical guideline/guidance in each regulatory region.



**Thank You!**

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