Microdosing studies: a consideration on analytical technology choice

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Abstract

Introduction: Microdosing has been used as an investigative pharmacokinetic tool for approximately 10 years. Initial skepticism of the value of these studies was followed by investigative clinical trials to understand the circumstances when they provide useful data and this has led to routine use. When first introduced, accelerator mass spectrometry coupled with LC fractionation (LC+AMS) was the only technology that could provide the sensitivity required for these studies. Over the years, LC-MS/MS sensitivity has improved so that it is now viable to use this technique for microdosing studies, and a decision needs to be made on what technique to use.

Methods: We have considered the relative merits of the technologies and the implications of using these techniques in terms of clinical study design along with the potential impact on the overall progress of the study. The authors' expertise in analyzing samples from microdosing studies by LC+AMS and LC-MS/MS from over 50 microdosing studies was captured to share this knowledge more widely.

Result: A decision tree is presented that allows the correct decision to be made on whether to use AMS or LC-MS/MS for these analytically challenging studies. The decision tree is further expanded with detailed information on the background to the questions and the facets that should be considered when determining the optimum pathway. It considers the impact of the clinical study design and the aims of the study and the challenges in that will be faced when using either of the instruments. The vagaries of both LC+AMS and LC-MS/MS will be compared to allow decisions to be made on the likelihood of success depending on the chosen pathway.

Novel Aspect / Conclusion: Previously, there has been a lack of an objective comparison between LC+AMS and LC-MS/MS. Sufficient experience has now been gained in these techniques. This has allowed this decision tree to be produced that will provide a valuable starting point when faced with the bioanalytical challenge presented by microdosing studies.

Introduction

A clinical microdose study is one in which a sub-pharmacologic dose is administered. For regulatory purposes, a microdose is ≤ 1/100th of the NOAEL and ≤ 1/100th of the pharmacologically active dose, up to a maximum of 100 µg. Microdosing provides insight to human pharmacokinetic (PK) data at the earliest opportunity and has been used to provide absolute and relative insight as follows:

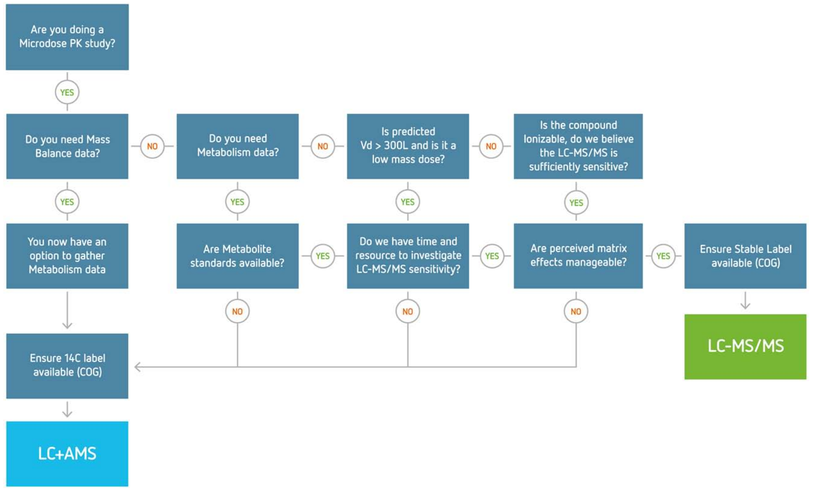
- If preclinical data are equivocal, which best represent the human in vivo situation?
- How does the human PK of the investigative compound compare to that of benchmark/competitor drugs?
- Does the compound reach the target tissue?
- What is the in vivo metabolic fate of the compound in human?
- What is the clinical relevance of potential drug-drug interactions identified through in vitro modulation of CYP450 activity?
- Which potential backup compound has the most appropriate PK profile, light of observed PK limitations of the lead compound?

Microdose studies are more predictive of the human in vivo situation than allometric scaling from preclinical species and are a cost-effective way to clarify or replace equivocal preclinical investigations. To understand the reliability of the data from microdose studies, three pharmaceutical industry studies have been conducted. These are:

- Consortium for Resourcing and Evaluating AMS Microdosing (CREAM) – 2004 [1]
- European Union Microdose AMS Partnership Program (EUMAPP) – 2006 [2]
- NEDO – Professor Yuichi Sugiyama – LC-MS/MS – 2009 [3]

These studies have shown that 70% of oral microdose studies properly predicted oral therapeutic PK and 100% of IV microdose studies properly predicted oral therapeutic PK. Where the PK parameters do not match within a factor of 2-fold, the shape of the PK curve is well predicted and this data is useful in determining the desired dosing level. The PK at pharmacologic doses of some drugs which are actively transported across the gut wall is not well represented by a microdose study. On-going research suggests that such compounds may be identifiable based on in vitro transporter affinity data.

Due to the low doses of compound administered, microdosing requires an extremely sensitive analytical platform. Modern LC-MS/MS instrumentation and LC+AMS are capable of providing adequate sensitivity. Both approaches have some pros and cons. In some cases, for some specific compounds it is necessary to utilize the unique attributes of LC+AMS to achieve the necessary limits of quantification. In addition, LC+AMS is a tracer technique and therefore can provide additional information, for example on the disposition of metabolites. On the other hand, the advantage of LC–MS/MS is that it is capable of analyzing non-radiolabeled drugs, and requires no special facilities. LC–MS/MS can be also applied to cassette MD studies with non-radiolabeled drugs to select drug candidates with appropriate PK properties in humans. The following decision tree, and the discussion around it, may be used to select the most appropriate analytical platform to answer the specific questions relevant to the molecule.



Mass Balance Data

Early mass balance data, or routes of excreta for the drug may enable project progression. This is most easily obtained by use of AMS as this does not require authentic standards to provide this data. AMS will rapidly provide data on:

- Amounts of ^{14}C excreted in urine and feces.
- Circulating levels of ^{14}C material in plasma and blood.

It is possible to obtain a significant amount of data by LC-MS/MS however this would require a good understanding of the metabolism of the compound, availability of standards, and the resource to develop multiple assays. As microdose studies are typically performed early in the development process, it is very unlikely that detailed information and metabolite standards will be available.

Quantitative Metabolism Data

The main source of information on metabolism at this stage will be in-vitro incubation in hepatocytes or microsomes, and LC-MS/MS data gained from these incubations. To quantify metabolites in the microdose study both LC-MS/MS and LC+AMS can be used.

- LC-MS/MS:
  - Due to the low circulating levels, triple quadrupole tandem mass spectrometry will be required to provide these data.
  - In-vitro incubations can be scaled up to provide metabolite standards. The resource required to perform the scale up, isolation and purification is not insignificant and hence the decision to perform this has to be compared to the information and resource for AMS.
  - Even with metabolite standards, there is the risk that not all significant human metabolites have been identified and this may result in incomplete recovery in the mass balance experiment when using LC-MS/MS.
  - If concentrations in different matrices are needed then additional resource will be required to ensure suitable LC-MS/MS methods are available
- LC+AMS:
  - by virtue of its' ability to quantitate any molecule containing a ^{14}C label, AMS can provide reliable information on the quantitative amounts of metabolites.
  - Minimal effort is needed to develop methods in different matrices as matrix effects are not seen with LC+AMS

Predicted PK and target LLOQ

- When designing a microdose study and considering LC-MS/MS or LC+AMS analyses, the dose administered has an impact on the decision. The maximum allowable microdose is 100 µg and the ease of detection following this dose will depend of the volume of distribution (V_D).
- For compounds with a large V_D, the concentration of the drug will be low and hence may test the LLOQ of LC-MS/MS. In these cases, LC+AMS will be the method of choice as it is likely that the PK curve can be better defined and will provide a more complete definition of the AUC. The amounts of ^{14}C administered can be changed, within boundaries, to maximize AUC definition.
- For compounds with a smaller V_D, the higher circulating plasma concentrations mean that LC-MS/MS may be able to provide the data for extensive AUC definition.
- Prior to determining which technique should be used, it is recommended that suitable in-vitro testing and modeling experiments are carried out. These data can be used to predict the AUC, and hence the desired LLOQ and the suitable analytical detector.

Lab environment

- The lab environment needs to be considered. Contamination is a potential issue when working at these levels and appropriate measures need to be taken to prevent contamination of the facility. Obviously, these measures are not particular to microdose studies but to any assay that is operating at the limits of analytical sensitivity.

Sensitivity

Originally AMS was the only technique capable of achieving the sensitivity required for microdosing. Modern LC-MS/MS instruments can now be used for these studies under optimum conditions. The following should be considered when deciding on LC-MS/MS vs LC+AMS:

- Does the LC-MS/MS response of the compound from pre-clinical assays indicate that the desired LLOQ may be achieved?
- Does the compound contain the correct structural features for efficient ionization leading to high sensitivity? If the compound does not contain the correct functionality would it be possible to derivatize the molecule to enable ionization? This may however add significant effort to method development.
- Can the chromatographic eluent be changed to promote efficient ionization? This may require extensive evaluation of different LC systems to ensure the optimum environment? The mobile phase may then be compromised such that optimum chromatographic efficiency and resolution may not be achieved. Loss of chromatographic efficiency may reduce the limits of detection achieved and more extensive method development may be required.
- Does the laboratory developing the assay have highly skilled staff to allow the development and application of these assays?

By comparison, the LLOQ of LC+AMS can be predicted with a fair degree of accuracy as its analyzes graphite derived from the original sample, and the LLOQ can be modified by the sample processing to achieve the desired sensitivity.

Matrix effects

- Whilst the initial assessment of sensitivity can be performed quite rapidly using LC-MS/MS, it is only when this is assessed in a biological extract that a true value will be obtained. Matrix effects can significantly impact the sensitivity obtained, and whilst a range of options exist to remove interferences that cause matrix effects, these will add to the method development time and the cost of analyses.
- In contrast, due to the destructive nature of the graphitization process that occurs prior to analysis, AMS does not suffer from matrix effects. As a result of this, simple sample preparation procedures such a protein precipitation can be employed.

Method development resource

When considering which analytical route to take, the time available prior to dosing has to be taken into account. As the LLOQ for LC+AMS can be predicted before any experimental work commences, the method development time can be predicted with a reasonable degree of accuracy. For LC-MS/MS, the resource taken to reach the target LLOQ will differ depending on the compound under investigation, Also, seeking a lab that is well equipped with high sensitivity instruments and in depth knowledge of method development will be key.

In addition to method development, a decision on what validation should be performed has to be taken. This may be a fully validated method, or follow the tiered approach suggested for MIST investigations [4].

Cost of goods

For both LC-MS/MS and LC+AMS, synthesis of an isotopically labelled compound may be required.

- LC-MS/MS assay will be operating at its limits and a stable labelled internal standard is recommended. This may already exist and have been used in assays for pre-clinical species.
- LC+AMS measures ^{14}C material and this is needed prior to dosing. This material may have been synthesized to enable early metabolic investigation.

Conclusion

- Since microdosing was first utlilized, LC-MS/MS sensitivity has increased to allow this to be used in addition to LC+AMS under certain circumstances.
- A decision tree is presented to help determine whether LC+AMS or LC-MS/MS is the most suitable analytical technique when conducting a microdose study.
- Discussion on the aims of the study, the predicted LLOQ, and the features of the two technologies are presented to aid the decision.

References

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