

## INTRODUCTION

Traditionally, absolute bioavailability (ABA) has been determined using a crossover study design, requiring administration of an IV dose expected to give plasma concentrations similar to those arising from the therapeutic extravascular (EV) dose.

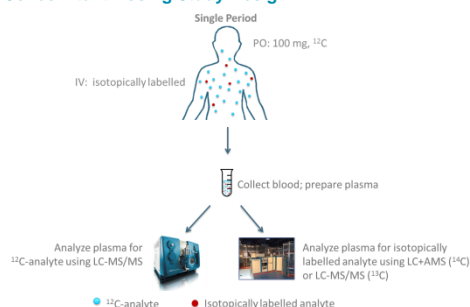
An alternative approach is to administer an isotopically-labelled IV microtracer dose concomitantly with the EV dose (see Figure 1). This methodology is both scientifically superior and more resource-efficient. With the inclusion of ABA data mandatory for marketing applications submitted to the Australian TGA [1] and other regulators increasingly asking for this information, a growing number of such studies are being conducted.

The concomitant dosing approach can be implemented using either a stable isotope (ie  $^{13}\text{C}$ ) or a radioactive isotope ( $^{14}\text{C}$ ). The utility of the methodology is underpinned by the availability of robust analytical methods, with sufficient sensitivity to detect the very low concentrations of labelled compound arising from the IV dose, for both isotopes and the choice is largely dependent on the physicochemical and pharmacokinetic characteristics of the compound. Here we assess the relative merits of using LC-MS/MS and a  $^{13}\text{C}$ -labelled tracer or using LC+AMS and  $^{14}\text{C}$ . We present a decision tree as an aid to making the decision.

## METHODS

### CLINICAL STUDY DESIGN

Figure 1: Concomitant Dosing Study Design



Non-labelled compound is administered by the therapeutic EV dose route, at a therapeutically relevant dose level (100 mg in this example). The isotopically-labelled IV dose is then administered at the expected  $T_{\text{max}}$  of the EV dose, ideally as a short (eg 15 min) infusion. Delaying administration of the microtracer dose and keeping the IV dose level as low as practicable, such that the predicted circulating concentrations are at least 100-fold lower than those expected from the EV dose, ensures that ensures that at all times the pharmacokinetics of the system are driven by the EV dose. The resulting low circulating levels of labelled compound minimizes any the potential for it to interfere with the LC-MS/MS assay for the non-labelled compound. Typical microtracer doses are 10  $\mu\text{g}/500$  nCi for  $^{14}\text{C}$ -labelled compounds and 100  $\mu\text{g}$  for  $^{13}\text{C}$ -labelled (typically incorporation of six  $^{13}\text{C}$  atoms per molecule is required to avoid isotopic interference in the LC-MS assay). As long as the IV dose does not exceed 100  $\mu\text{g}$ , it can be treated as a microdose [2] and consequently no safety toxicology data are required. In most cases, the dose can be kept sufficiently low that it can be formulated in a simple (aqueous), biocompatible solution, minimizing the time and cost required for formulation development and avoiding the need for local tolerance testing. Because both doses are administered at the same time, equal clearance is assured and there is no possibility of temporal variation in PK between the dosing occasions, eg due to changes in subjects' health status.

## ANALYTICAL PLATFORMS

### LC-MS/MS

The combination of high-performance liquid chromatography and mass spectrometry has created an ideal analytical tool for drug separation and quantitation. It has had significant impact on the drug development process over the past few decades. The technique is specific and robust, and advances in hardware and software have made method transfer easy and analysis of both non-labelled therapeutics (ie from the EV dose) and stable isotope-labeled analytes (arising from the IV dose) quite straightforward. Due to the low dose of the microtracer administered in ABA studies, high detection sensitivity is required. With the continuous improvements in LC-MS interface technologies, the assay sensitivity in the region of 1 pg/mL are now achievable for many compounds/matrices.

Whichever type of isotope is selected, circulating levels of the  $^{12}\text{C}$ -analyte arising from the non-labelled EV dose will be measured using a conventional bioanalytical method. In principle, this assay can be adapted to measure  $^{13}\text{C}$ -analyte by monitoring ion(s) with the appropriate mass shift. This, coupled with the convenience of not having to deal with radioactive material in the clinic, is attractive. However, consideration must be given to whether the sensitivity of the assay is sufficient, given the constraints on the total mass of compound that can be administered as the tracer dose. Moreover, many LC-MS assays use a  $^{13}\text{C}$ -labelled analogue as an internal standard (ISTD) and synthesizing three labelled compounds (ie ISTD for EV dose,  $^{13}\text{C}$ -analyte for microtracer and ISTD for  $^{13}\text{C}$ -analyte) that are mass spectrometrically distinguishable, without isotopic interference, may not be straightforward (or cost-effective).

### LC+AMS

HPLC separation with off-line accelerator mass spectrometry detection (LC+AMS) is now a well-accepted frontline bioanalytical technique [3]. The accuracy and precision of the technique are comparable to those obtained using LC-MS and the inherent sensitivity of the detector facilitates assays with extremely low LLOQs (for a 4  $\mu\text{g}/500$  nCi dose, the LLOQ is between 50-120 fg/mL). Because sample processing for AMS analysis involves conversion of the analyte to elemental carbon or  $\text{CO}_2$ , the technique is independent of chemical structure and class, and is not susceptible to matrix effects caused by ion suppression. A reliable prediction of the achievable LLOQ can therefore be calculated before starting method development commences and, if necessary, the assay and/or the specific radioactivity of the dose can be adjusted to ensure that it is below the predicted concentration at the last sampling time point.

An advantage of using a  $^{14}\text{C}$ -labelled tracer dose is that additional information on metabolism and tissue distribution can be obtained for minimal additional effort or cost:

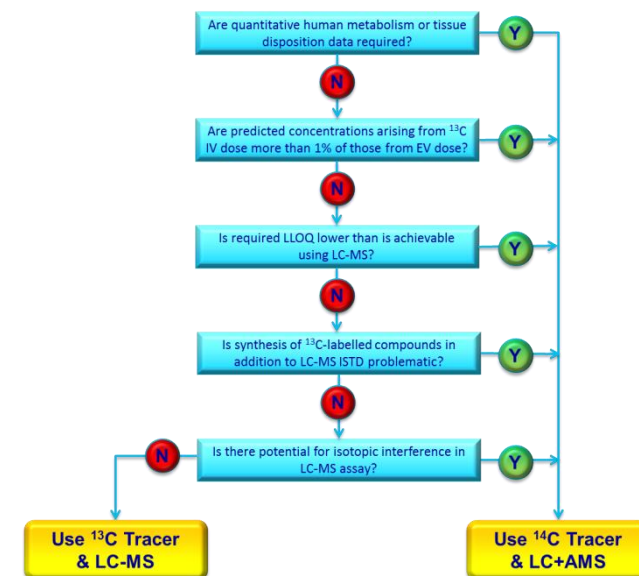
- circulating concentrations of all compound-derived material (ie parent plus any metabolites) arising from the IV dose;
- routes and rates of excretion of the IV dose (excretion *via* feces quantifies biliary excretion);
- concentrations of compound-derived material in accessible tissues;
- quantitative metabolite profiles in plasma, excreta and tissues.

Although it is important to remember that the data obtained reflects systemic metabolism only (ie any pre-systemic, first pass metabolism will be missed), such data can be invaluable, especially when obtained early in the drug development process.

## DISCUSSION AND CONCLUSIONS

The decision tree shown in Figure 2 has been developed based on the authors' extensive experience of LC MS/MS and LC+AMS analysis in tracer studies. It

Figure 2: Decision Tree for Selection of Isotopic Label



builds on that published by Xu *et al* [4] by including an assessment of the relative predicted concentrations arising from the IV and EV doses. It is essential to the integrity of the concomitant administration approach that the pharmacokinetics of the system are at all times driven by the EV dose. Keeping the IV dose as low as possible can also facilitate IV formulation of insoluble compounds.

Irrespective of the isotopic label chosen, the concomitant administration study design should be considered the "gold standard" for determination of ABA, since it ensures the clearance of drug arising from both the IV and EV doses is equal. This is not necessarily the case for a crossover study design, where circulating concentrations may be quite different on the two dosing occasions. Equal clearance is the key assumption in the equation for ABA, and without it the calculated value may be erroneous.

## REFERENCES

1. Australian Department of Health Therapeutic Goods Administration (2014). Regulatory Guidelines for Prescription Medicines: Mandatory requirements for an effective application (version 2.1).
2. ICH Topic M3 (R2) Note for guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals. CPMP/ICH/286/95 (2009).
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4. Xu, X, Jiang, H, Christopher, LJ, Shen, JX, Zeng, J & Arnold, ME (2014). Sensitivity-based analytical approaches to support human absolute bioavailability studies. *Bioanalysis* 6(4), 497-504.