

# Metabolite Profiling

## AMS study design considerations



Purpose Regulatory authorities have provided some of the substantive reasons underlying the conduct of metabolite profiling studies. The FDA guidance for "Safety Testing of Drug Metabolites" provides recommendations to industry on when and how to identify and characterize drug metabolites whose nonclinical toxicity needs to be evaluated. The safety of drug metabolites may need to be determined in nonclinical studies because these metabolites are either identified only in humans or are present at disproportionately higher levels in humans than in any of the animal species used during standard nonclinical toxicology testing. One of the best ways to determine the presence and proportion of unique human metabolites is through the conduct of an AMS-based metabolite profiling study. These studies permit the quantitation of a  $^{14}\text{C}$ -labeled drug product and its  $^{14}\text{C}$ -metabolites in plasma, urine and feces. Structural confirmation is performed by comparing the chromatographic retention time of the  $^{14}\text{C}$  fraction with that of a known unlabeled reference standard spiked into the same sample. Structure identification of unknown metabolites requires additional, and sometimes elaborate, procedures beyond pure AMS measurement. This AMS-based approach provides a solid approach that addresses guidelines described by regulatory authorities.

Accium has successfully delivered on a wide range of AMS-based metabolite profiling studies. Each study was customized to address particular challenges that were specific to each program. An outline of the various approaches we have employed in the design and conduct of these studies is shown below:

### Clinical Design

1. Six to eight subjects.
2. Single dose at, or near, the pharmacologic dose.
3. Radiolabel dose is around 100 nCi if agent is well absorbed and a limited number of metabolites are expected.
4. Radiolabel dose is around 1 uCi if agent is well absorbed and a large number of metabolites are expected.
5. Radiolabel dose may increase up to 100 uCi if agent is poorly absorbed and a large number of metabolites are expected.
6. Collect 1-2 mL plasma for AMS pharmacokinetics and metabolite profiling.
7. May need to collect 2-4 mL plasma at the later time points for metabolite profiling.
8. Collect urine and feces if profiling those matrices.

### Bioanalytical Design

#### Pre-study Phase

1. Transfer extraction and HPLC procedures to Accium.
2. Transfer unlabeled parent drug and any metabolites, if available, as reference standards.
3. Demonstrate acceptable chromatography conditions by UV-HPLC (using spiked references).

#### Study Phase

1. Prepare and analyze all plasma samples by AMS to obtain total  $^{14}\text{C}$ /mL plasma.
2. Report ng-eq/mL plasma concentrations to Sponsor to aid in the selection of time points for metabolite profiling.
3. Prepare plasma pools across subjects at Sponsor-selected time points (typically four to six time points, may include predose).
4. Extract pooled plasma, spike unlabeled drug reference(s), HPLC and collect all fractions across the chromatogram.
5. Prepare and analyze an aliquot of each HPLC fraction by AMS.
6. Report UV chromatogram along with DPM/mL in each HPLC fraction.

### Advantages

- Radiolabeling permits tracing of all known and unknown  $^{14}\text{C}$ -metabolites.
- Reduction of isotope to nCi range eliminates dosimetry requirements.
- Ultrasensitive AMS analysis can definitively quantify the appearance of rare metabolites.

### Disadvantages

- Requires  $^{14}\text{C}$ -labeled drug product.
- Ideally, requires unlabeled reference standard for each known metabolite.
- AMS analysis of large number of fractions can be costly, hence the plasma pooling strategies. (see '2012 Fees' flier to learn how Accium has addressed any cost issues).

