Bridging in vitro data to clinical outcomes with MetID

XenoTech
A BioIVT Company

AGILEX BIOLABS

8 March 2022

SPEAKERS

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MAIN OVERVIEW

In vitro met ID overview

- In vitro met ID in drug development
- Definitions
- Regulatory standards for met ID
- Timing of in vitro met ID
- In vitro test systems
- In vivo studies
- Quantitative information
- In vitro met ID endpoints

General Introduction

- **FDA, CDER, Guidance for Industry, Safety Testing of Drug Metabolites**

- MIST (Metabolites In Safety Testing)
  - FDA encourages the identification of any differences in drug metabolism between animals used in nonclinical safety assessments and humans as early as possible in development

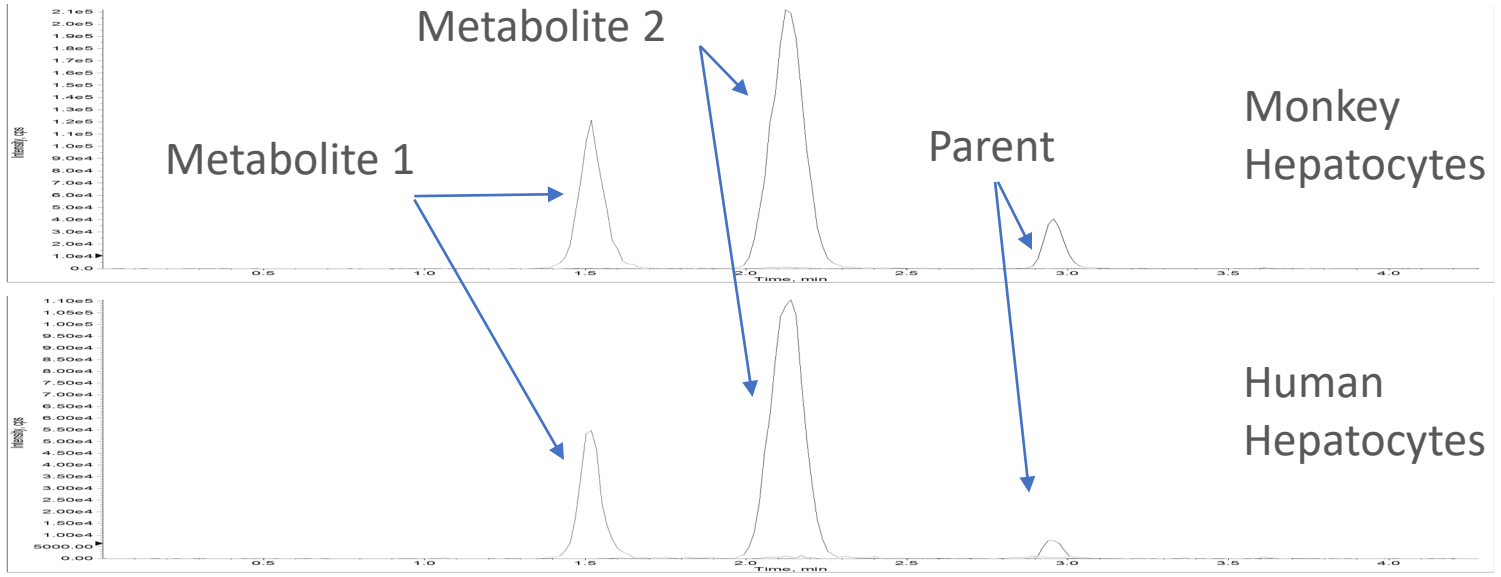
How many metabolites are formed?
What are they?
Are they human-specific or disproportionally higher in human than any of the toxicity species evaluated?

  - Metabolite profiles vary across species (qualitatively + quantitatively)
  - Metabolites can be pharmacologically active and/or chemically reactive

DEFINITIONS

Q: Metabolite profiling/characterization/identification - what should I call it?

A: Profiling - how many metabolites are formed in each species/test system



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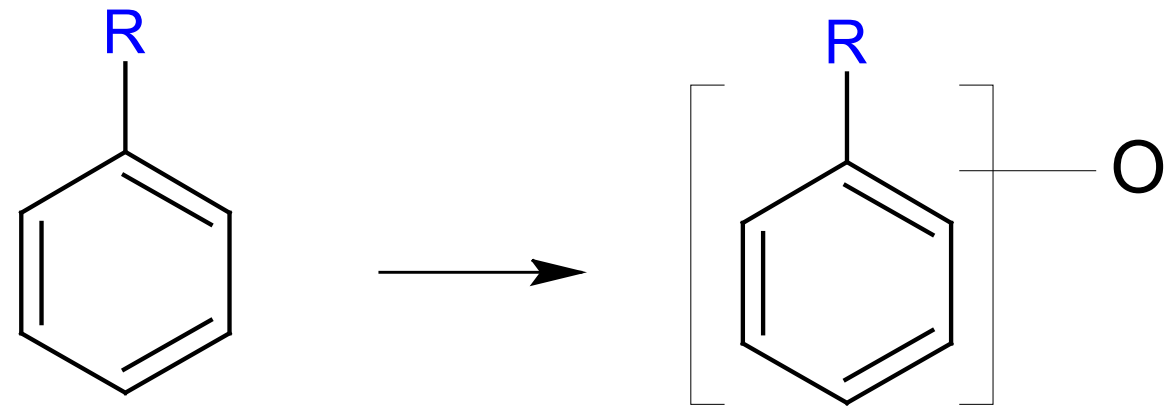
DEFINITIONS

Q: Metabolite profiling/characterization/identification - what should I call it?

A: Characterization – determination of the molecular weight and elemental composition of a metabolite



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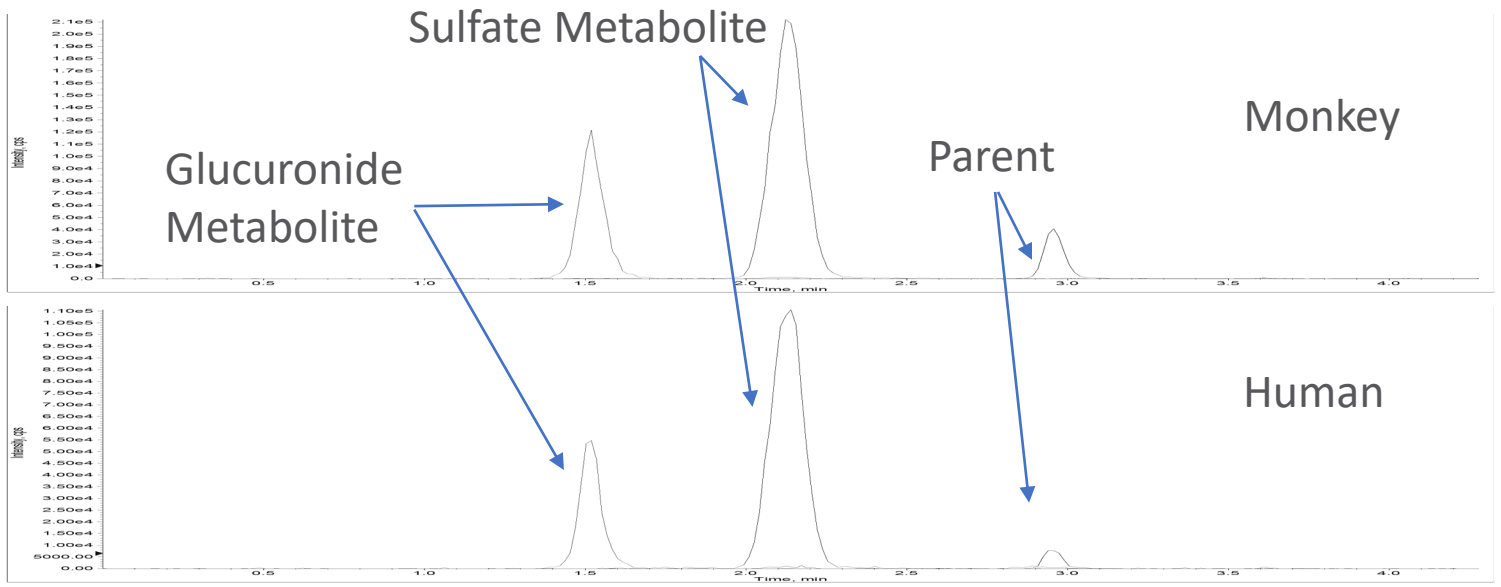

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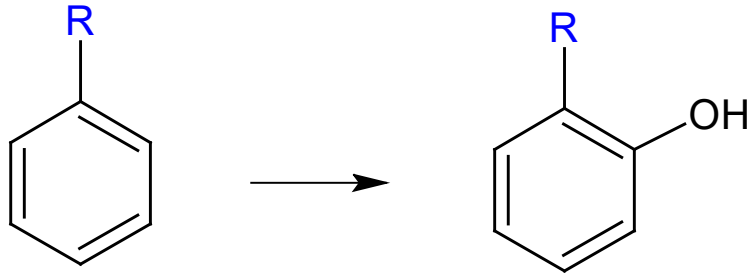
DEFINITIONS

Q: Metabolite profiling/characterization/identification - what should I call it?

A: Identification – definitive structural assignment

Typically accomplished by an exact match when comparing the retention time and MS/MS fragmentation with an authentic reference standard

(Or by NMR analysis - definitive chemical structure)



Our studies are performed as Metabolite Characterization

If metabolite reference standards are provided, we will use them to confirm the identity as applicable.

Metabolite characterization does not need to be GLP

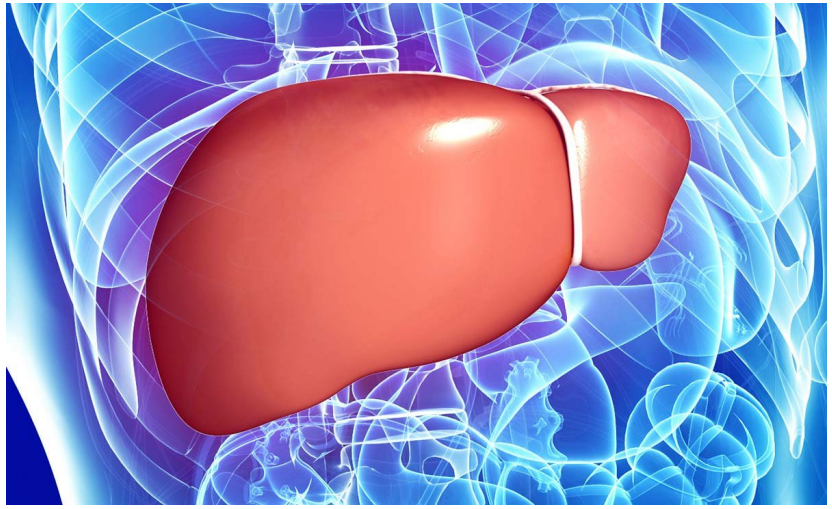
- GLP (Good Laboratory Practice) compliance is not required or applicable for in vitro drug metabolism studies.
- XenoTech Metabolite Characterization studies are performed as non-regulated studies
    - Conducted in accordance with applicable standard operating procedures (SOP) of our facility that were developed based on the high standards of record keeping as outlined in the FDA GLP regulations, 21 CFR Part 58 (FDA).

In vitro Met ID timing in drug development

- As early as possible in the drug development process
- Cross species in vitro met ID to assist with tox species selection
- In vitro met ID to determine any potentially toxic or reactive metabolites
- Prior to reaction phenotyping studies

Test system for met ID studies

Cryopreserved hepatocytes are the most commonly used test system.



- Intact hepatocytes contain the major hepatic drug-metabolizing enzymes required to study the four categories of xenobiotic biotransformation:
  - Hydrolysis
  - Reduction
  - Oxidation
  - Conjugation
- Other options:
  - Microsomes or S9 (need appropriate cofactors, NADPH/UDPGA)
  - Attachable (plated) cryopreserved hepatocytes

Metabolite characterization of in vivo samples

We routinely perform studies with in vivo samples from preclinical and clinical studies using cold or radiolabelled test articles

- Plasma
- Urine
- Fecal homogenate
- Various tissue homogenates
- Test articles labeled with ^{14}C or ^{3}H

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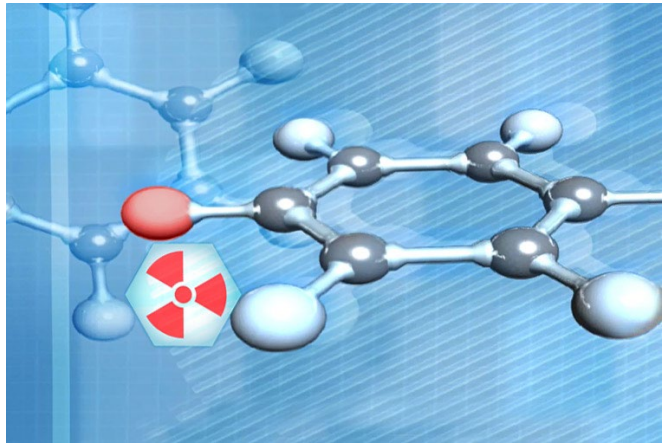
Quantitative metabolite characterization studies

- Generally, met ID studies are not quantitative
- Unless it is a radio-labeled study.
- Radio-labeled studies will provide quantitative information for each metabolite formed as long as the metabolite retains the radio-isotope

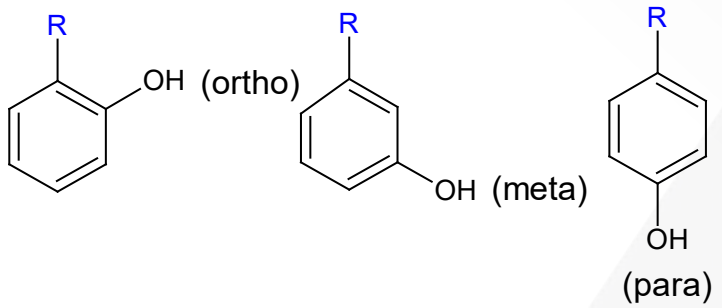


Why metabolite characterization studies are not quantitative

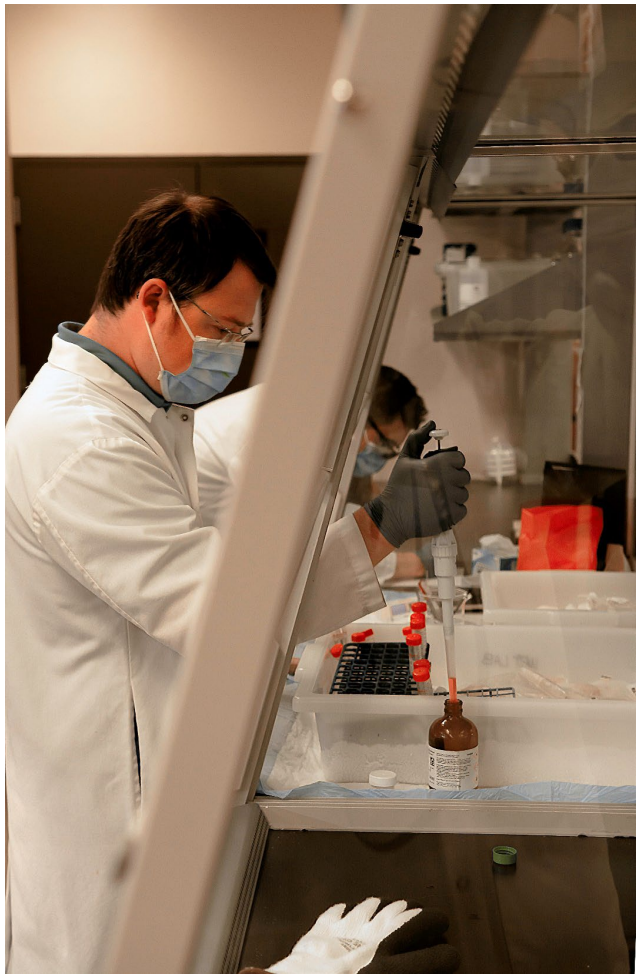
Mass spectral data are not reliably quantitative due to inherent differences in ionization efficiency between the test article and metabolites.

- For example, equimolar concentrations of 3 structural isomers (ortho, meta and para hydroxylations on a phenyl group) do not all ionize equally
- UV response is not as definitive as radiometric detection, but can be more representative of abundance than MS signal (assuming that the metabolite chromophore is not substantially altered relative to the parent)

Isomer	ortho	meta	para
Peak height (MS)	2.4×10^{5}	4.3×10^{5}	2.2×10^{5}
Peak height (UV)	1.1×10^{-2}	1.0×10^{-2}	9.5×10^{-3}

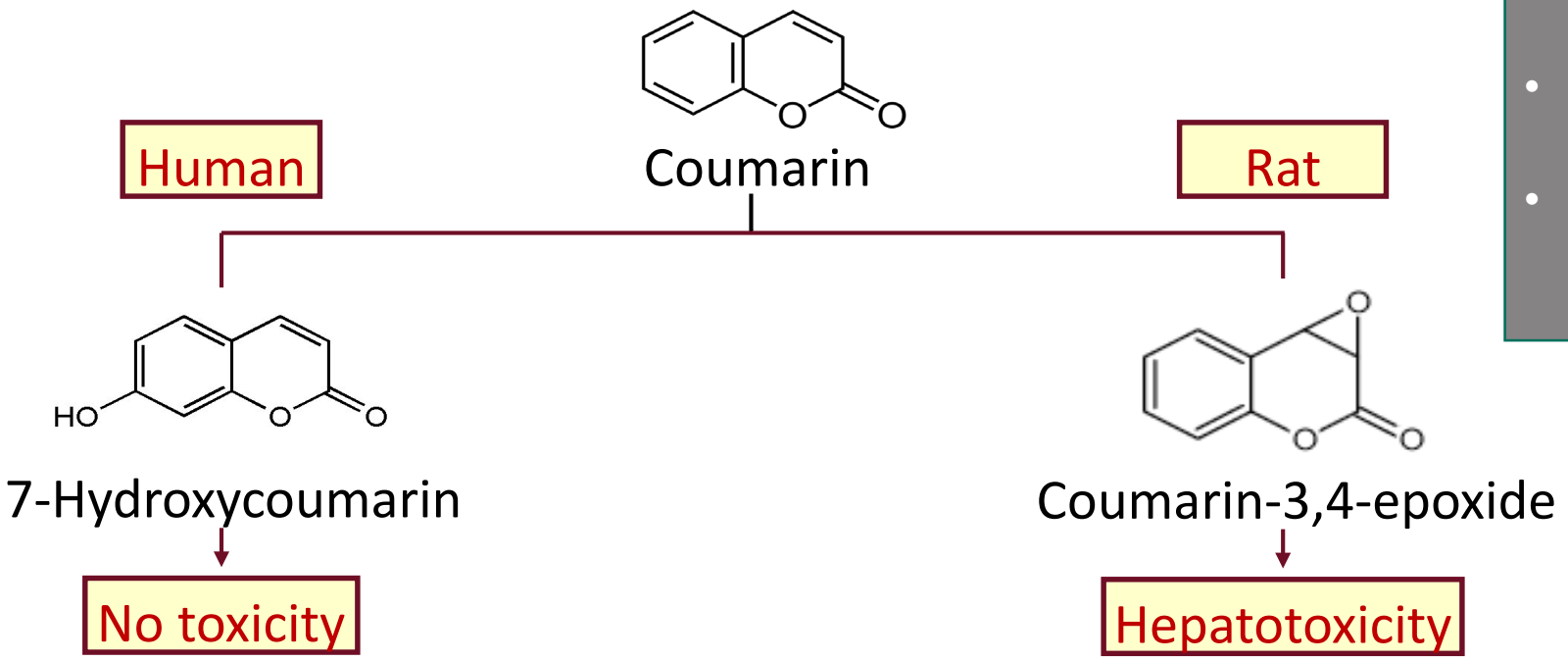


Met ID study endpoints



- How many metabolites were detected in each species.
- Proposed biotransformation that led to their formation.
- If there are any human-specific metabolites.
- If any metabolites were detected at disproportionally higher levels in human than other species.
- UV peak areas for parent and metabolites, as applicable
- Proposed metabolite structures and fragmentation assignments

Drug Metabolism: Inter-Species Comparative Metabolite ID



Goals:

- Complete profile of metabolites
- Are there human specific metabolites?
- Which other species have a similar metabolic profile?

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Cross-Species Met ID

Component	Retention time (min)	Mass shift	Proposed biotransformation	Mouse	Rat	Dog	Pig	Human
C1	3.43	255.9889	Sulfation + glucuronidation	+	+	+	+	+
C2	3.63	354.0783	Di-glucuronidation + hydrogenation	+	+	+	+	+
C3	3.78	159.9135	Di-sulfation	+	+	+	+	+
C4	4.00	258.0045	Sulfation + glucuronidation + hydrogenation	+	+	+	+	+
C5	4.41	161.9298	Di-sulfation + hydrogenation	+	+	+	+	+
C6	4.44	194.0428	Glucuronidation + oxygenation + hydrogenation	ND	ND	ND	+	+

OVERVIEW- Metabolite BA in pre-clinical and clinical

- Bioanalytical metabolite analysis
- Options for bioanalytical metabolite analysis
- What information is helpful for the bioanalytical laboratory
- Case studies from when metabolites have been investigated at various stages throughout a clinical program.

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Metabolites in Bioanalytical Analysis

- The extent of this assessment of metabolites can be dependent on variety of factors:
  - The activity or potential activity of the identified metabolites
  - Available information regarding the drug
  - The type of delivery system used (i.e. if the dosed drug isn't active, more is generally known about the active metabolites)
  - Clinical endpoints for the drug and metabolite
  - Current phase of the program (i.e. later phase program will have more metabolite information available)
  - Metabolic pathway where genomic testing indicates that the drug can be metabolized differently (i.e. fast/slow metabolisers)

Metabolites in Bioanalytical Analysis

- Metabolites are assessed to varying degrees in both pre-clinical and first in human trials.
- This can range from:
  - Monitoring of the metabolite
  - Measurement of the metabolite using a fit for purpose (FFP) assay
  - Measurement of the metabolite using a fully validated assay
- Noting that each metabolite is treated as a unique analyte and while analytical methods may be combined, each analyte is treated individually against the required acceptance criteria

Need to monitor metabolite – choices

- Monitoring of the metabolite
  - No reference material available
  - Metabolite not fully characterized
  - MRM transition included in primary analyte analysis
  - No clinical endpoints
  - No regulatory or safety decisions to be made from data
  - Limited scope and robustness of data
  - Not performed within regulatory guidance

Need to monitor metabolite – choices

- Measurement of the metabolite using a fit for purpose (FFP) assay
  - FDA guidance includes the concept of FFP where the level of validation should be appropriate for the intended purpose of the study
  - Full characterisation of the metabolite to have occurred
  - Reference material available
  - Limited validation is performed fit for the purpose and use of resulting data
  - Only exploratory clinical endpoints
  - Expect no regulatory or safety decisions to be made from data

Need to monitor metabolite – choices

- Measurement of the metabolite using a fully validated assay
  - Fully validated assay to the standards of FDA and EMA guidances
  - Full characterisation of the metabolite has occurred
  - Reference material available
  - Full validation is performed
  - Could be used for clinical endpoints
  - Regulatory or safety decisions could be made from data

Don't need to monitor metabolite

What info should I share with BA lab?

- As much as possible
- Can help to identify potential analytical concerns early in the method development
  - Stability – metabolites can often be unstable and can revert to the parent drug therefore not providing an accurate or reproducible indication of the parent drug in the sample
  - Selectivity or specificity – method development is performed with spiked reference standards and potential interference from metabolites may not be detected until sample analysis commences
  - Both of these issues would require the potential repeat of the development and validation resulting in loss of time and money

Don’t need to monitor metabolite

But when?

- FDA says - As early as possible in the drug development process
- The metabolic pathway can differ between non-clinical species and humans
- Human metabolites that can raise a safety concern are those present at greater than 10 percent of total drug-related exposure at steady state (reference – FDA Safety testing of drug metabolites guidance for industry)
- If the met-ID is performed late in the drug development process and identifies a metabolite that must be assessed, this can result in significant additional time and money from repeat analysis or even repeating of the clinical study

Assess metabolites after starting human trials

- As information evolves throughout a program Phase I to Phase II/III, the level of metabolite analysis may also vary.
- If metabolite analysis is required throughout the clinical program:
  - Develop an assay and determine the clinical collection procedures
  - If no changes to the existing procedures are required, the already collected samples can be re-assayed for the metabolite and stability retrospectively established
  - If changes to the existing procedures are required, the already collected samples may not be able to be assayed and studies may need to be repeated

CASE STUDY -metabolite in pre-clinical studies

- Metabolite identified prior to commencement of pre-clinical studies
- Assay was developed in rat and dog plasma
  - Metabolite was very unstable in dog blood
  - The use of additives and fast processing of the blood were able to stabilize this metabolite.
- With the dog samples, additional met-ID was performed and identified another metabolite
  - This metabolite was incorporated into the human assay and required a custom blood collection tube in order to stabilize all analytes

CASE STUDY -metabolite analysis of already collected samples

- In the late stages of Phase II/III trials, it was determined that a metabolite was required to be measured
- An assay was developed, the analytes were stable and samples from the Phase I trial were re-assayed for the metabolite
- Retrospective stability was required to be established for up to 3 years to support the metabolite data

CASE STUDY -metabolite analysis during the FIH trial

- Metabolite identified early in the human FIH
- Assay updated to include the metabolite, validated and analysis continued throughout the trial

Question and Answers

GET IN TOUCH

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