XENOTECH

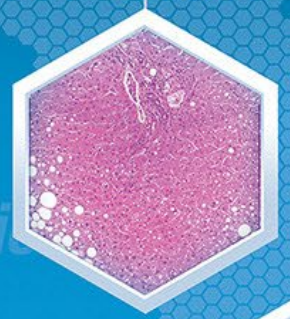
A BioIVT Company

25 Years

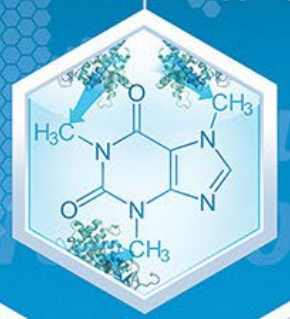
ADME | DRUG-DRUG INTERACTION | DMPK

CONTRACT RESEARCH & TEST SYSTEM EXPERTISE

Cell & Tissue-Based Products

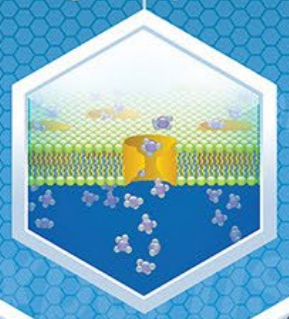


Reaction Phenotyping

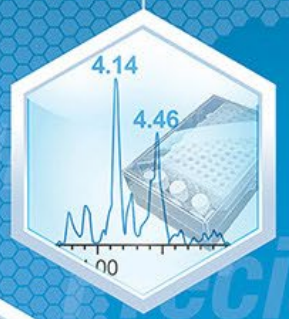


Enzyme Induction & Inhibition

Drug Transporters



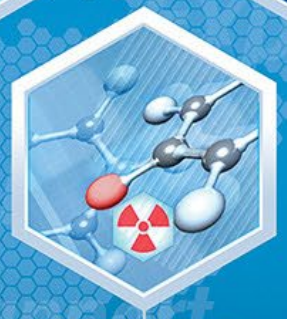
Metabolite ID & Production



Screening

Pharmacokinetics & QWBA

Radiolabeling



Bioanalytical

FAQ: Metabolite Characterization Studies

Mark Horrigan
Principal Scientist, Program Oversight
XenoTech

Overview

- Met ID in Drug Development
- Definitions
- Regulatory expectations
- In vitro test systems
- In vivo studies
- Radiolabeled vs cold test article
- Quantitative information
- Interpretation of results

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?

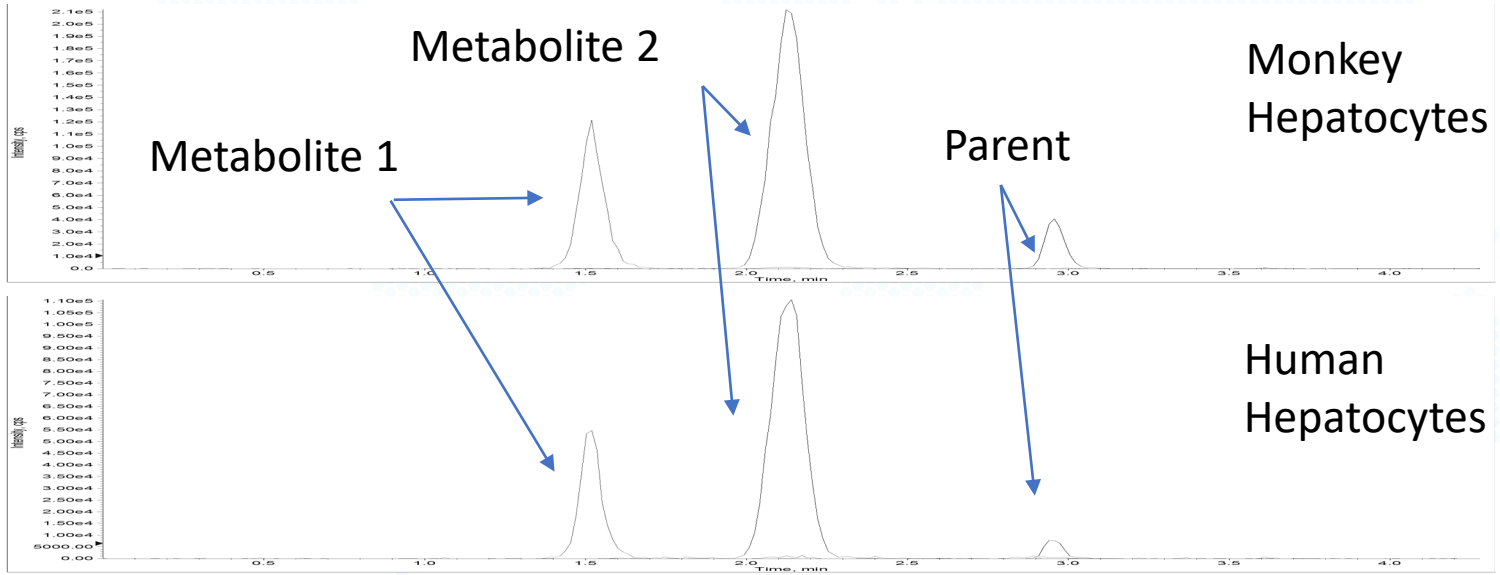
Are they human-specific or disproportionally higher in human than any of the toxicity species evaluated?

  - 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?

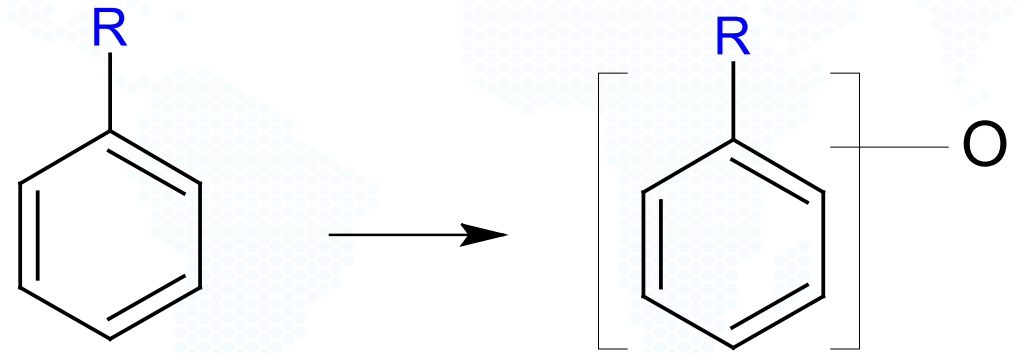
A: Profiling - how many metabolites are formed in each species/test system



Definitions

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

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



mw = X

mw = X + 15.9949 amu

Definitions

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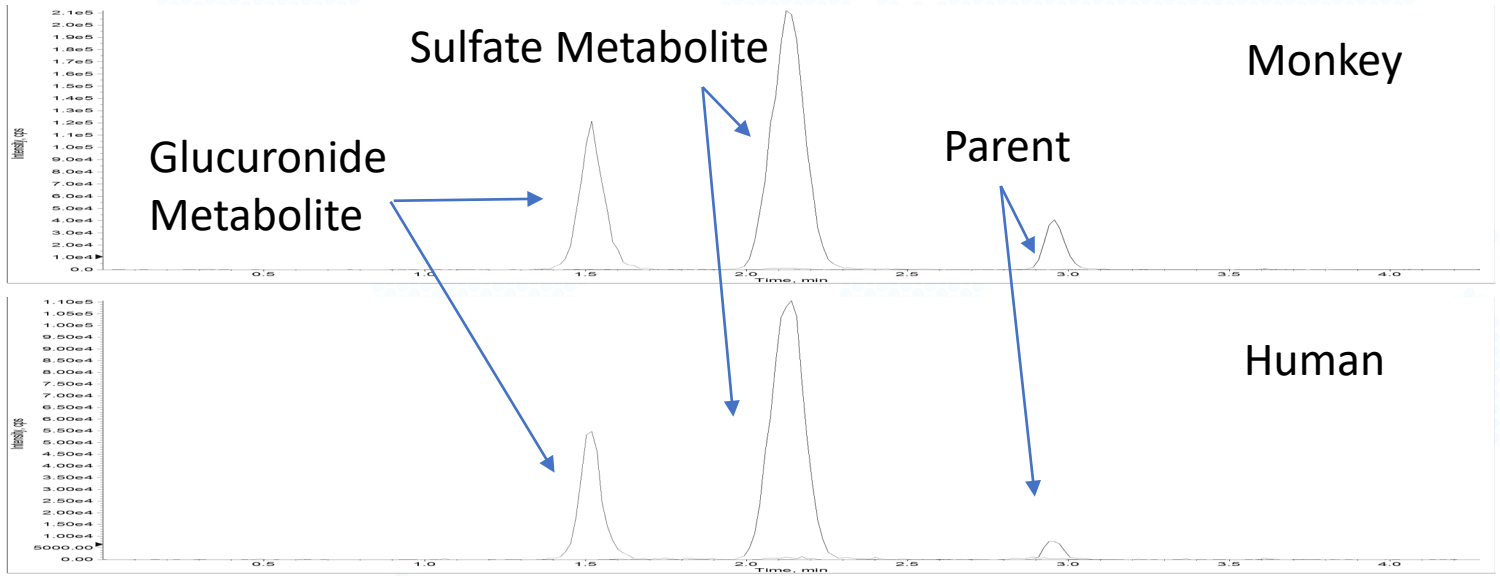


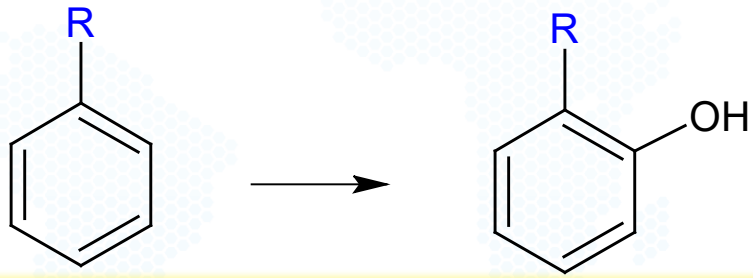
Definitions

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.

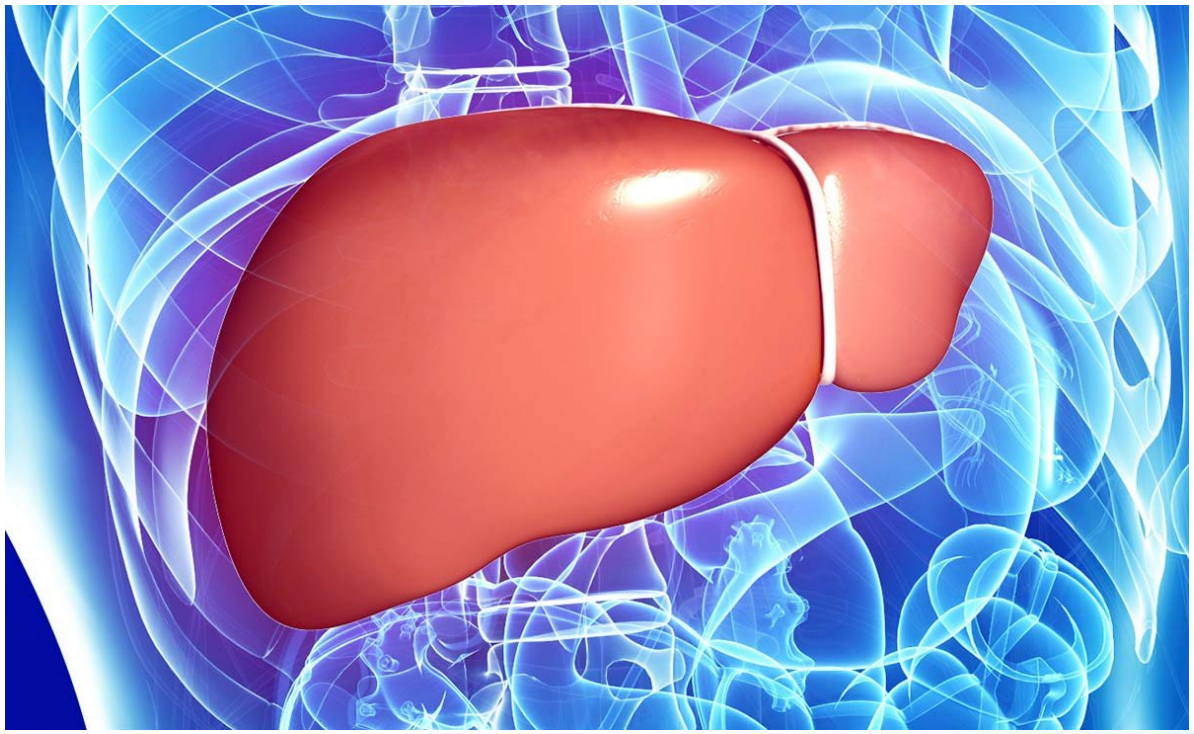
Q: Can my metabolite characterization study be performed as a GLP compliant study?

A: No

- GLP (Good Laboratory Practice) compliance is not required or applicable for in vitro drug metabolism studies.
- XT 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).

Q: Which in vitro test system is best for my metabolite characterization study?

A: 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

Q: Can XT perform metabolite characterization of in vivo samples?

A: Yes

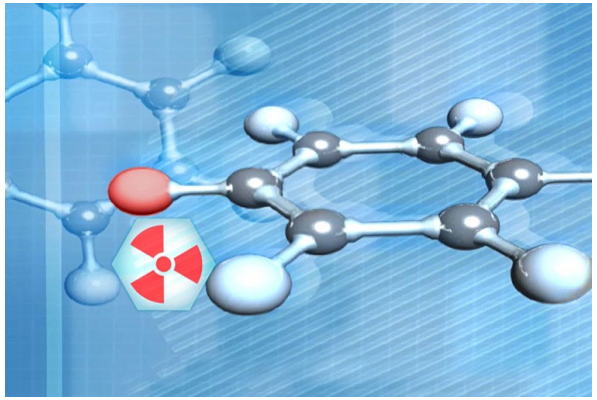
We routinely perform studies with in vivo samples from preclinical and clinical studies using cold or radiolabeled test articles

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

Q: Will I get quantitative information about the metabolites detected in my study?

A: No

- 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



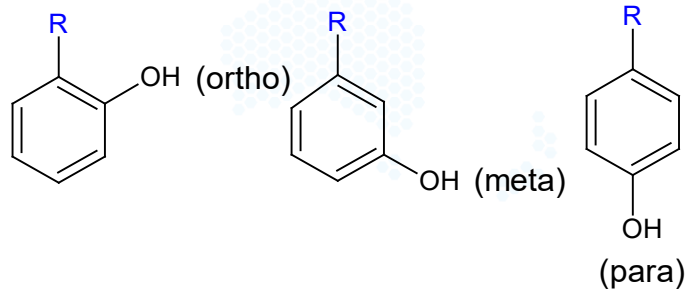
Q: Will I get quantitative information about the metabolites detected in my study?

A: No

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)

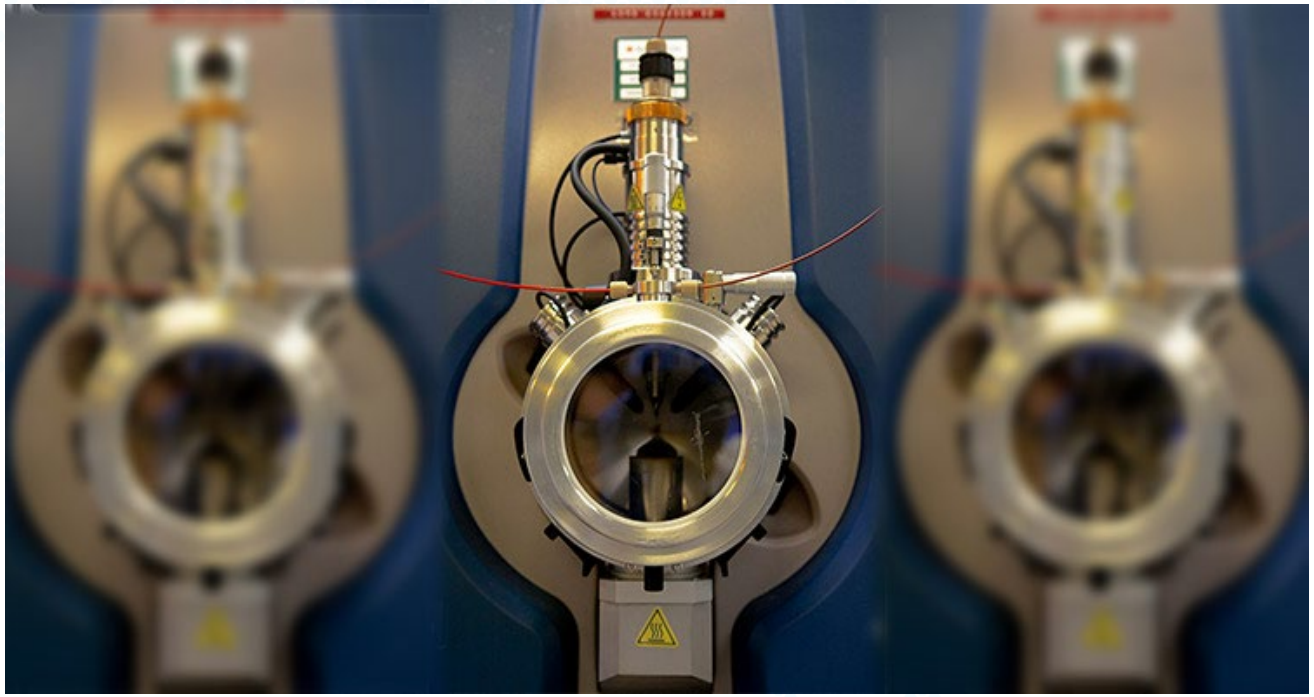
Isomer	ortho	meta	para
Peak height (MS)	2.4×10^5	4.3×10^5	2.2×10^5
Peak height (UV)	1.1×10^{-2}	1.0×10^{-2}	9.5×10^{-3}



Q: My test article isn't radiolabeled. Can I still run a metabolite characterization study?

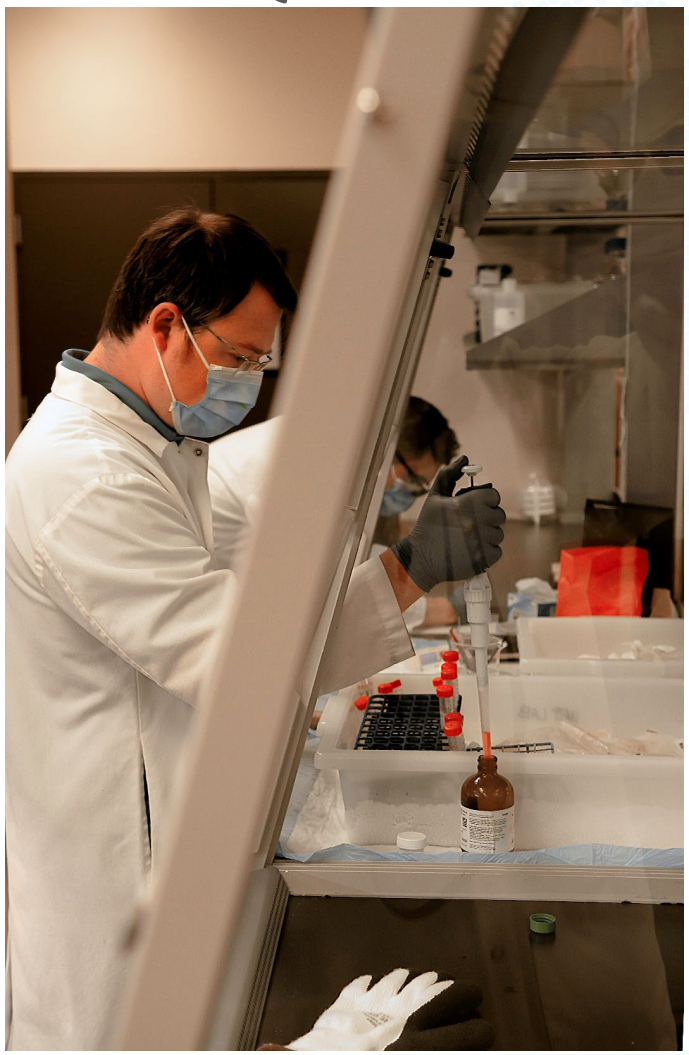
A: Yes

- The majority of metabolite characterization studies at XT are performed with unlabeled test articles (< 1000 mw)
- Sample analysis is performed using LC-MS/MS techniques with in-line UV detection



Please see our website for details regarding our analytical capabilities

Q: What can be concluded from my study?



- How many metabolites were detected in each species.
- Proposed biotransformations 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

Q: What XT needs from you prior to a metabolite characterization study?

- The test article structure
- Any known or suspected metabolite reference standards
- Any relevant in-vitro data (e.g., metabolic stability, reaction phenotyping)
- Analytical method, if available (LC-MS/MS)
- If it is an in vivo study, please provide the PK data so we may select appropriate time points for pooling

Thank you for watching!

Please contact your regional account manager if you are interested in a placing a Metabolite Characterization study with us

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- Protein Binding
- Metabolite Identification
- ADME Screening
- Toxicology

In Vivo ADME/PK & Distribution
- QWBA
- Microautoradiography
- Excretion / Mass Balance
- Tissue Distribution
- Blood / Plasma & Lymphatic Partition Rate

Bioanalytical

Pharmacology
- In Vitro Ligand Binding & Radioreceptor Assays
- Immunoassays

Chemical Synthesis
- Radiolabeled Synthesis
- Metabolite Synthesis
- Peptide Synthesis

Consulting...

Cellular Products
- Hepatocytes (Cryo/Fresh, Genotyped...)
- Non-Parenchymal Cells (Kupffer Cells)

Subcellular Fractions
- Liver Microsomes
- S9 Fractions
- Cytosol
- Homogenate
- Lysosomes & Tritosomes
- Mitochondria
- Extrahepatic Fractions

Custom Products
- Various Species, Tissues & Preparations

Research Biobank
- Normal & Diseased Tissue Samples

Recombinant Enzymes

Substrates & Metabolites

Metabolite Production Kits

JCRB Cell Lines...