

SIMPLE AND COST-EFFECTIVE COMPARISON OF ANIMAL AND HUMAN METABOLITE PROFILES BY MATRIX MATCH METHOD

Introduction

The purpose of metabolite profiling is to find all drug metabolites in humans, and to assess whether these metabolites were present in animal species that were used for the toxicological risk assessment of the drug. Discovery of disproportionate human metabolites or metabolites that are human-unique requires additional nonclinical toxicology studies. This can be burdensome and costly and causes delays in clinical development.

The Matrix match Method (MmM) is a simple and cost-effective bioanalytical approach to evaluate whether nonclinical toxicology studies adequately cover human metabolites. Combining MmM with AMS radiolabeled studies enables quantitative assessment of metabolite coverage in one go. This saves time and money and does not require method transfers.

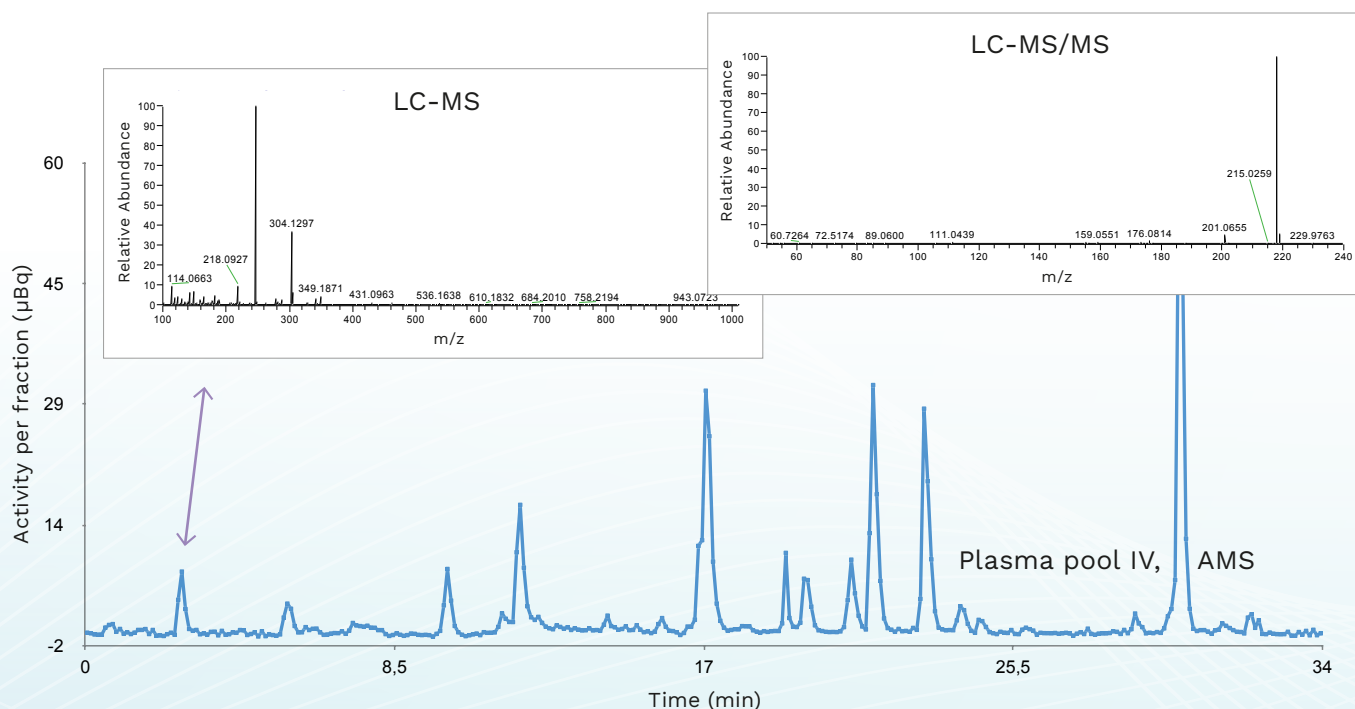
Metabolite profiling is a regulatory requirement

Metabolites identified only in human plasma, or metabolites present at higher levels in humans than in the animal test species should be considered for safety assessment. Such metabolites are called *disproportionate drug metabolites*. Human metabolites that can raise a safety concern are those present at > 10% of total drug-related exposure at steady state.^a

The earlier the better

The MIST guidance encourages identifying differences in drug metabolism between animals and humans - as early as possible - during the drug development process.

Early metabolite profiling is enabled by microtracer radiolabeling of drug products. Microtracer studies only use 1 μCi of radiation, which is classified as safety Class I.^b Microtracer studies can therefore be executed from Phase 1.



Metabolite profiling studies generate LC-MS/(MS) data, which can be matched with quantitative early-stage AMS results on metabolite abundance. The AMS results support the selection of unexpected, unique, or abundant metabolites for in-depth analyses and MIST related decision making.

ACCELERATE. DE-RISK. REDUCE COSTS.

EARLY ASSESSMENT OF METABOLITE COVERAGE IN TOXICOLOGICAL SPECIES PREVENTS LATE-STAGE SURPRISES AND DELAYS

Matrix match Method is simple and quick

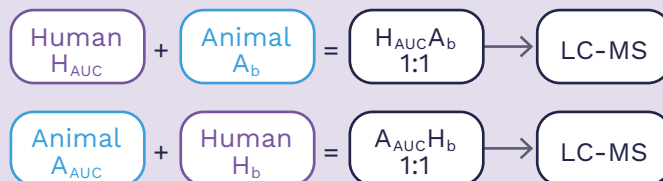
- Plasma is collected after drug exposure in human (H) and animal (A) studies.
- Pooled plasma of one species; H_{AUC} or A_{AUC} , is mixed 1:1 (v/v) with blank plasma ($_b$) from the other species.
- This is done both ways, generating two samples for LC-MS: $H_{AUC}A_b$ and $A_{AUC}H_b$.
- Mixing of the samples 1:1 takes away the matrix effects of the species-specific plasma samples.
- If the $H_{AUC}A_b$ mixture shows additional metabolites or much higher peaks for specific metabolites, further investigations are required, for instance, animal tox studies for human unique metabolites.

Combining LC-MS with AMS for targeted deep-dives

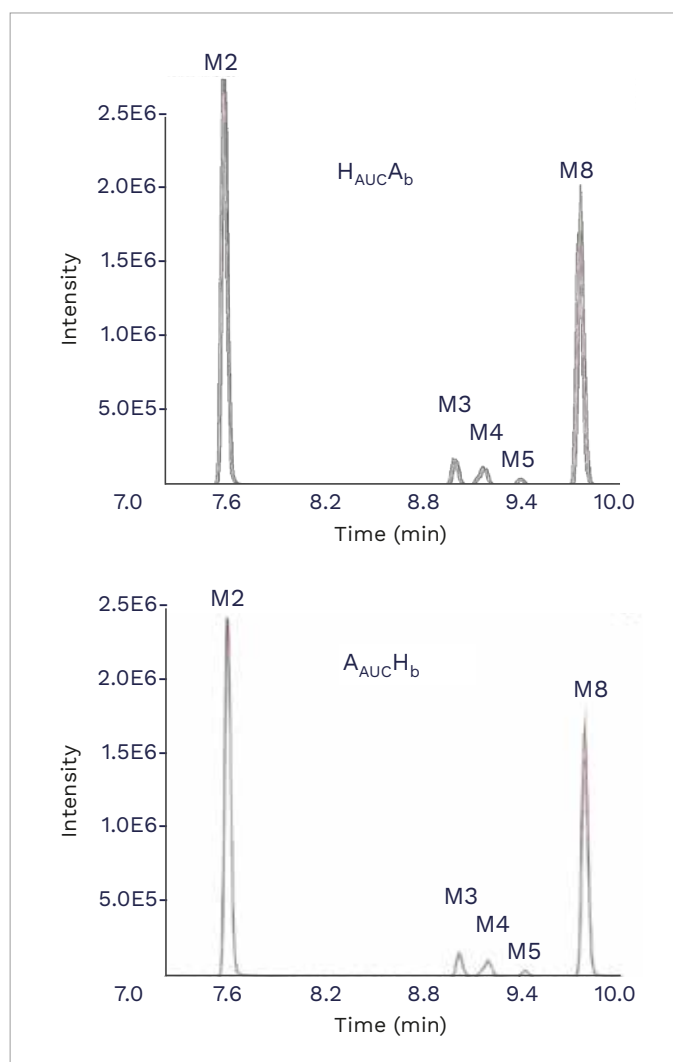
- LC-MS/MS identifies unexpected and human unique metabolites that are not always apparent in preclinical studies.
- Abundant human metabolites are shown in the AMS profiles.
- Combining LC-MS and AMS data enables data-driven selection of human relevant metabolites for further analyses. Or, more importantly, provides waivers for further toxicological assessment.

Early data for timely risk mitigation

- The sensitivity of AMS technology enables microtracer studies.
- Microtracer studies are considered ethical from Phase 1.
- Early-stage identification of disproportionate drug metabolites allows for timely de-risking and clarifies whether additional toxicological assessments are required or can be waived.



MmM workflow



Example of replicates of LC-MS chromatograms for plasma mixtures in the MmM.

^a MIST guidelines apply to small molecule drugs under FDA Safety Testing of Drug Metabolites Guidance and ICH M3(R2)

^b Radiological Protection in Biomedical Research, ICRP Publication 62

