AMS: A smarter way to cut through the MIST

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RMI-Pharmacokinetics

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Objectives

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- Brief overview of Accelerator Mass Spectrometry (AMS) and its history
- Reminder of the MIST guidelines and their impact:
  - ☞ The traditional program
  - ☞ An accelerated program using AMS (Microtracing)
- Examine further ways AMS can accelerate early drug development programs:
  - ☞ Determination of clearance and design of dosage regimens
  - ☞ Determination of absolute bioavailability
  - ☞ Microdosing

What is Accelerator Mass Spectrometry (AMS)?

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- Pioneered by Muller in the 1970s and 1980s for the direct detection of ^{14}C and other long-lived radioisotopes.
- Initial focus was for accurate radiocarbon dating using small sample sizes
  - ☞ Extremely high sensitivity and accuracy required
- Biomedical applications initially explored by Vogel and coworkers at the University of California
- Biomedical applications further developed by scientists in the US and Europe with three main centers in the US:
  - ☞ Vitalea Science, Davis, CA
  - ☞ Xceleron, Germantown,MD
  - ☞ Accium, Seattle, WA
- AMS is a form of isotope ratio mass spectrometry determining the ratio of the trace isotope (^{14}C) to total carbon (C) at the parts per quadrillion level.
- No structural information is obtained with AMS alone, therefore used for the detection and quantitation of trace radioisotopes.
- Extremely high sensitivity: 4 to 5 orders of magnitude greater than liquid scintillation counting (sub-attomole (amol) region).

AMS: The Instrument

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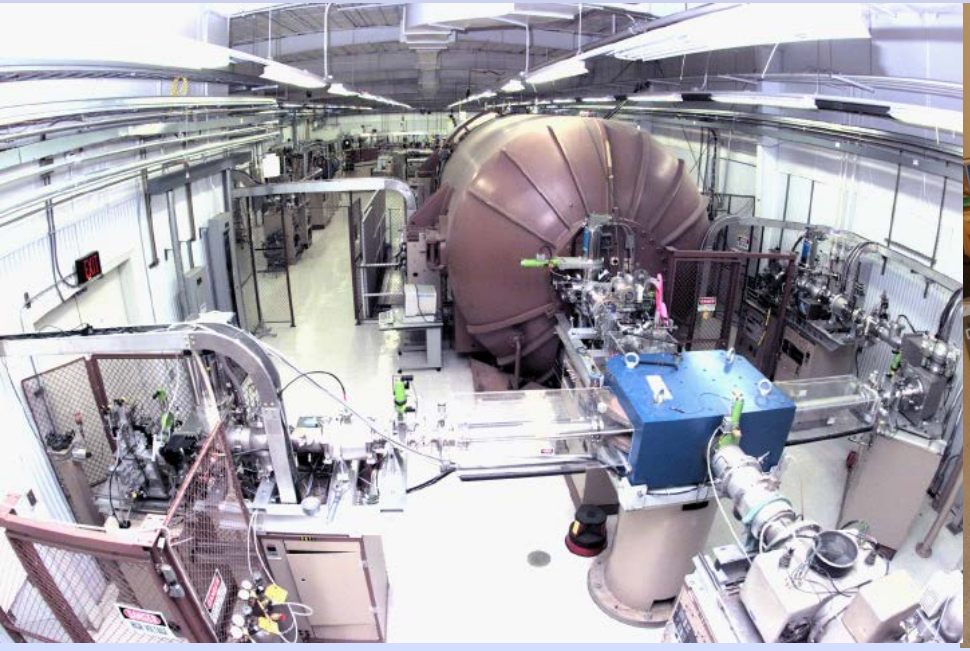
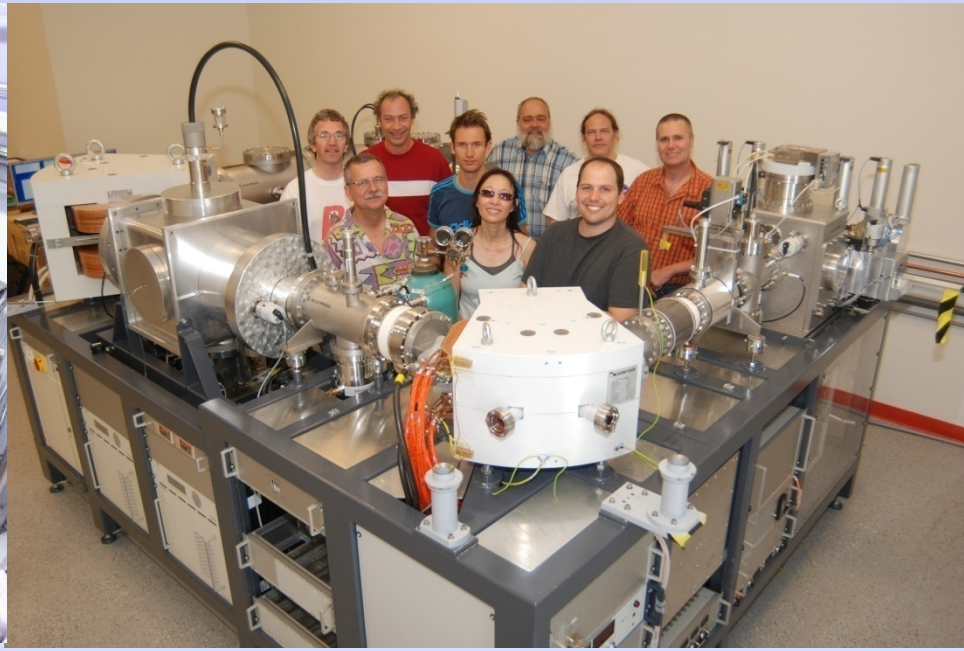


10 MV AMS at Lawrence Livermore National Laboratory



200KeV BioMICADAS AMS at Vitalea

Courtesy of Vitalea Science

AMS: The Instrument

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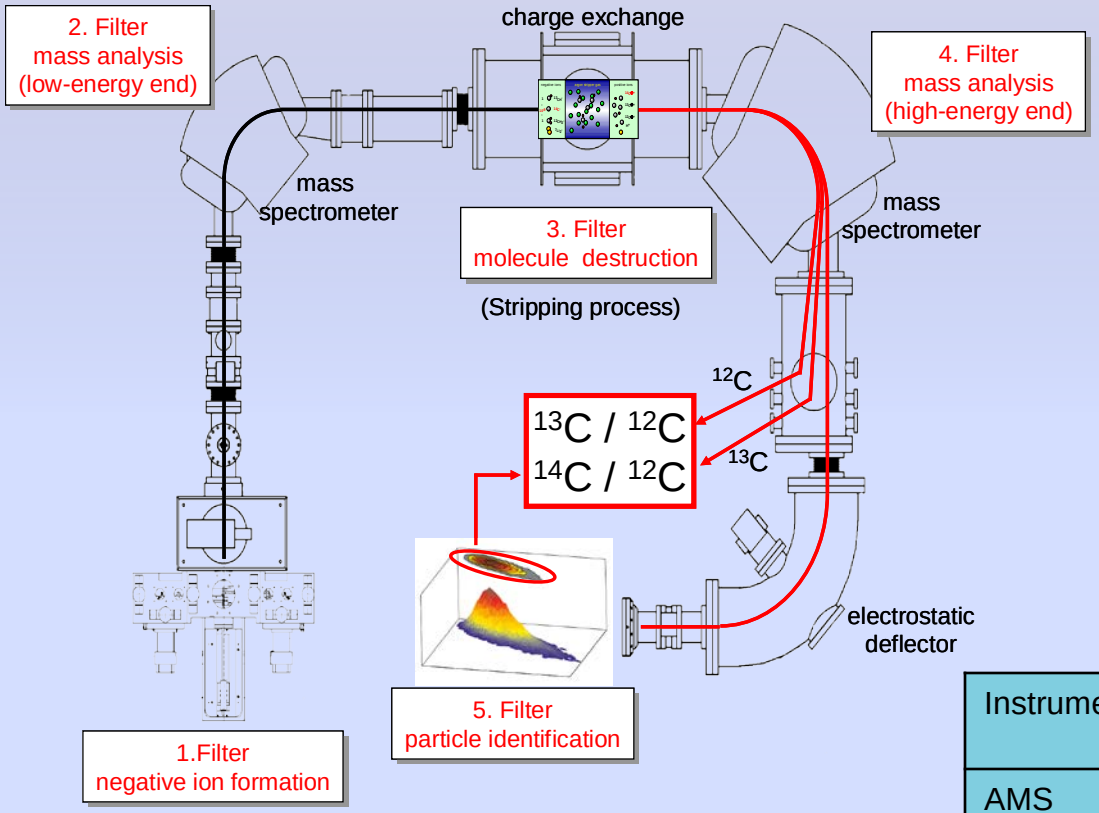

- Ions spluttered into AMS by focused beam of cesium ions
- 1st Magnet bent by 90° to select mass (12, 13 & 14 amu)
- Enter acceleration chamber with high +ve voltage in center
- Negative carbon atoms stripped of valence electrons and become +ve
- Positive atoms repelled from accelerator at speeds a few % lower than that of light
- Pass through an analyzing and switching magnet to select mass of interest
- ^{12}C and ^{13}C measured off axis in Faraday cups
- ^{14}C passes into an electrostatic analyzer and counter using gas ionization or a particle detector

Instrument	DPM at LLOQ	Mole Quantity at LLOQ
AMS	0.001-0.005	10-15 amol
TOP COUNT	2-5	16-40 fmol
Off-line LSC	5-50	40-400 fmol

BioMICADAS™ accelerator Mass Spectrometer (*courtesy of Vitalea Science*)

Excretion: Urine and feces and expired air

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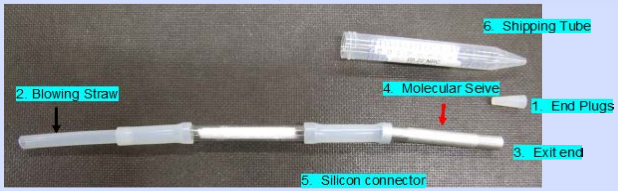
Feces is prepared by slurry formation in cans at 1:1 ratio with solvent

+

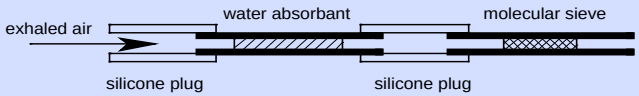
Urine is analyzed after carbon dilution using validated protocols

+

Expired Air captured using Vitalea device



	Mass	Carbon in 24 hr void	LLOQ dpm/g	LLOQ %administered dose
Urine	1.5 g	10	0.01	0.001
Feces	500 g	25	0.07	0.003



Balance = >99%

AMS: Sample Preparation Overview

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- AMS measures total ^{14}C so parent drug and metabolites need to be separated by HPLC/UPLC prior to sample preparation and analysis if specific quantitation required
- Samples have to be converted to graphite to be introduced into the AMS:
  - ☞ Carrier carbon added followed by oxidation with copper oxide at ~600-900^0
  - ☞ Then reduced to a fullerene at 500^{o}C using zinc, titanium oxide and cobalt
  - ☞ Fullerene compressed into small cathode for loading into AMS
  - ☞ Fullerene very stable over several years
- Process is highly reproducible
- Approximately 150 samples can be prepared daily

MIST Guidelines

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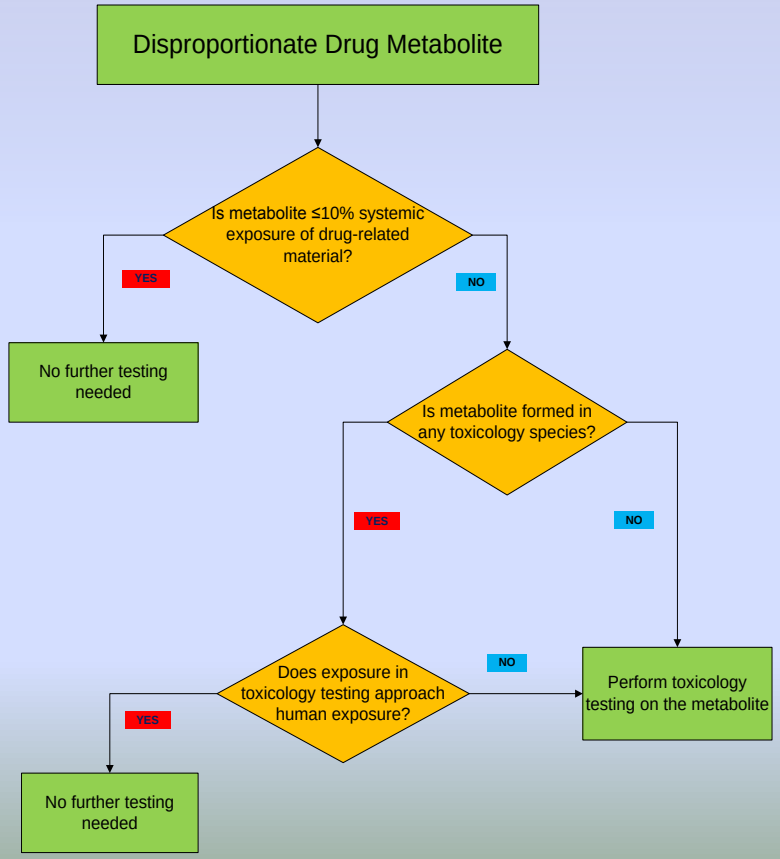
- PhRMA "White Paper" published 2002 (Baillie *et al,* Toxicol Appl Pharmacol, **182** 188-196, 2002)
- FDA Guidelines on safety testing of metabolites, published 2008
- FDA Guidelines M3(2) Nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals, published 2010

FDA MIST DECISION TREE



Consequences of MIST

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- Toxicokinetics need to be established for each metabolite with a human exposure >10% drug-related material
- Identity of human metabolites needs to be established much earlier than previously
- The clinical exposure (C_{max} and AUC) to the metabolites needs to be established as early as possible so that if needed, they can be synthesized, bioanalytical methods developed and validated prior to pivotal toxicology study
- Ideally should be completed at a stage where toxicology species can be changed or toxicology performed on metabolite if there is inadequate exposure with current species

Traditional methods for human metabolite identification

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- *In vitro* incubations and LC-MS/MS identification
  - ☞ Advantage: quick and can be completed early since it does not require human dosing
  - ☞ Disadvantage: may give indication on extent of formation of metabolism but gives no information on the elimination of metabolite; both are necessary to determine exposure
- LC-MS/MS "fishing" for metabolites using human samples from FIH study
  - ☞ Advantage: can be performed early in development
  - ☞ Disadvantage: difficult to obtain a mass-balance and ensure all major metabolites are accounted for
- Radioactive study in human
  - ☞ Advantage: Full excretion-balance obtained and all drug-related materials are accounted for. Meets MIST requirements
  - ☞ Disadvantage: Lengthy enabling studies required
    - ⇨ Radiosynthesis
    - ⇨ Plasma total radioactivity profiles in two (toxicology) species
    - ⇨ Excretion-balance studies in two species

    } Usually also incorporating metabolite i.d

    - ⇨ Tissue distribution studies (e.g. QWBA) in rodent species for dosimetry
    - ⇨ Dosimetry calculations and regulatory approval

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WE NEED A METHOD THAT INCLUDES THE ADVANTAGES OF EACH OF THE TRADITIONAL METHODS WITHOUT THEIR DISADVANTAGES:

- Can be performed early in drug development so that it can influence TK studies
- Provides a mass-balance so that all drug-related material is accounted for

Alternative Approach

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- Isotopically label the compound with a long-lived isotope (^{14}C) so that all drug-related compound can be quantitatively measured
- Use a radioactive level so low that it falls within normal background radiation levels (Microtracing)
  - ☞ No dosimetry or regulatory approval needed
    - ⇨ ≤1µCi for US, Belgium & Switzerland
    - ⇨ ≤0.27µCi UK
  - ☞ Preclinical radioactive enabling studies, therefore, unnecessary
- Use a highly sensitive, accurate and precise bioanalytical method to determine the radioactivity
  - ☞ AMS
- Perform study as early as possible e.g. FIH

Microtracing Method

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- If possible, use a dose level at or about the anticipated clinical dose
- Spike the non-labeled dose with ^{14}C-labeled material at a radioactive level permitted by the country where the clinical phase is undertaken
  - ☞ Ensure dose is homogenous
  - ☞ GMP
- Collect urine, feces and plasma samples as per a normal radiolabeled study
- Measure total radioactivity in urine and feces using AMS to obtain a mass-balance and undertake metabolism identification as described below
- Determine levels of total radioactivity in plasma samples using AMS and compare with parent drug levels determined by AMS or traditional methods e.g. LC-MS/MS
- If the difference in exposure (C_{max} and/or AUC) >10%, perform chromatographic separation (HPLC or UPLC) with fractionation of drug-related material, quantitating each metabolite using AMS
  - ☞ Reference standards not needed since AMS is a direct measure of ^{14}C
  - ☞ Check the mass-balance of radioactivity at different stages of the process to correct for losses
  - ☞ Internal standard can also be used to correct for losses in separation process
- Identify those metabolites representing >10% of parent using conventional LC-MS/MS

Human Mass Balance of Ixabepilone

(Beumer *et al*, Invest. New Drugs, 2007)

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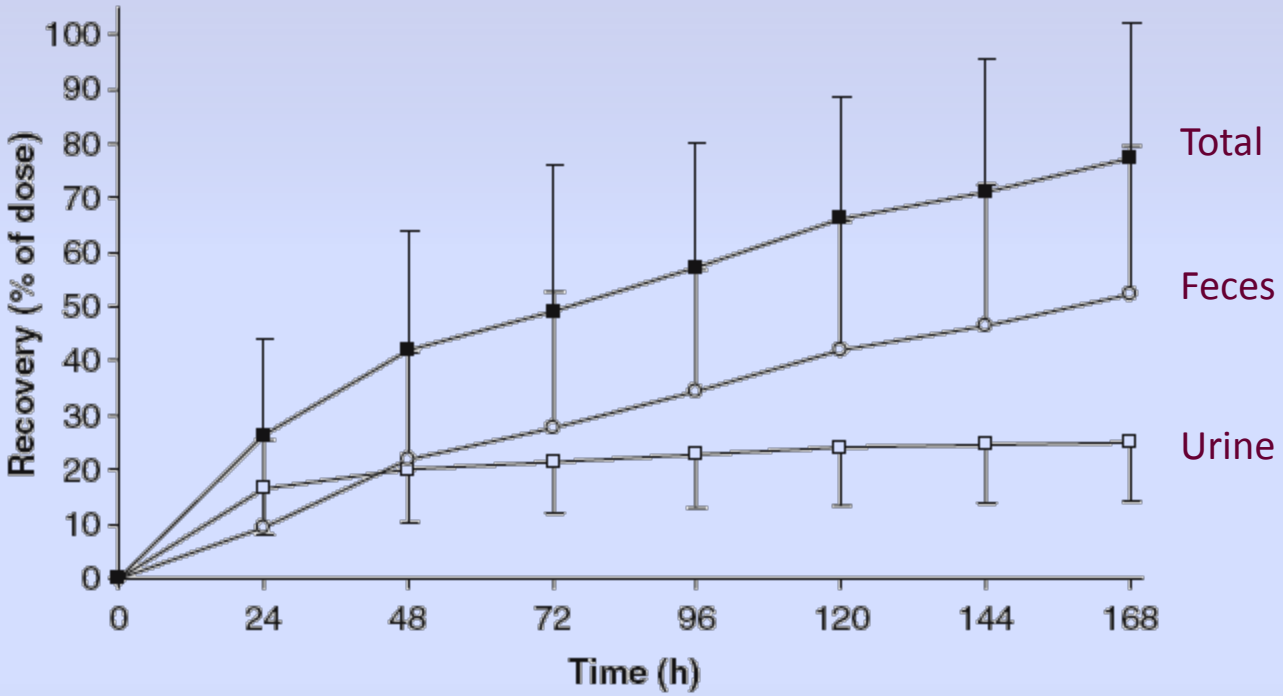

AMS performed at Xceleron

UPLC/AMS Metabolite Profiling of Excreta & Plasma

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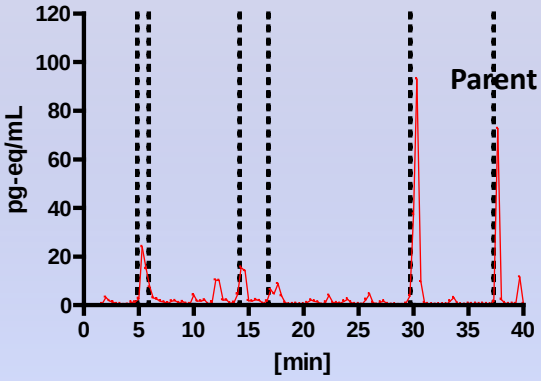
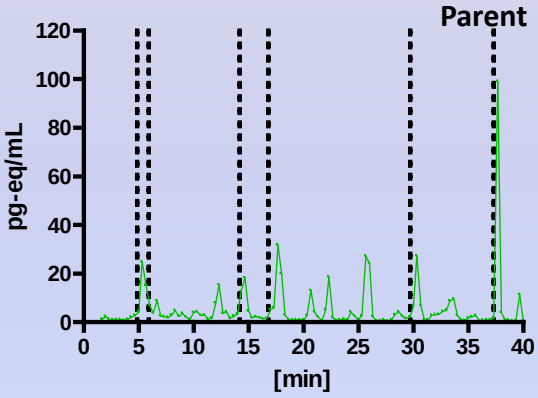
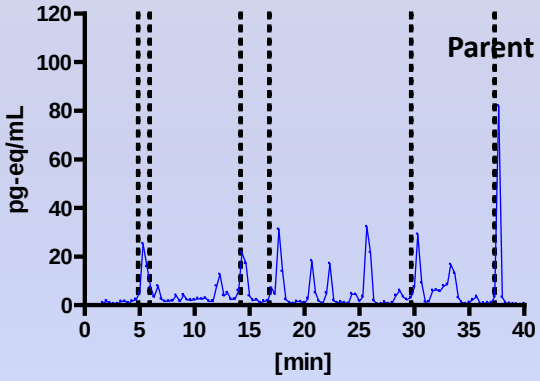


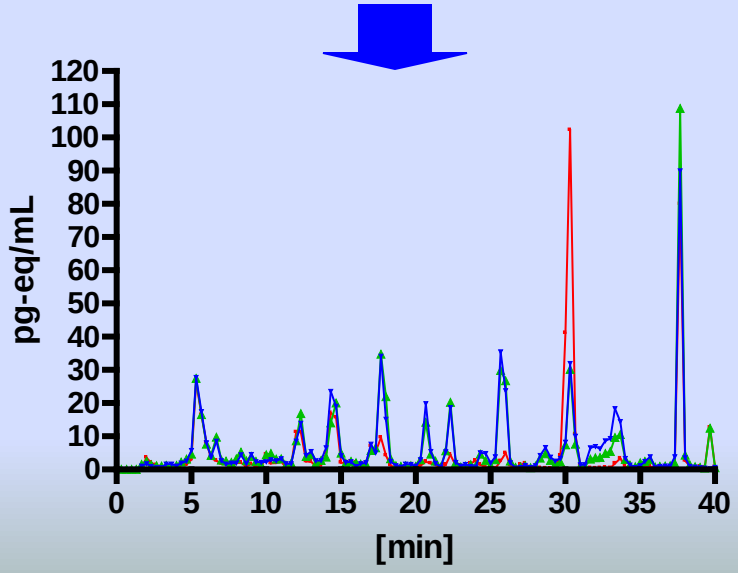
Plasma



Urine



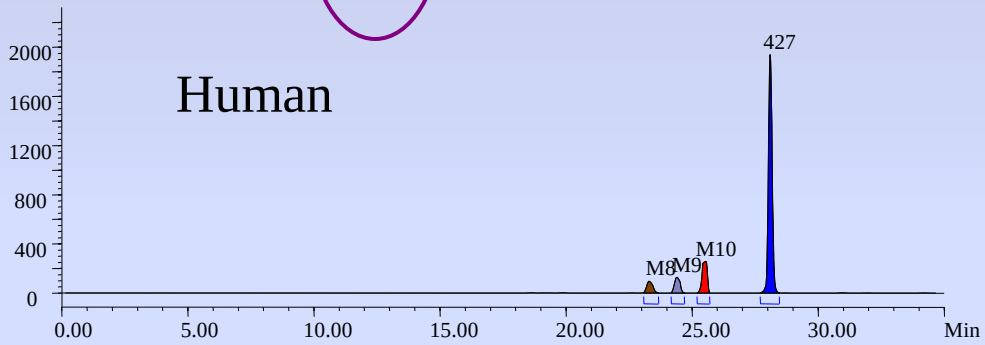
Deconjugated Urine

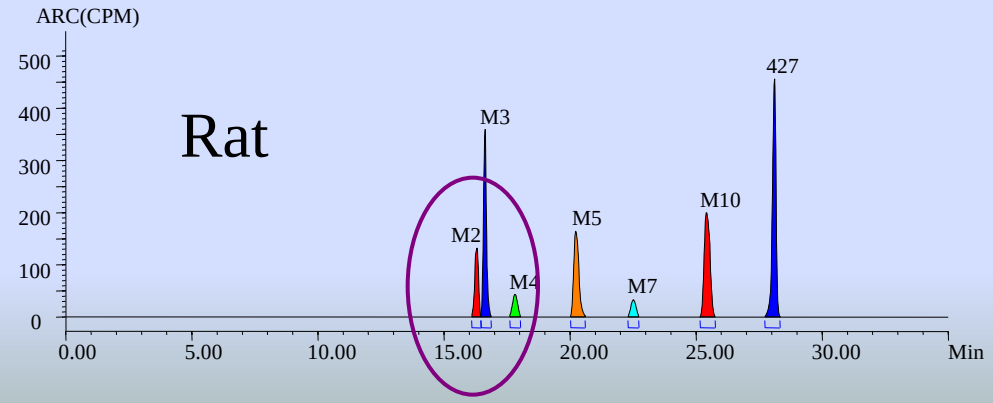


Courtesy Vitalea Science

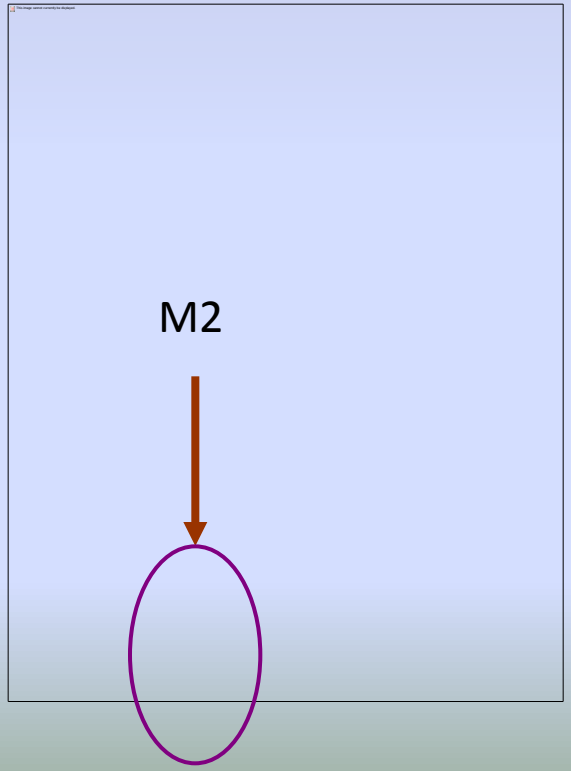
Metabolism: Species Differences – M2 not predicted from in vitro experiments

Species	% Radioactivity											
	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	621427	Total
Rat		10.8	20.2	1.9	14.1		1.5			17.5	33.1	99.1
Dog		0.7				3.8		4.9	17.6	1.5	69.7	98.3
Monkey						2.1		3.5	10.6	22.1	60.4	98.6
Human								3.2	4.5	10.4	81.9	100





M2 major metabolite in vivo/human



Courtesy Vitalea Science

Initial Assessment of Metabolite burden to Comply with MIST Guidelines

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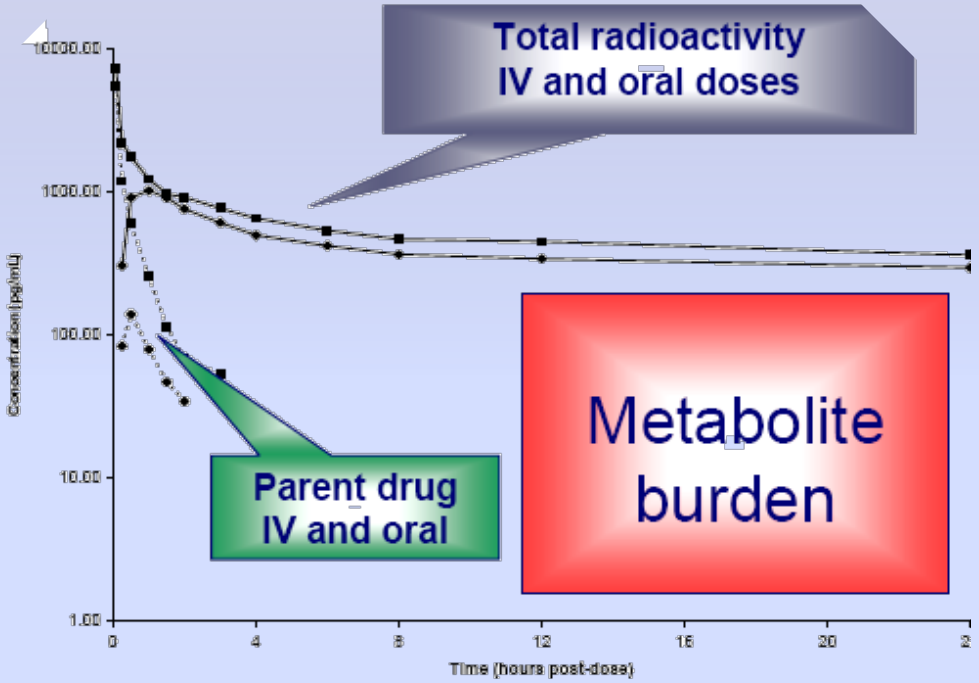

Courtesy of Vitalea Science

Metabolite profiling of plasma using AMS

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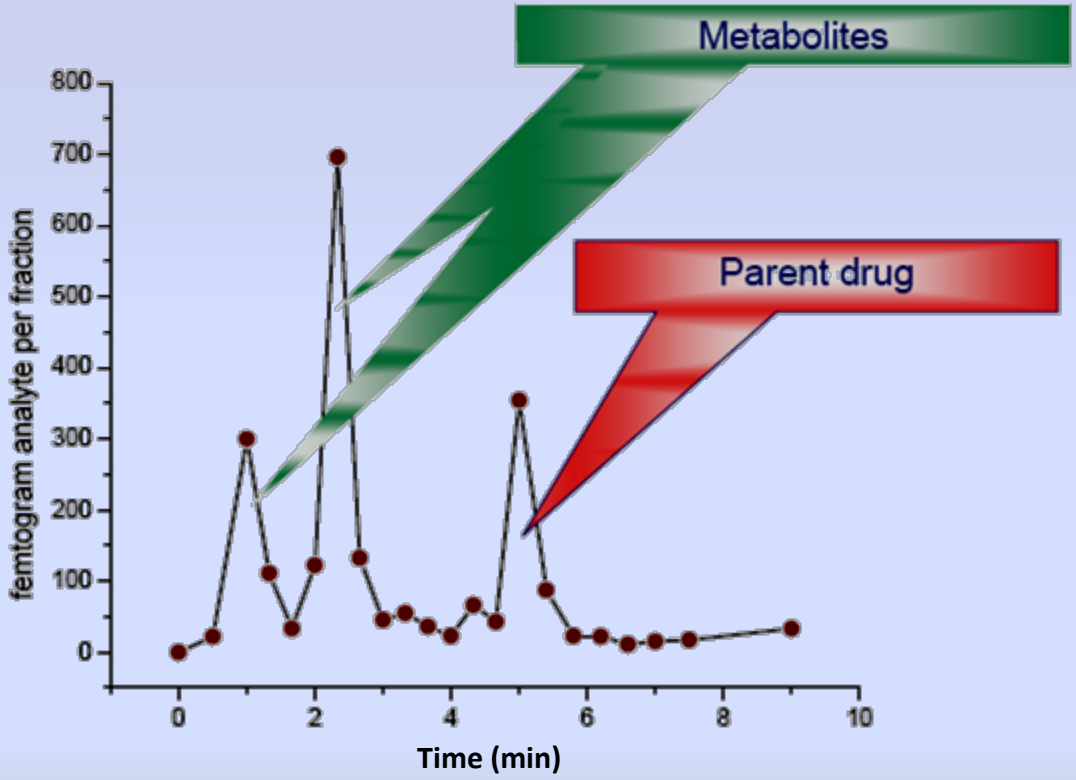
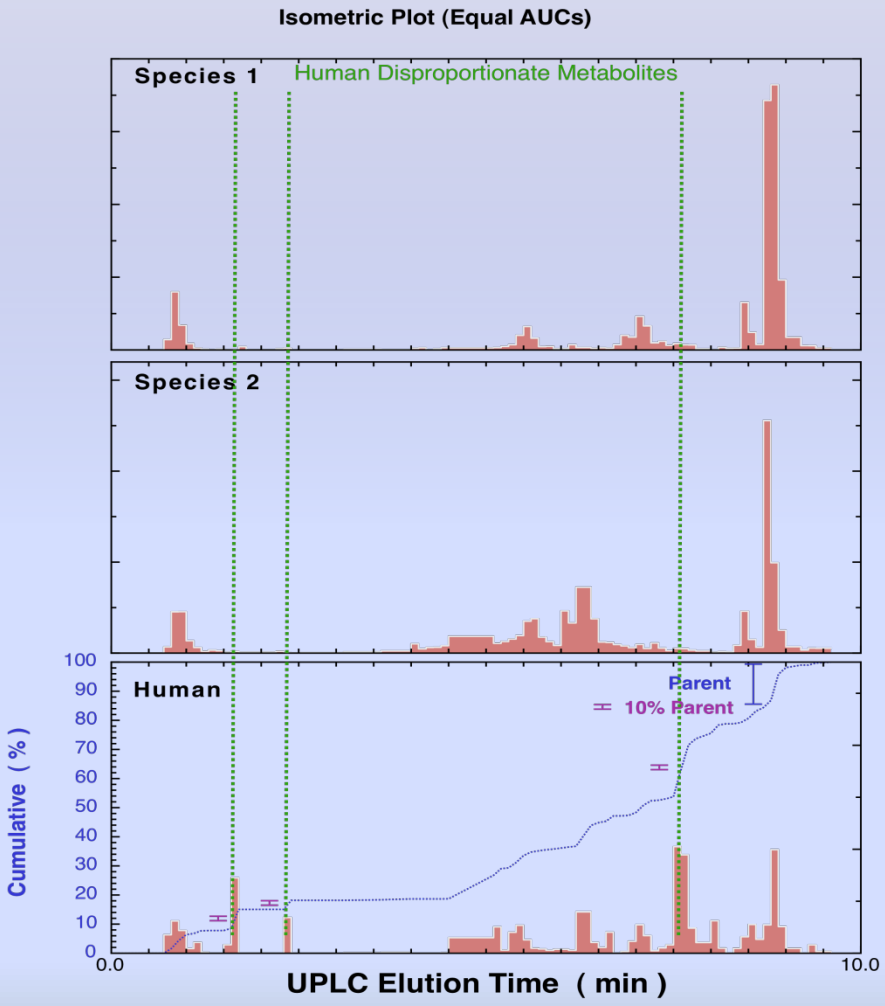


Courtesy of Vitalea Science

Metabolism: Species profiles reveal human disproportionate metabolites



- Disproportionate metabolites (DM) readily quantified (green dotted line)
- 10,000:1 linear AMS range reveals DM in animals

Courtesy of Vitalea Science

MY HOBBYHORSE

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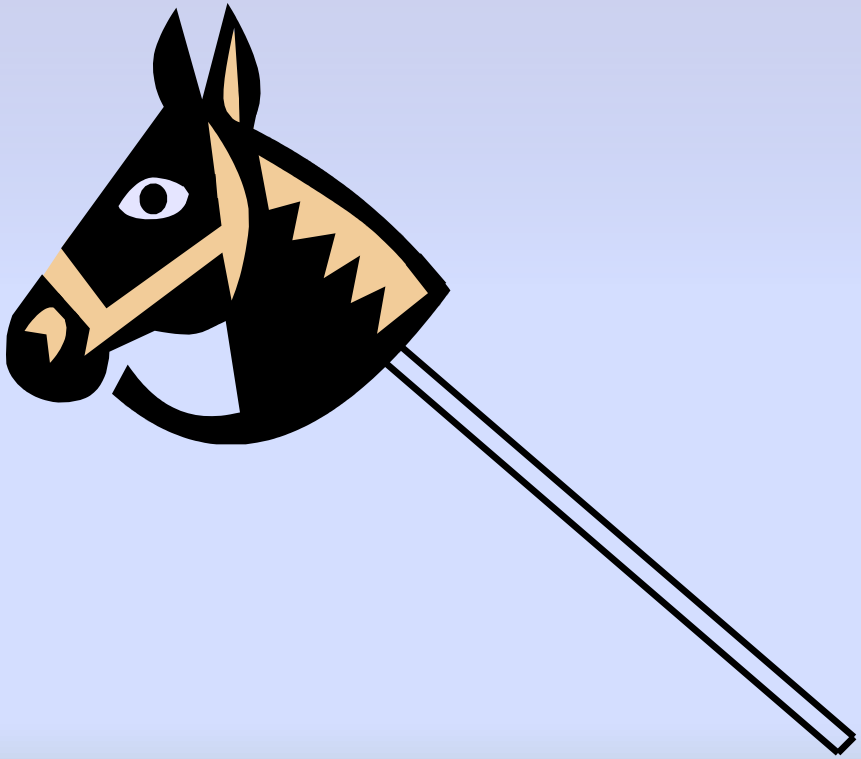

Dosage regimen design: Single dose showing the concept of a therapeutic window

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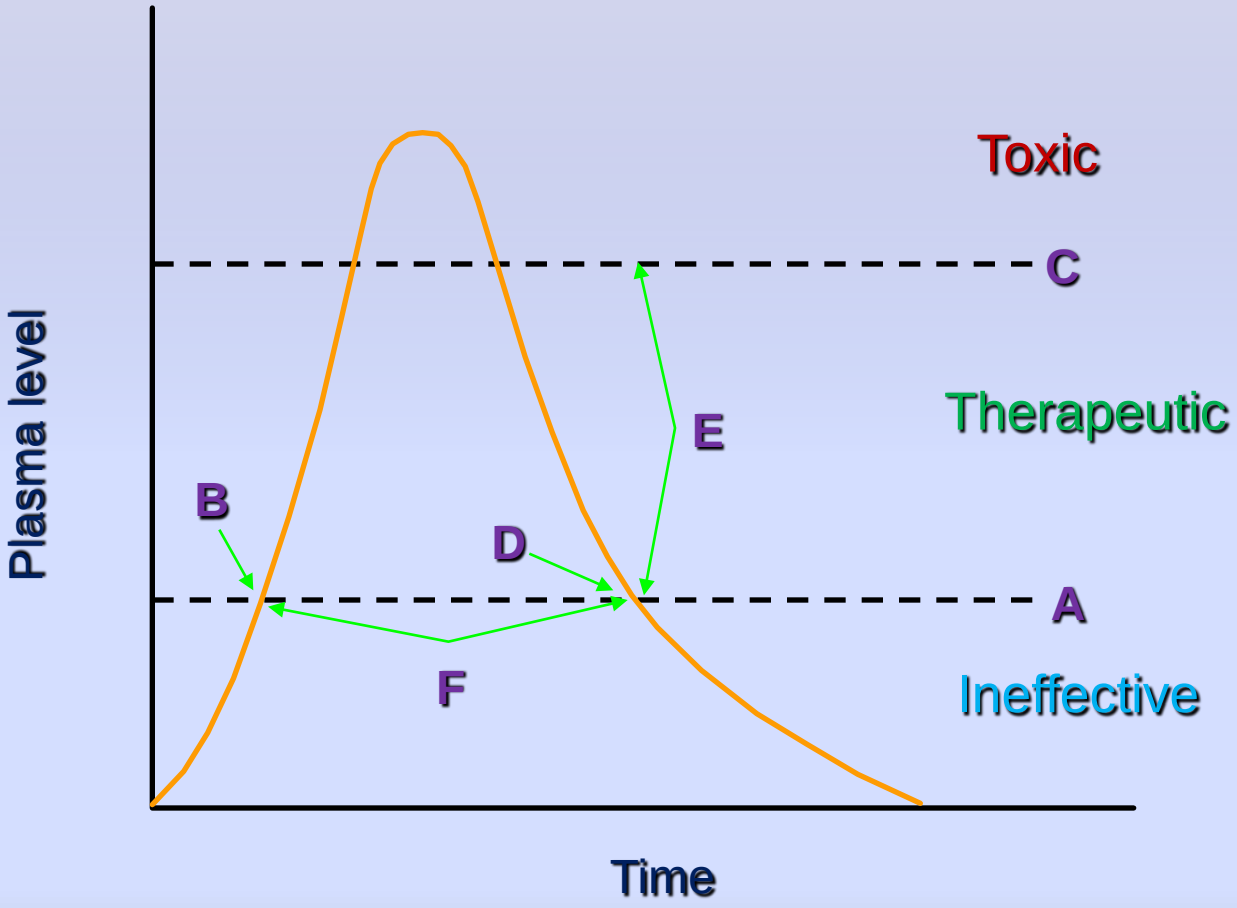
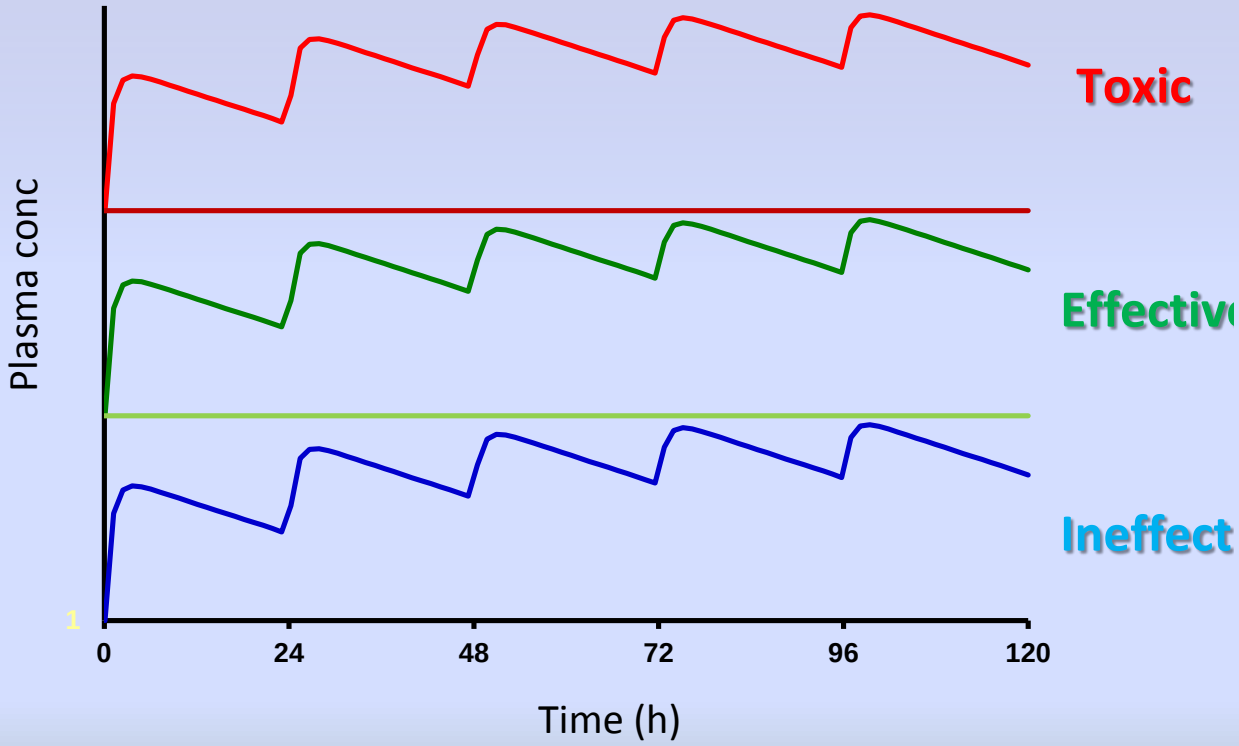


Dosage regimen design: Repeat dosing showing the concept of a therapeutic window



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HOW CAN WE DESIGN AN EFFECTIVE DOSAGE REGIMEN?

CLEARANCE & ABSOLUTE BIOAVAILABILITY!

Clearance

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- Clearance (Cl) is defined as the volume of the matrix being evaluated (e.g. plasma, serum or blood) completely cleared of the total or unbound drug per unit time.

- It is the proportionality constant that relates the rate of elimination at any one time to the concentration.
  - ☞ For linear kinetics the rate of elimination ∝ concentration
  - ☞ Rate of elimination = Cl * C
  - ☞ At steady-state rate of elimination = Cl * C_{ss}
  - ☞ Also once at equilibrium: rate in = rate out = Cl * C_{ss}

- Clearance is calculated from: $Cl = \frac{F * Dose}{AUC}$

- The only time we when we know F is when it has been administered intravenously directly into the systemic system i.e. when F=1

Calculation and adjustment of dosage regimen

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- At steady-state

$$\text{rate in} = \text{rate out}$$

$$= \text{Clearance} * C_{ss}$$

- Thus, for clinical dosage regimen design all that is needed is knowledge of human clearance, absolute bioavailability & target concentration
- How do we get absolute bioavailability (F)?

Calculation of absolute bioavailability

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$$\text{Absolute bioavailability} = \frac{AUC_{oral} * Dose_{iv}}{AUC_{iv} * Dose_{oral}}$$

Ideal features of absolute bioavailability studies in humans

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- Determine intravenous and oral pharmacokinetics in the same subject to avoid intersubject variability.
- Determine intravenous and oral pharmacokinetics at the same time (avoid period effects)
- Have virtually identical plasma levels by both intravenous and oral routes to avoid possible effects of non-linearity
- Must be cost effective in time and money considering prior enabling studies (pharmaceutical development and toxicology for intravenous dose)
- Safe method avoiding undue inconvenience to subjects

Intravenous microtracing study design

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- Dose subjects with normal dose of oral formulation
- At a selected time (e.g. anticipated C_{max} of oral dose) give a microdose of ^{14}C-labelled drug intravenously
- As the intravenous formulation will be a microdose it will only require a very simple and abridged development
- Measure the plasma levels of the oral dose by conventional methods (e.g. LC-MS/MS) and measure intravenous dose from the radioactivity using LC for specificity and Accelerator Mass Spectrometry (AMS) for quantification of radioactivity.
- Obtain full intravenous profile for robust calculation of clearance, volume distribution, absolute bioavailability, $t_{1/2}$ as well as examining absorption function using deconvolution, if required.

MICROTRACING APPLIED TO ABSOLUTE BIOAVAILABILITY DETERMINATION

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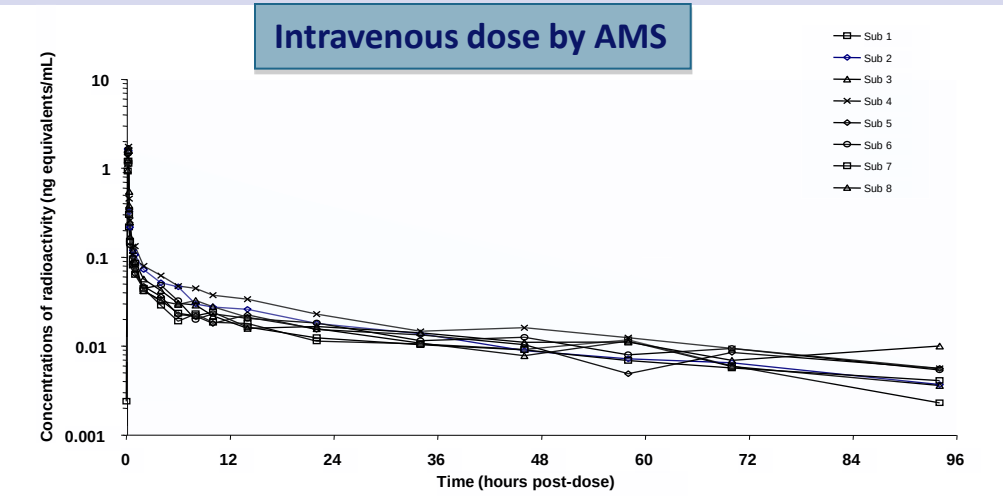

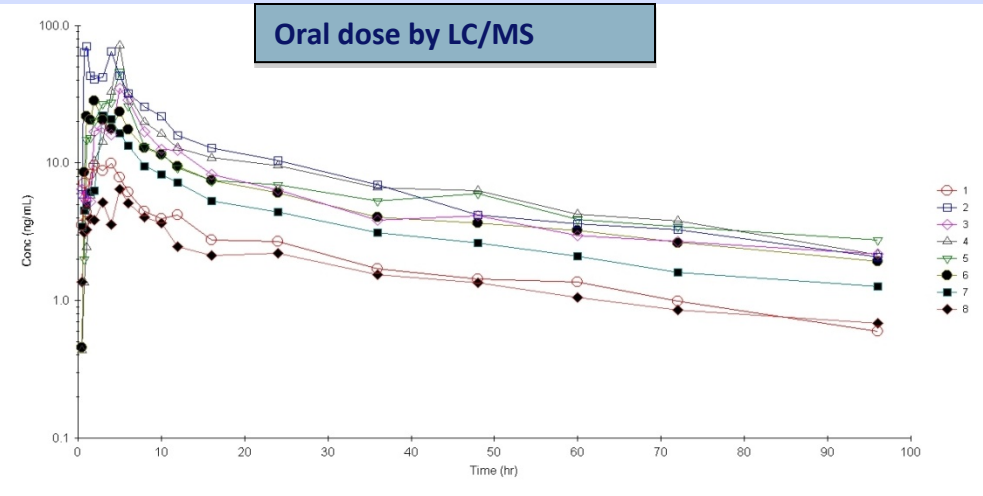

Parameter	i.v.	Oral
$t_{1/2}$	40.36	46.61
F (%)	NA	32.4

Courtesy Lloyd Stevens via Vitalea Science

WHY NOT COMBINE MIST & ABSOLUTE BIOAVAILABILITY STUDIES?

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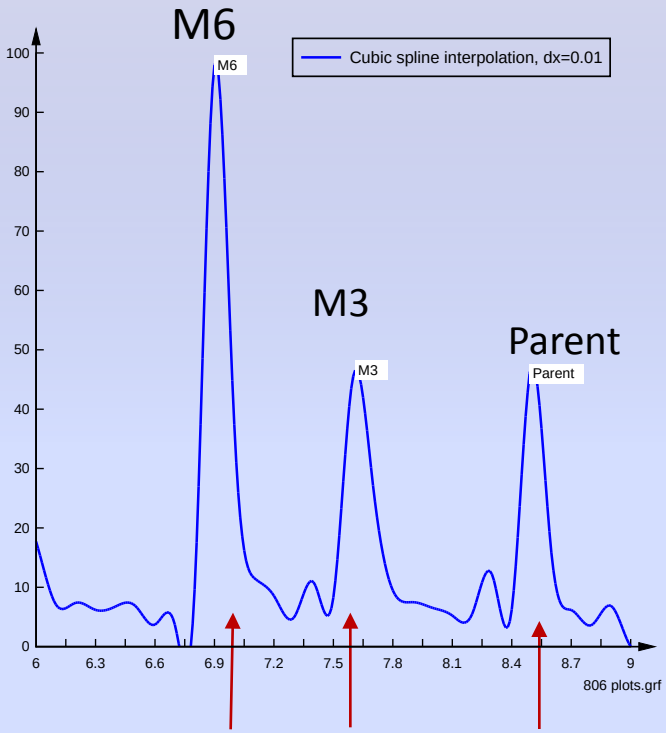
- When at an appropriate dose level, e.g. at or about the clinically anticipated dose level:
  - ☞ Add an intravenous ^{14}C microdose to the oral dose as in an absolute bioavailability study
  - ☞ Determine excretion-balance using AMS
  - ☞ Determine the urinary, fecal and plasma parent drug and metabolites as for the MIST studies using HPLC/UPLC fractionation, AMS and LC-MS/MS for identification
  - ☞ Determine the clearance and absolute bioavailability using the intravenous data and AMS
- If performed early in clinical program (e.g. FIH study), it can have a profound beneficial impact on the future development program
- The combined study is not recommended if there is substantial pre-hepatic (gut lumen or gut wall) loss e.g. metabolism

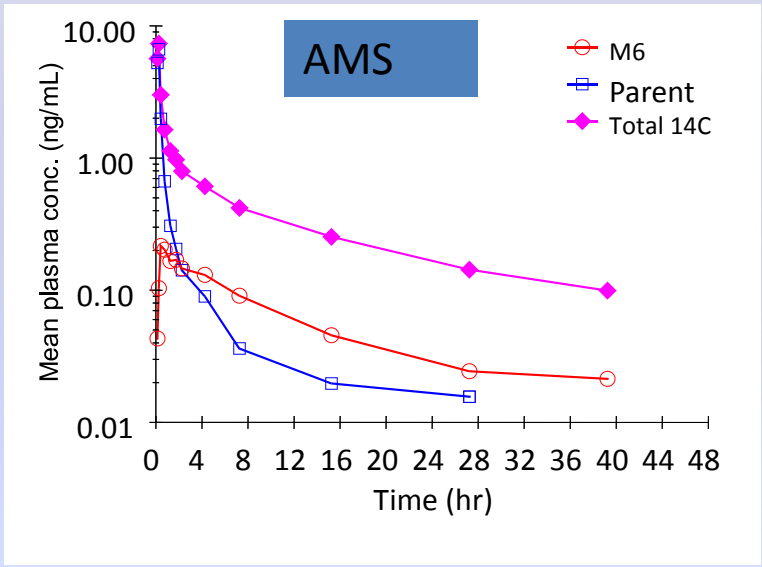
Example of pre-hepatic loss



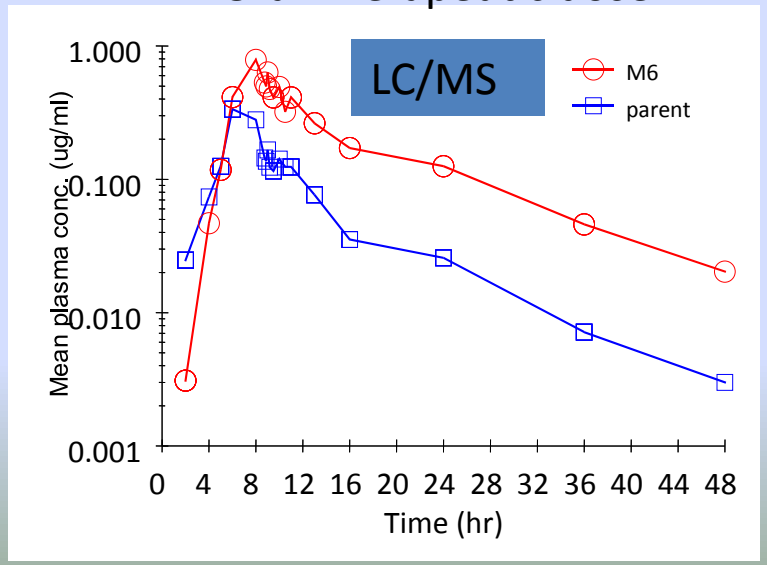
Collect individual fraction off UPLC and quantify for 14C/C

Courtesy Vitalea Science

I.V. tracer



Oral Therapeutic dose



Summary

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- AMS has been overviewed, emphasizing its value to DMPK by providing extremely high sensitivity with good accuracy and precision
- The MIST guidelines have been described showing the need of obtaining human metabolite exposure data, early
- Examples given of how AMS provides early clinical metabolite data
- The advantages of obtaining intravenous data using an intravenous microdose and AMS for the determination of clearance and absolute bioavailability is described and how it can be used for dosage regimen design
- The possibility of combining MIST and absolute bioavailability studies proposed