“LC+AMS” for Clinical Microdose/Microtracer support – an evolving science

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Outline

- **AMS (Accelerator Mass Spectrometry)**
  - Instrumentation and sample prep.
  - What and how many instrument or process standards /controls….?
- **LC+AMS Validation – similarities and differences (!) to LC-MS**
- **Clinical study designs supported & enabled by AMS**
  - What are these?
- **What kind of data do we achieve….acceptance criteria**

AMS

(Accelerator Mass Spectrometry)

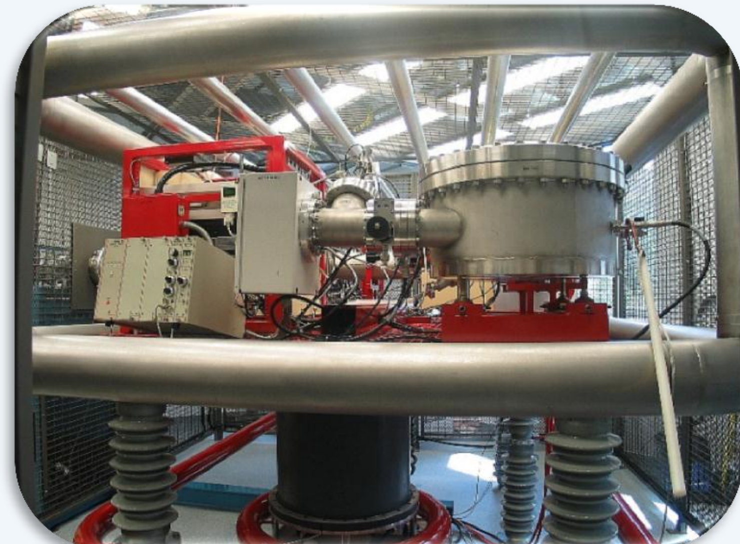
- General Background

AMS (Accelerator Mass Spectrometry) – the basics

- 2 Mass Spectrometers and an Accelerator in series
  - major focus on ^{14}C detection (carbon dating)
  - samples "converted" to fullerene graphite [gas introduction also possible]
  - 10^3 to 10^6x more sensitive than Liquid Scintillation Counter
  - ~1000x more mass sensitive than Mass Spec.
  - 10^{10} more sensitive for ^{14}C than for ^{13}C

Ion source (of GSK 250kV SSAMS)

Side view of High Voltage Deck



What is AMS?

- Quantification, but **NOT** structural identification of ^{14}C labelled analytes !
  - Sensitive Isotope Counter would be a clearer name for the technique
  - GSK has demonstrated mass sensitivity into the low fg/mL range
- Measures extremely small quantities of rare isotopes
  - ^{14}C incorporated into molecules as a tracer
  - direct analysis of neat/diluted sample or following analyte isolation
- Isotope ratio provided by the AMS – from combusted sample!
- Carbon content of samples provided by elemental analyser
- Result can be expressed as disintegrations per min./mL
  - aids comparison to other radioactive content data
  - converted (using specific activity) to unit of mass/volume eg. pg/mL

Sample preparation for AMS….

graphitisation

"flames, furnaces and cryogenics"

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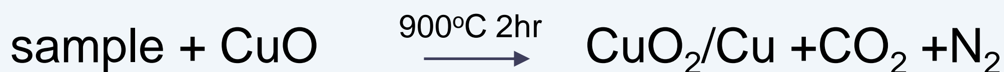
The graphitisation process….. flames, furnaces and cryogenics

The chemistry of graphitisation

Combustion stage

Sample eg. plasma, urine, LC fraction is flame-sealed in a … d quartz tube
Quartz tube placed in furnace:



sample + CuO $\xrightarrow{900^{o}C\ 2hr}$ CuO_2/Cu +CO_2 +N_2

Reduction stage

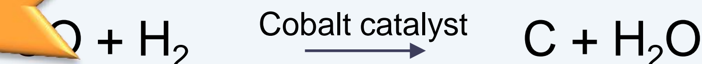
- CO_2 is cryogenically transferred an … into a borosilicate tube
- Borosilicate tube placed in furn…

reaction is carried out at 500ºC … and 550ºC for 6 hrs.
Zn is present to become ox… s is TiH_2 as a source of hydrogen.

TiH_2 → $2H_2$ + Ti

CO… → CO + H_2O

… + Zn → CO + ZnO



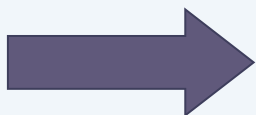
…O + H_2 $\xrightarrow{\text{Cobalt catalyst}}$ C + H_2O

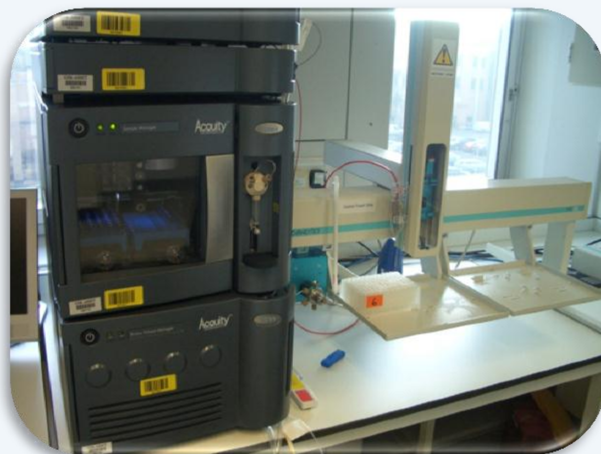
3 day process !! – batch size of 200 samples

Analyte isolation sample prep: LC followed by off-line analysis by AMS ["LC+AMS"]

Parent peak isolation by chromatography

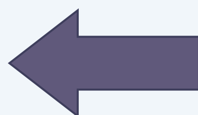
Human plasma sample or extract

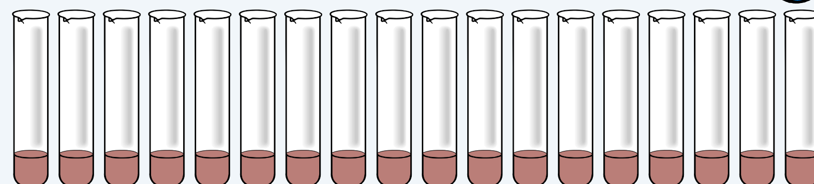




Parent fraction collected from each sample

Fractions graphitised prior to analysis by AMS





What standards/QCs are run?

- **Instrument standards**
  - Pooled ANU (sugar harvested in 1950s) graphite for instrument ratio normalisation [x3]
  - Synthetic graphite for instrument background check [x3]

- **Graphitisation process standards**
  - Carbon carrier blanks [x6 (or more dependent on batch size)]
  - ANU graphites [x6 (or more dependent on batch size)]

- **Analyte peak isolation standards (initial sample prep. & LC) and QCs**
  - Spiked analyte recovery constant biological matrix (eg. plasma) standards [x5 (single concentration)]
  - Spiked analyte QCs in biological matrix (eg. plasma) [x9 (3 @ 3 concs.)]
  - Total of >=32 standards or QCs of one sort or another (>= 24% of a 134-cathode wheel)

LC+AMS Assay “Validation”*

- viva la diferencia !?

* Much of the following content is taken from a recent publication[1] in *Bioanalysis*, Vol. 3, Issue No. 4, Feb 2011

Key differences between LC-MS & LC+AMS

Consideration	LC-MS	LC+ AMS
Tandem and off-line techniques	Hyphenated technique	➢ Off-line technique ➢ Manual processing ➢ Extended timeframe
Measurement	MS measures m/z value; Differentiates analytes by virtue of mass/charge	➢ Sample prep. destroys structural information ➢ No mass discrimination (other than ^{12}C:^{13}C:^{14}C)
Data output	Relative concentration ; Differs from analyte to analyte	➢ Absolute isotope ratio ➢ Irrespective of nature of analyte prior to graphitisation
Interference	Matrix effects; Ion suppression	➢ No matrix effects ➢ If appropriate carbon diluent amount added – no effect of other analytes; assuming chromatographic isolation eg. from ^{14}C metabolites
Detector response type	Calibration curve with use of Int. standard throughout process	2 distinct influences: (1) AMS instrument (2) Sample prep - analyte isolation
Environment	Contamination from the environment not generally a concern – except very sensitive assays (?)	Precautions required to ensure contamination is limited – some issues are inevitable

Validation of LC+AMS assay – general principles

- **Fitness for purpose**
  - Appropriate scientific rigour should be applied in lieu of formal guidance
  - Assay will likely be used only once, to support a specific clinical study
    - at one analytical facility; no method transfer to another AMS facility
  - Build on analytical expertise for particular analyte
    - pertinent existing validation data – eg. stability & freeze thaw
    - fresh samples may be generated to check integrity of analyte isolation
- **Total ^{14}C data gathered from same samples as Parent PK**
    - acts as a reality check and aids definition of dilution scheme
    - if necessary PK and total ^{14}C data can be checked via metabolite profiling

Validation of LC+AMS assay – common themes consistent with BMV

- **Reference materials**
- **Selectivity**
- **Accuracy**
- **Precision**
- **Stability – possibly rely significantly on prior knowledge?**

Notable items –

- **AMS instruments are linear across several orders of magnitude[2,3]**
  - **very little inter-instrument variation[4]**

- **Once sample combusted to harvest graphite – all "the same"**
  - No matrix effects to consider
  - Graphite sample stability not of concern (5730 year half-life !)

Clinical study designs supported & enabled by AMS

Clinical study type, primary output, decisions and "customer(s)"

Study Type	Primary output	Decisions	Main Customer(s)
^{14}C Nanotracer (aka "light label") & conventional Human ADME study (HRS); Dosed via therapeutic route	ADME info. (incl. mass balance & extractability)	• Assessment of metabolic liabilities • Aid design of QTc study	• DMPK • Safety Assessment • *Regulators*
Microdo... (stand al... *[Cold or ^{14}C...* dose of ≤ 100µg ... PO rou...			...m Team
IV ^{14}C tracer + Oral therapeutic (concomitantly)	• Absolute Bio. % • Metabolism info.	• Direct formulation effort? • Project progression?	• Project Team • Clinical PK/DMPK • Formulation scientists • *Regulators*

Note that none of these studies can strictly be classified as Safety or Efficacy studies…..

Assay quality

- What is appropriate?

- What do we need?

- What is achievable?

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What kinds of assay quality do we achieve?

Compound No.	^{14}C Specific activity	Assay range (LLoQ-HLoQ)	QC acceptance criteria; actual	No. of subjects
1 (DDI microdose)	[9.3kBq (250nCi)/50µg]x2	4.3423 - 1400 pg/mL (0.0548dpm/mL [CV = 14.5%] - 17.7dpm/mL)	**+/- 30%** ; 70% of QCs in 3 assay batches within limits	10M Healthy
2 (early microtracer)	10kBq (270nCi)/100µg	0.882 pg/mL - 441 pg/mL (0.0045 to 2.25 dpm/mL)	**+/- 25%** ; >70% of QCs in 4 assay batches within limits	6 M Healthy
3 (early microtracer)	10kBq (270nCi)/100µg	12.3 - 1820 pg/mL (0.0684 to 10.1 dpm/mL)	**+/- 20%** ; 90% of QCs in 2 assay batches within limits	8M Healthy
4 (early microtracer)	9.3kBq (250nCi)/100µg	30.2 - 1460 pg/mL (0.16 to 7.7 dpm/mL)	**+/- 20%** ; 86% of QCs in 2 assay batches within limits	7F Healthy
5 (“regulatory” microtracer)	7.4kBq (200nCi)/50µg	1.8 - 470 pg/mL (0.0163 to 4.17dpm/mL)	**+/- 20%** at High and Mid; **+/- 25%** at Low ; study ongoing	4 M/F Cancer Patients
6 (“regulatory” microtracer)	7.4kBq (200nCi)/5µg	1.1 - 104 pg/mL (0.1 to 9.1dpm/mL)	**Study ongoing**	6 (?) M/F Cancer Patients

AMS Technology development: Laser interface – gas analysis

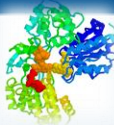
AMS Technology development
- the GSK/NEC/MIT collaboration for interface development

- Collaboration initiated October 2007
  - Based upon MIT's patented design for laser interface to generate CO_2 from dried HPLC/UPLC fractions[1]
  - MIT continue to provide intellectual and practical input to the project
- NEC is licensed to continue the development and commercialisation of the interface
  - providing Engineering/Physics expertise and resource
  - MIT interface design adapted for use on NEC instrumentation
  - 96-well format adopted
  - prototype interface built and undergoing testing
- GSK providing financial support to MIT and scientific input to the project
  - to purchase first production model; acceptance tests agreed
  - poster providing further information presented at AMS-12 conference (March 2011)

1. "An interface for direct analysis of ^{14}C in non-volatile samples by Accelerator Mass Spectrometry", Liberman RG *et al.*, Anal. Chem. 2004, 76, 328-334

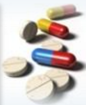
AMS through time..........

1970's:
AMS Invented



1990s: First use in biochemistry

2000 : First use in drug development – Mass Balance and Metabolite Profiling



Several studies ongoing…assay validation procedures being refined

1970 1980 1990 2000 2010

2011: White Paper on assay validation

1970-1980s
AMS first used for carbon dating

2009: GSK's First experience of use of AMS as a true Bioanalytical tool

Summary

- No formal guidance exists for application of AMS in bioanalysis
- Use in determining intravenous PK in humans justifies that <u>appropriate</u> scientific rigour is applied to AMS methods and their validation
- Purpose of the study should play a part in determining quality criteria
- Bioanalytical community input being gathered by EBF Topic Team
- Global Bioanalysis Consortium have an AMS harmonization sub-team….

Acknowledgements

- GSK Management – foresight in investing in the technology & support ever since….
- VITALEA Science and Simbec Research for support of one of the IV microtracer studies detailed
- NEC and MIT staff involved in the laser interface collaboration
- Xceleron for support of some of the other studies
- Co-authors of the validation and method papers in Bioanalysis:
  - Graham Lappin and Mark Seymour of Xceleron Ltd.
  - David Higton of AstraZeneca
  - Howard Hill of Huntingdon Life Sciences

Thank you for your attention

References

1. "AMS method validation for quantitation in pharmacokinetic studies with concomitant extravascular and intravenous administration" ,Lappin G et al., Bioanalysis, 2011, 3(4), 393-405
2. "Analytical validation of accelerator mass spectrometry for pharmaceutical development", Keck B et al., Bioanalysis, 2010, 2(3), 469-485
3. "Accelerator mass spectrometry best practices for accuracy and precision in bioanalytical ^{14}C measurements", Vogel J et al., Bioanalysis, 2010,2(3), 455–468
4. "Comparison of a 250 kV single-stage accelerator mass spectrometer with a 5 MV tandem accelerator mass spectrometer – fitness for purpose in bioanalysis. " Young G et al., Rapid Commun .Mass Spectrom. ,2008, 22;4035–4042

The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents.

Back-ups

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Novel clinical study design for PK
– IV Microtracer

IV Microtracer + Therapeutic Oral – The Purpose

Aim

- to understand reason(s) for variable systemic exposure in humans following oral dosing

- poor/variable absorption?
- first pass metabolism?
- Progress or not…re-formulate?

Attraction of the approach

- rapid evaluation study (relatively low effort)
- possibility of "piggy-backing" to another Phase 1 study….enhanced study
  - Age versus gender
  - Formulation assessment
  - Food effect

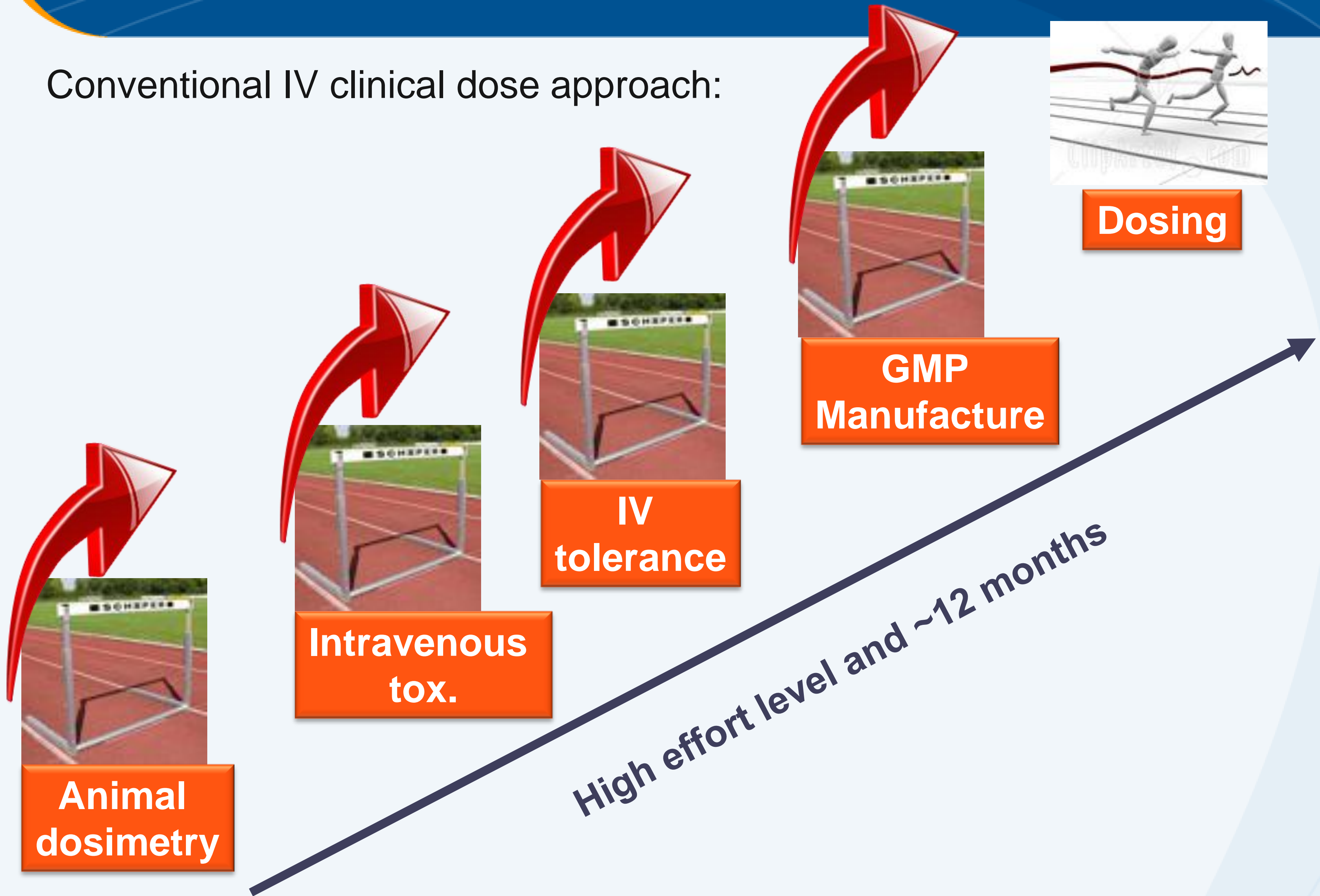
IV Microtracer + Therapeutic Oral Dose – The Design

Study design

- IV ^{14}C-tracer dose administered concomittantly at T_{max} of Oral therapeutic dose in a single dosing period
  - Design established in 1970's; ^{13}C-drug by IV route, non-labelled by oral route, **both** at therapeutic levels
  - IV ^{14}C-dose at $\leq$ 1/200th of oral dose @ <270nCi ^{14}C
  - Conventional dose of ^{14}C is >200x greater

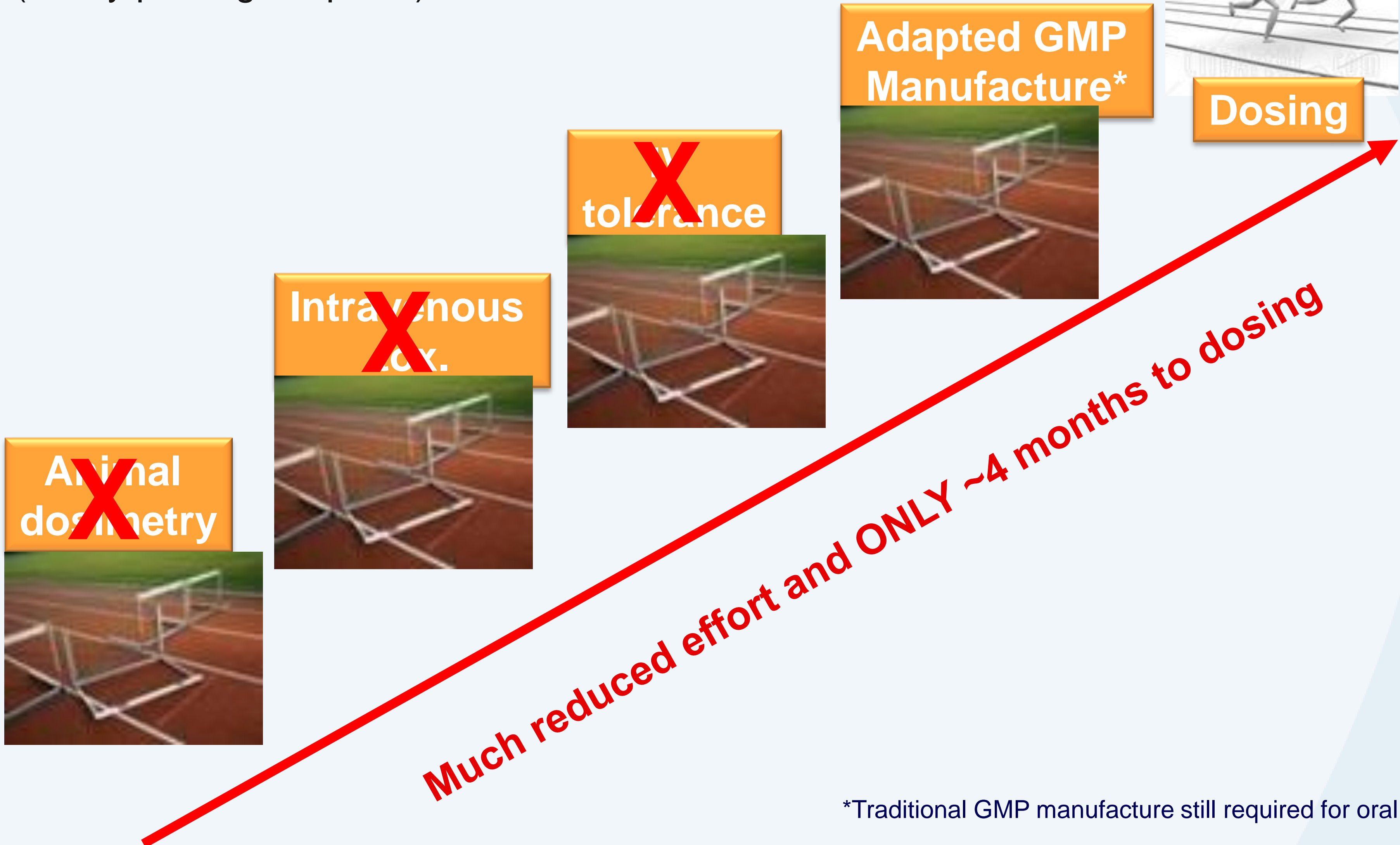
IV Microtracer + Therapeutic Oral Dose

Conventional IV clinical dose approach:



IV Microtracer + Therapeutic Oral Dose

Microdose **but** as tracer administered with oral dose……
(safety package in place)



*Traditional GMP manufacture still required for oral dose

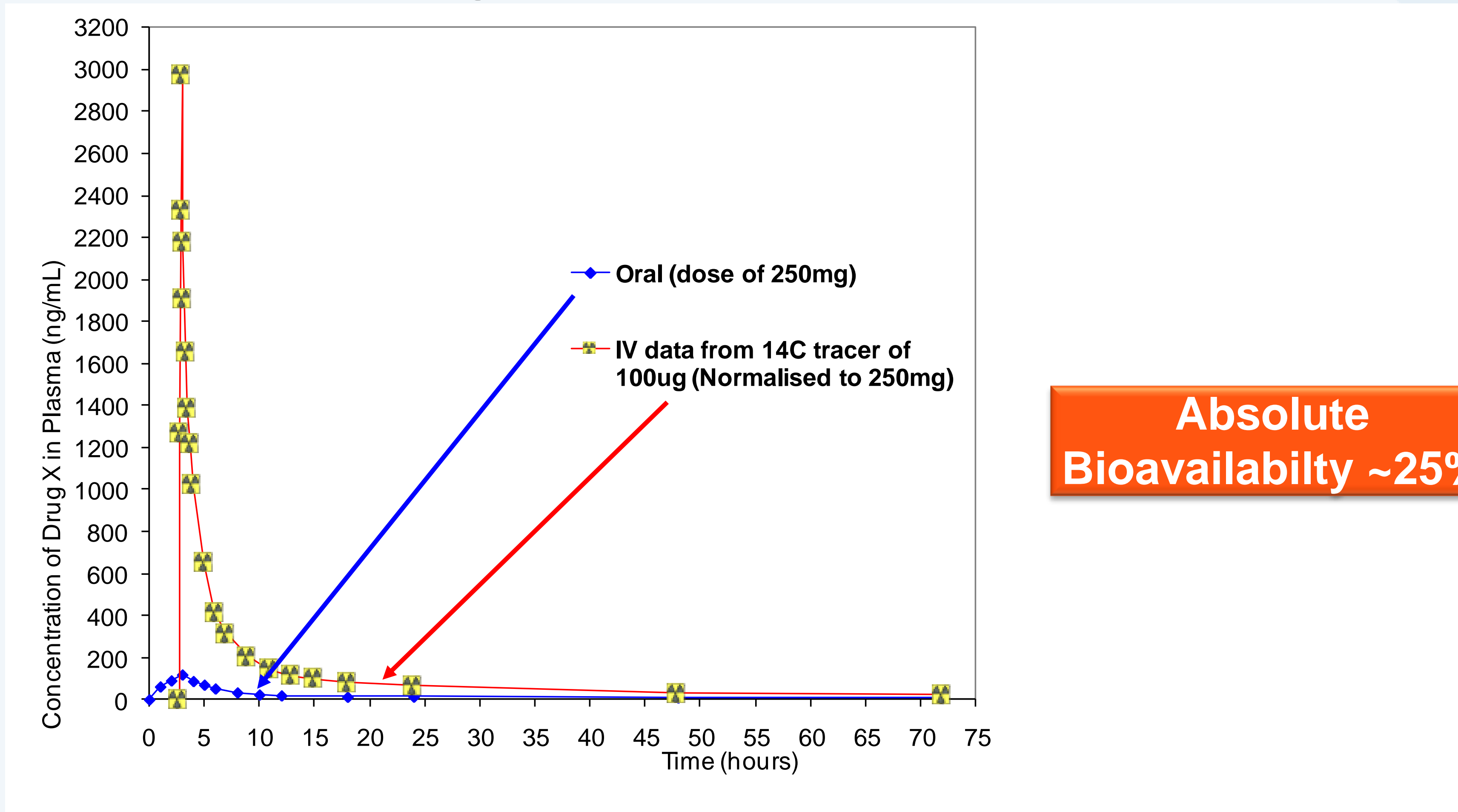
IV Microtracer – GSK study example

IV tracer (^{14}C) @ 100μg + oral therap. dose (^{12}C) @ 250mg; 8 ♂ humans

- IV dose given at T_{max} of oral dose (2.75-3 h), as 15 minute infusion
- Blood collections for plasma, to 72 hours; urine collection, 0-24 hours
- IV “followed” by AMS (total ^{14}C-drug & metabolites, parent PK)
- PO dose “followed” by LC/MS/MS (parent PK)

IV Microtracer – GSK study example

Comparison of plasma concentrations of Drug-X following IV (100µg) and Oral doses (250mg); IV data normalised to equivalent dose



Provided confidence for progression of molecule…..

Reference materials

- Several principles common to BMV guidance
- Specific activity and purity of the ^{14}C material must be established
  - Pivotal to successful quantitation
- Non-labelled analyte may be used as chromatographic marker or as an Int.Std. ; should be assessed for ^{14}C content
- Carbon carrier used as isotopic diluent should be certified or assessed within the AMS facility
  - fraction carbon and isotope ratio

Selectivity

- Check of background ^{14}C in blank/control matrix
- Selectivity of LC+AMS; conferred by the chromatographic separation
- Authentic standards used to develop LC method
  - if available, can also use in vitro/animal (even human) samples containing metabolites to check integrity of analyte isolation
- Ultimately needs to be confirmed using clinical samples
  - possible use of secondary chromatographic system or "2D" of analyte peak fraction
  - selectivity may be checked via metabolite profiling analysis
- Check of carryover – LC separation or on the AMS itself

Accuracy and precision

- Several principles common to BMV guidance
  - Due to intensive manual processing involved – wider limits on acceptance may be appropriate
- How to address “recovery” may be somewhat different
  - AMS qualification and analyte recovery are 2 separate activities
- Direct analysis of spiked standards/QCs is possible at several levels by virtue of the ^{14}C label; so 2 independent weighings is not necessary

Incurred sample reanalysis (ISR)?

- Is this assessment truly warranted for this specific assay type?
  - bearing in mind effort involved and specialist nature of the assay
  - data is not directly providing safety or efficacy data……
  - not pivotal studies; enabling

- Case by case approach?

Lower LOQ definition

- Several principles common to BMV guidance

- LOQ definition integral to the study design as dictated in part by specific activity of the dose
  - Lower LOQ achievable with higher specific activity

Stability

- Sample storage stability typically established as part of validation of conventional bioanalytical assays
- Stability of ^{14}C-analyte established during manufacture
- Stability in spiking solutions & LC fractions should be established for LC + AMS assay