

Accelerator Mass Spectrometry (AMS) in Drug Development at GSK

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Outline

- Background to AMS
- GSK's in-house AMS capability
- History of use of AMS in GSK
 - Example study data
- Future of AMS in GSK
 - potential impact on Drug Development
 - potential advances in AMS technology

Background to AMS

Background to AMS - What is AMS?

- 2 Mass Spectrometers and an Accelerator in series
- Allows measurement of extremely small quantities of rare and radioactive isotopes e.g. ^{14}C , ^3H , ^{41}Ca , ^{10}Be , ^{26}Al .
 - Major focus on ^{14}C (carbon dating)
- $\sim 10^3$ to 10^6 x more mass sensitive than Liquid Scintillation Counter
 - all biomedical samples will have detectable levels of ^{14}C , even controls!
- Quantification, but **NOT** direct 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
- AMS measures ^{14}C content of graphite samples

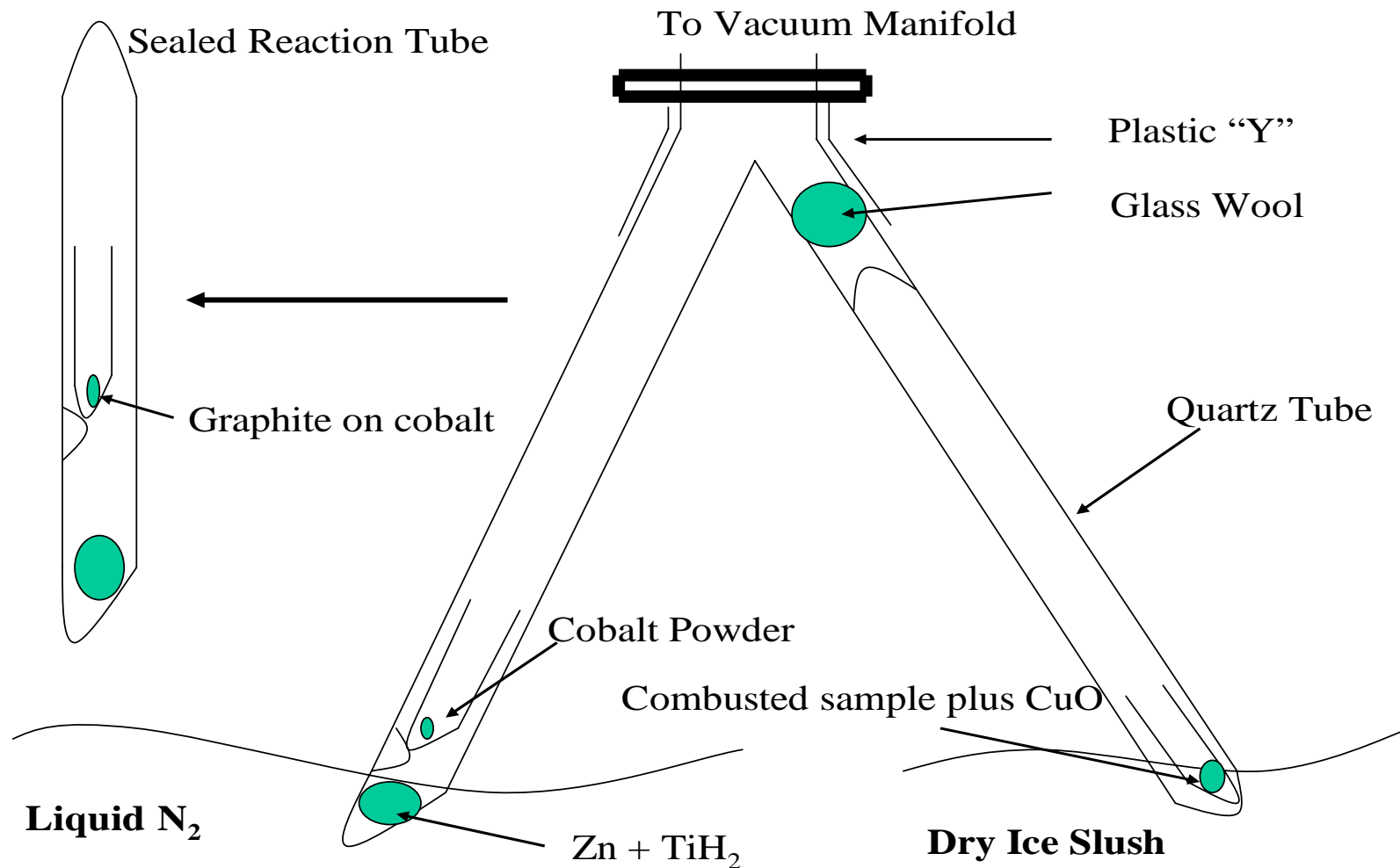
Background to AMS – Making graphite

AMS sample preparation procedure :

1. Samples placed in a quartz tube with CuO wire and dried under vacuum
2. Baked for 2 hours (in a Carbolite furnace) at 900°C to produce CO₂
3. CO₂ cryogenically transferred to reaction tube

Background to AMS – Making graphite

Schematic of stage 3 (cryogenic transfer)



Ref : Vogel *et al*, *Nucl. Instrum. Methods Phys. Res. Sect B* (1984), 5, 289-293

Background to AMS – Making graphite

AMS sample preparation procedure (ctd...):

4. Baked with Cobalt, Zn + TiH₂, at 500°C for 4 hours
5. Baked again for a further 6 hours at 550°C
6. Graphite harvested and pressed into aluminium cathodes (at 200 psi)

Background to AMS – Graphite analysis

- Graphite cathodes loaded onto AMS sample wheel
- Graphite impacted by Caesium gas ion beam
- Negative ion beam of $^{12,13,14}\text{C}^-$ ions produced
- Electrons stripped to produce $^{12,13,14}\text{C}^{1+}$ to $6+$ ions
- C^{X+} ions selected (C^{4+} for large AMS and C^{1+} for “compact” AMS)
- Isotope ratio of $^{14}\text{C}/^{12}\text{C}$ measured (^{13}C beam also monitored)

Background to AMS – Data output

- Isotope ratio provided by AMS – converted to a pMC value [pMC = percent of Modern Carbon]
- Total carbon content provided by C,H,N analyser
- Data from AMS and C,H,N combined
- Result can be expressed as dpm/mL or dpm/g
 - units easily recognised by the Pharmaceutical industry

GSK's in-house AMS capability

GSK's in-house AMS capability

- GSK has (2x) Single Stage Accelerator Mass Spectrometers (250 kV)
 - 1 for Ware site in the UK
 - 1 for Upper Merion site in the US

- SSAMS is designed solely for radiocarbon analysis
 - larger instruments are capable of accelerating larger ions!

- Throughout 2005, staff received training in sample prep and AMS operation
 - Specialised sample preparation suites and instrument labs. were constructed

- Instruments were delivered in summer 2005
 - Accepted on 1st November 2005
 - Validation, confidence building and documentation production in 4Q05-1Q06
 - Operational for study support, by end 1Q06

GSK's in-house AMS capability

- Instruments have 2 ion sources ; 134 graphite samples per wheel (per source)
- ~12 (non-biological) standards are run, of known pMC (122 unknowns/wheel)
 - ANU sugar (150.61 pMC)
 - Processed synthetic graphite (<5 pMC) ; very process/lab dependent
[For Ref : Human Control plasma (110 pMC ; 0.45 dpm/mL)]
- At present, ion sources cannot be run sequentially (without manual intervention)
 - working with NEC (AMS manufacturer) to address this

GSK's in-house AMS capability



**SINGLE STAGE AMS
134 MC-SNICS DUAL INJECTORS**

**General Purpose
Acceleration tube**

**Molecular
Dissociator**

High Voltage Deck

**Analysing
Magnet**

**Analysing
Magnet**

**C12/C13
faraday cups**

**Low Energy
Beam Line**

ESA

ESA

5.7 M

C14 Detector

**MC-SNICS 1
(Multi-Cathode Source of Negative
Ions by Caesium Sputtering)**

MC-SNICS 2

9.8 M

SSAMS schematic reproduced by kind permission of NEC ; labels added



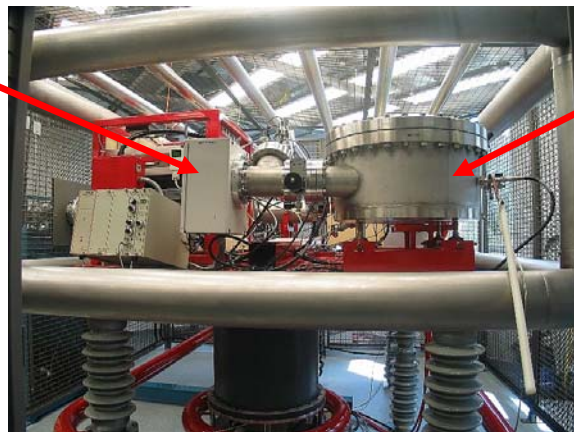
GSK's in-house AMS capability

SSAMS at Ware, UK

Side view of High Voltage Deck

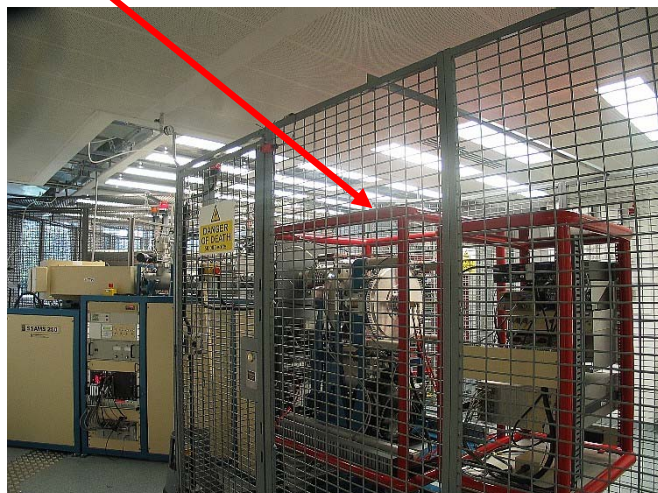
14C SSB detector

ESA



MC-SNICS 1

MC-SNICS 2



History of use of AMS in GSK

History of use of AMS in GSK

- All historical GSK “AMS” studies supported by Xceleron Ltd. (aka CBAMS Ltd.), using 5MV Tandem Pelletron AMS
 - GSK was one of the co-founders of CBAMS Ltd.
- Some initial sample prep. carried out by GSK
 - Including fractionation by HPLC for metabolite profiling
- Dedicated laboratories for AMS work used throughout
 - temporary laboratory used for pilot and early studies
[although sample dilution sometimes carried out in a conventional ADME lab.]
 - part of conventional ADME lab converted into AMS prep lab
[AMS sample prep. suites now established at GSK]

History of use of AMS in GSK (continued)

- AMS has been used for “atypical” projects due to :
 - Cost constraints (especially in relation to HPLC-AMS)
 - Throughput limitations
 - Lack of confidence, in early days, of this “new technique”

History of use of AMS in GSK (continued)

Studies in 2 general categories : ***AMS studies & hybrid studies***

1. ***AMS studies***

- those where level of radiocarbon dosed/used was very limited

Studies have fallen into this category due to :

- dosimetry limitations due to tissue retention
- potent pharmacophore
[insufficient ¹⁴C label could be administered ; low total dose]

History of use of AMS in GSK (continued)

2. *hybrid studies*

- where conventional level of radiocarbon used, but **some** samples generated were low in radiocarbon

Studies have fallen into this category when :

- very limited absorption
- investigations of later timepoints (eg. plasma)
[ie. conventional methodologies such as LSC, were inadequate]

History of use of AMS in GSK

- Example study data

1999 / 2000 :
Pilot Low Radioactive Dose Clinical study

Example study data (ctd..)

1999 / 2000 : Pilot low radioactive dose clinical study- published work*

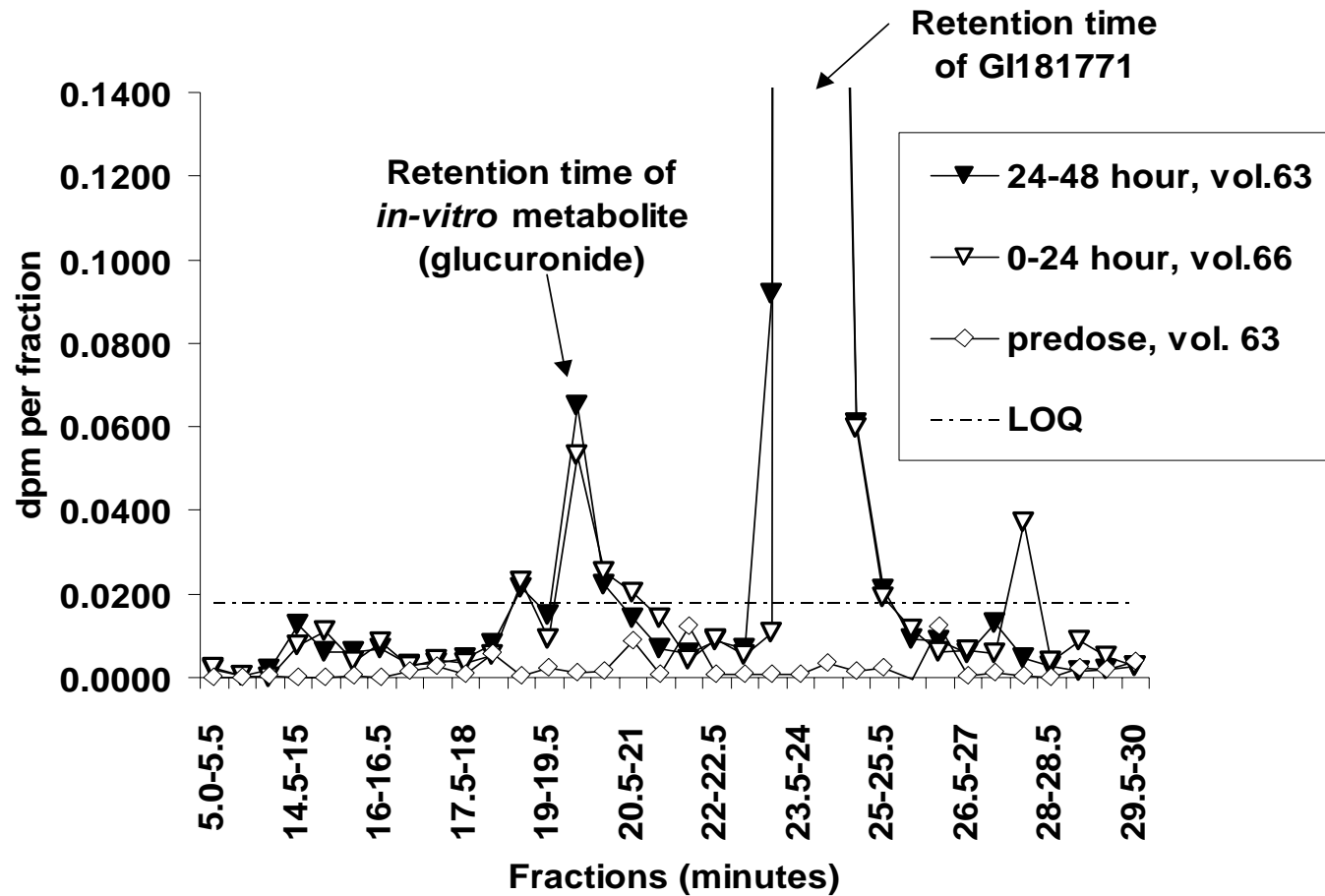
- Oral dose of ^{14}C -GI181771, 2.7mg , 3.3nCi (122Bq) to 6 male volunteers
 - Translated to only 40ng of ^{14}C labelled GI181771
 - Exposure to radiation of only 0.06 μSv (due to compound properties)
i.e. 15-fold less than the 1 μSv “cut-off” for an exempt study
 - Samples taken
 - predose + faecal and urine collections (5 days)
 - 15 blood samples (0 - 24hours) for serum

* Young G. *et al.*, *Xenobiotica*, 2001, 31 (8/9), p619-32

Example study data (ctd..)

1999 / 2000 : Pilot low radioactive dose clinical study (ctd..)

Profile of faecal extract – HPLC fractions analysed by AMS



Example study data (ctd..)

1999 / 2000 : Pilot low radioactive dose clinical study (ctd..)

- Mass balance of radioactivity achieved
- Total radioactivity in plasma achieved (after protein crash)
- Successful metabolite profiling of faecal extracts
- Provided information on systemic exposure to drug-related material
- AMS tested to its limits for biomedical use
 - Profiling of plasma was not achieved!!

2001 :
Serum Metabolite Profiling for
“Conventional Dose” Clinical study

Example study data (ctd..)

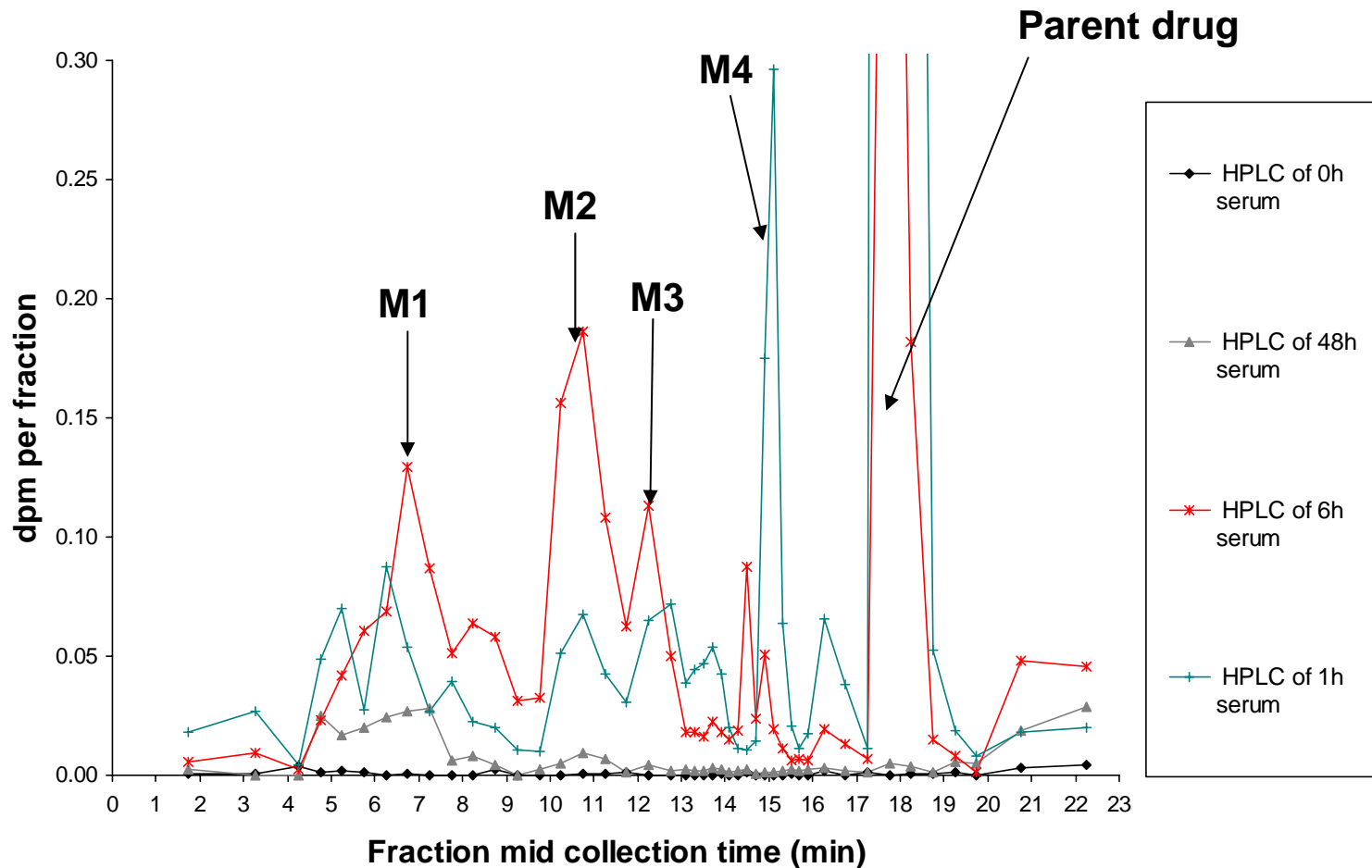
2001 : Serum metabolite profiling for “conventional dose” clinical study

- 75 μ Ci administered to healthy male volunteers
 - Most study support conducted via LSC
 - Late timepoint serum samples too low for normal radioprofiling (diluted 25x for AMS !)
 - Profiling conducted by HPLC with fraction collection then AMS (10 – 0.2dpm on HPLC column)
 - Example of “*Hybrid*” study

Example study data (ctd..)

2001 : Serum metabolite profiling for “conventional dose” clinical study (ctd..)

Reconstructed radiochromatogram showing retention times of known metabolites (each serum was diluted 25x with water)



Example study data (ctd..)

2001 : Serum metabolite profiling for “conventional dose” clinical study (ctd..)

- Experience gained in profiling over range of 10 – 0.2dpm on HPLC column
- AMS answered question
 - what RDM (radioactive drug related material) in late timepoint serum samples?

2001 :
In Vitro Blood Cell Binding study

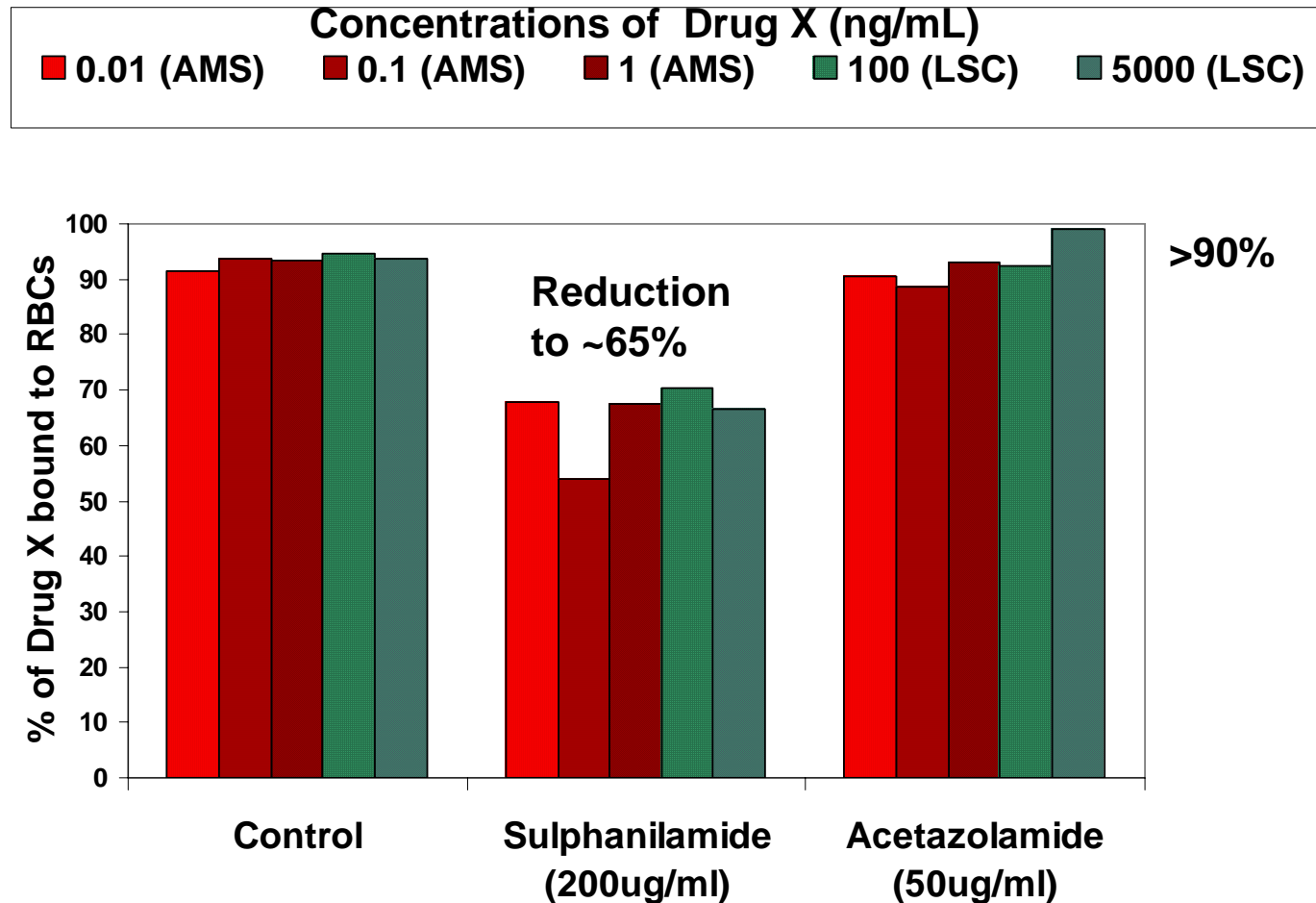
Example study data (ctd..)

2001 : in vitro blood cell binding study (displacement by competitor)

- In vitro blood binding study for a potent NCE (primary sulphonamide)
- Prior work conducted at elevated concentrations ; analysis by LSC
- AMS used to support work conducted at (low) relevant therapeutic concentrations

Example study data (ctd..)

2001 : in vitro blood cell binding study (displacement by competitor)



Note : Good agreement achieved between high and low concentration work

**2003 / 2004 :
Non-clinical studies**

Example study data (ctd..)

2003

Dog excretion balance study ; conducted using new metabolism cages

- low dose 1µg/kg (and low radio dose ; 100nCi/kg) due to potency of NCE
- successful balance by LSC, combined with AMS
- successful profiling by HPLC - AMS

2003 / 2004

Dog plasma and rat tissue metabolite profiling studies

- low doses (and low radio doses) due to potency of NCE
- successful profiling by HPLC - AMS

2004 :
Low Radioactive Dose Clinical study

Example study data (ctd..)

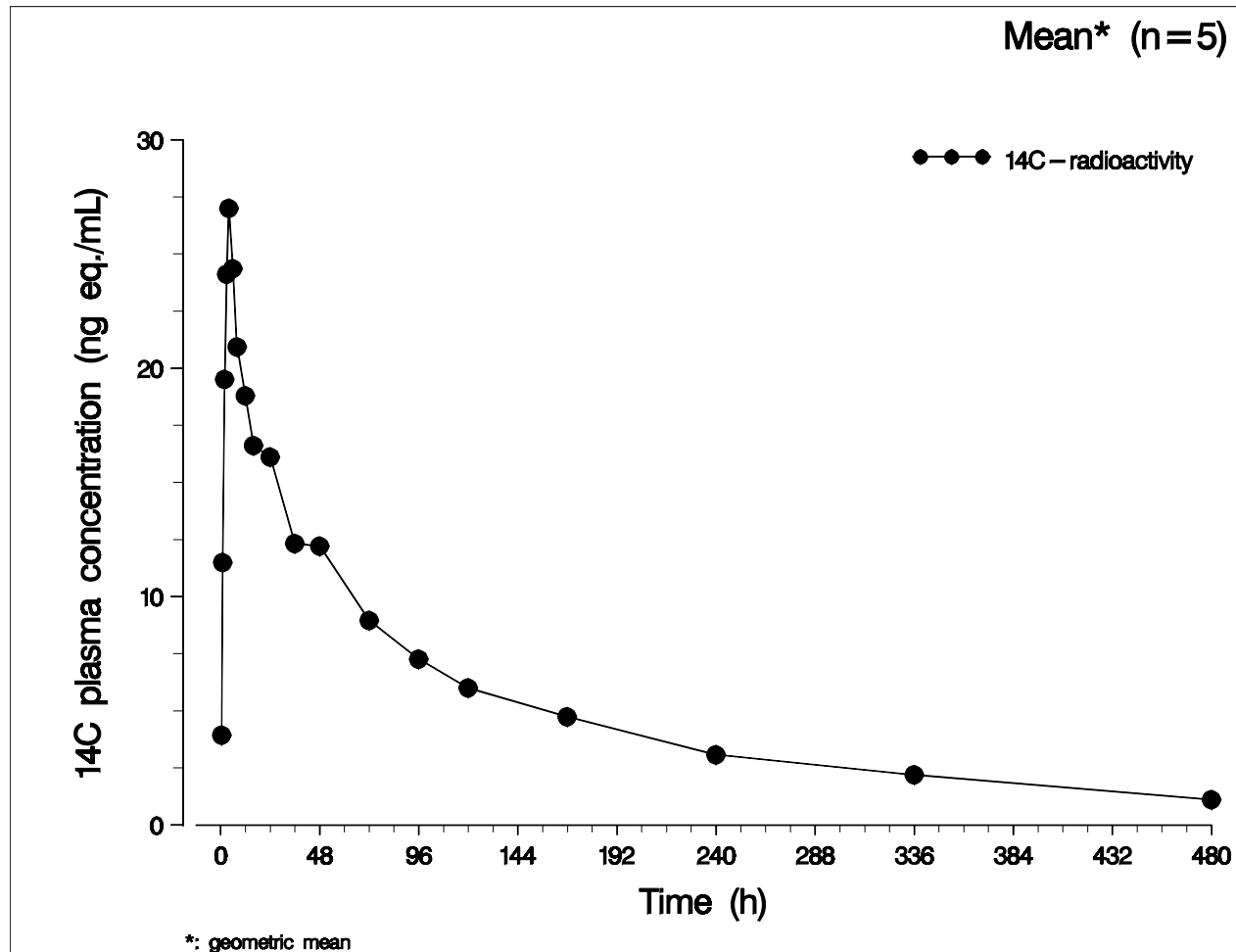
2004 : Low radioactive dose clinical study

- 500nCi administered to healthy male volunteers
 - dose necessitated by predicted protracted recovery (based on animal data)
 - collections made over several weeks following dosing
 - excretion balance and total radiocarbon in plasma measured by AMS

Example study data (ctd..)

2004 : Low radioactive dose clinical study (ctd..)

Plot of total radiocarbon in plasma with time after oral dosing



Note : long $t_{1/2}$ of radioactive drug-related material in plasma ; protracted recovery

2004 / 2005 :
Metabolite Profiling of Samples from
Low Radioactive Dose Clinical study

Example study data (ctd..)

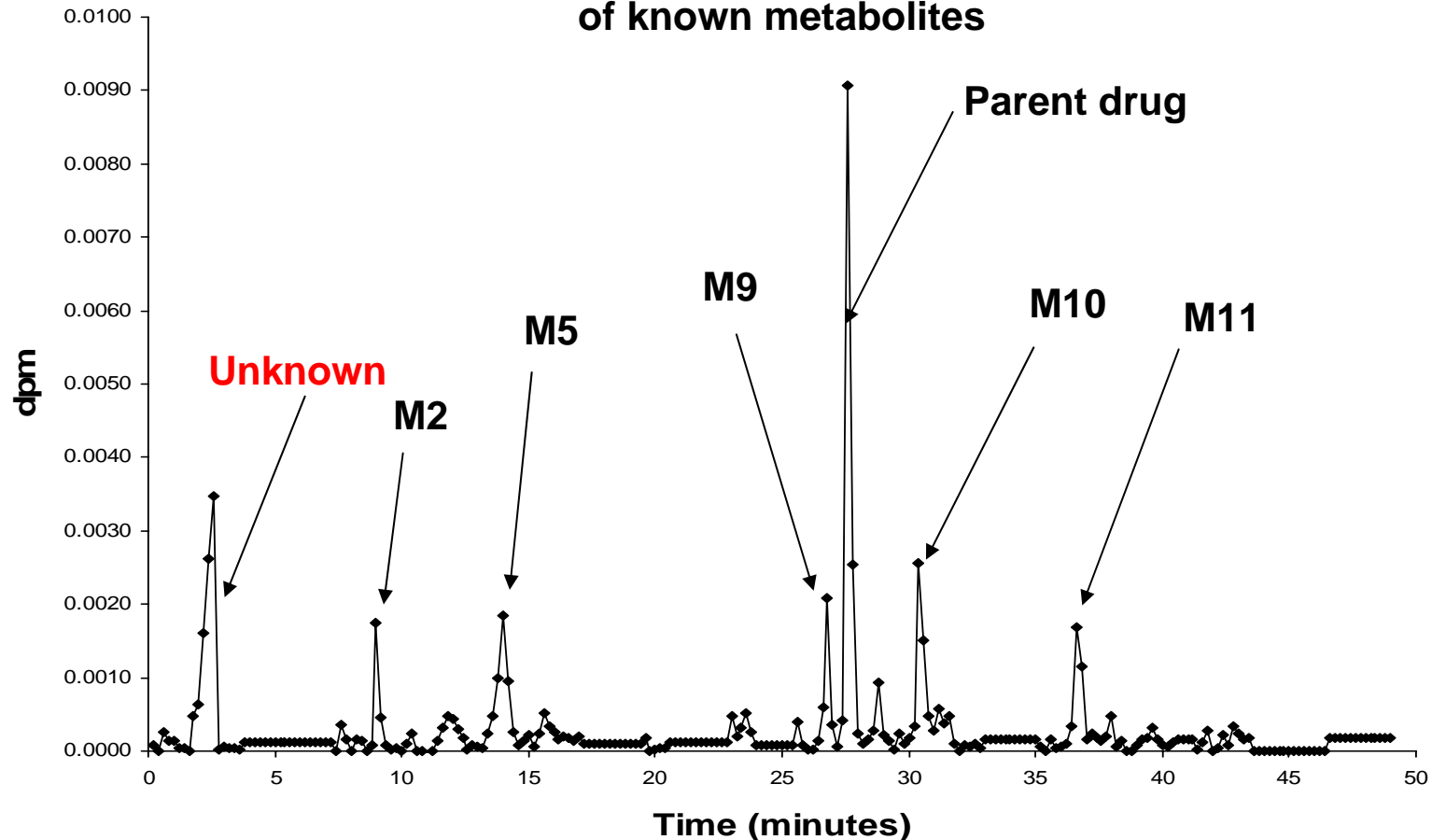
2004-2005 : Metabolite profiling of samples from low dose clinical study

- Profiling of plasma and urine generated from the above study
 - HPLC fractions (5 – 0.02dpm on column) analysed by AMS
 - ~250 fractions generated with some pooled to reduce no. analysed by AMS

Example study data (ctd..)

2004-2005 : Metabolite profiling of a post-dose plasma sample
(0.06 dpm on column)

Reconstructed radiochromatogram showing retention times
of known metabolites



Note : Most major peaks observed co-chromatographed with known metabolites

Future of AMS in GSK

- potential impact on Drug Development

Future of AMS in GSK - potential impact on Drug Development

Limitations of AMS

- AMS provides no direct structural identification
 - needs to be coupled with info. from MS and/or NMR for full structural id [achievable if doses are sufficiently high]
- Robust chromatography is of paramount importance in profiling by AMS
 - use of metabolite chromatographic markers is a distinct advantage
 - pre-clinical work often advantageous [consider application of AMS to animal work also]
 - use of 2 chromatography methods may be prudent

Future of AMS in GSK - potential impact on Drug Development

Limitations of AMS

- Many “AMS” studies may not be exempt* studies
 - although drive towards reducing radioactive doses
 - & lead to greater flexibility in study designs

* Exempt from approval via ARSAC (Administration of Radioactive Substances Advisory Committee, UK) - For many compounds this would result from a dose of <100nCi

Future of AMS in GSK - potential impact on Drug Development

Current situation

- GSK use of AMS currently focussed on HRS support :
 - Inhaled & Intranasal compounds; Dermal compounds
 - low dose (potent) oral compounds
 - poorly absorbed oral compounds

Future of AMS in GSK - potential impact on Drug Development

Short term

- Harnessing the sensitivity of AMS
 - Study designs may be impacted due to availability/accessibility of AMS
 - Use in non-clinical studies and in-vitro studies where AMS sensitivity is enabling
 - Applications outside of DMPK remit
- Conventional HRS studies still conducted
 - early AMS study adds value to conventional HRS
 - Enable early conduct of HRS
 - Avoid surprises in the late stage HRS
[understand metabolism of NCE's in humans earlier in development]
 - Early “validation” of the selected toxicology species

Future of AMS in GSK - potential impact on Drug Development

Longer term

- AMS applied to typical compounds as well as to atypical!
 - supporting early profiling and quantification of circulating metabolites
- Possibility of use for microdosing studies
 - Compound selection, or de-selection
 - Definition of IV pharmacokinetics in cases where drug solubility is dose limiting [assumption of linear PK across doses]
- Reduction of ¹⁴C dose in some preclinical studies

Future of AMS in GSK - potential impact on Drug Development

Potential impact of data

[simplified view]

- if no significant circulating metabolites in man then no effect on comp. progression
 - if significant circulating metabolites then compound possibly placed on hold
- BUT** – better to discover this early in development, rather than late!!

Future of AMS in GSK

- potential advances in AMS Technology

Future of AMS in GSK – potential advances in AMS Technology

- Advances in AMS sample preparation technology
 - automation of graphitisation process (throughput bottleneck)
- or
 - identify new graphitisation process
- Interfacing of AMS with either on-line or off-line HPLC
 - GSK aware of several groups who are looking into these aspects
- Adaptation of current AMS vs. Adoption of “next generation” AMS
 - GSK recognises that AMS technology is evolving quickly

Summary

- AMS used in increasing frequency and widening range of applications
 - clinical, non-clinical & in-vitro for “*AMS*” and “*hybrid*” studies
- Cost of future CRO use predicted to be high
 - move towards in-house capability
- Objective to elucidate metabolism in man early in development
- Further widening of applications as AMS becomes established in GSK?
- Active interest in development of AMS, particularly sample preparation and interfacing

Acknowledgements

- Xceleron Ltd. for their assistance in all AMS studies presented
- GSK colleagues
 - Will Ellis for all of his practical and theoretical assistance
 - GSK, & particularly DMPK, Management for their support

Back-ups

Future of AMS in GSK

- potential impact on Drug Development

Radiation Exposure Risk Categories.....where AMS “fits in”

Level of risk	Risk Category	Effective dose range (mSv)	Typical radio doses ¹	Comments
Exempt (ARSAC)	NA	<0.001 (<1 µSv)	<100 nCi	AMS required – all aspects ²
Trivial	I	<0.1	1 – 10 µCi	Mainly LSC – AMS for profiling
Minor	IIa	0.1 – 1	10 - 100 µCi	LSC (AMS use for late timepoint profiling)
Intermediate	IIb	1 – 10	>100 µCi	eg. PET studies
Moderate	III	>10	> 1 mCi	

¹ As may be used in HRS

² For some compounds, even AMS may not allow metabolite profiling for plasma to be achieved

Low ^{14}C dose - matrix influences on sensitivity

Sample description	Matrix	Volume or weight analysed	Mean predose or bkg	SD (pooled)	CV %	LOQ	
						after predose or background has been subtracted	
						Best estimate	95 % confidence interval
Faeces	homogenate	50mg	0.60	0.17	28	0.86	0.58 - 1.97
Urine	neat	200 μl	0.11	0.040	36	0.20	0.15 - 0.37
Serum	neat	60 μl	0.57	0.087	15	0.43	0.28 - 0.96
Serum extract	supernatant	800 μl	0.025	0.0042	17	0.021	0.017 - 0.026

						LOQ	
						including background	
HPLC fraction of serum extract ⁴	mobile phase	150 μl	0.0011	0.0016	145	0.009	0.008 - 0.012



GlaxoSmithKline

History of use of AMS in GSK (continued)

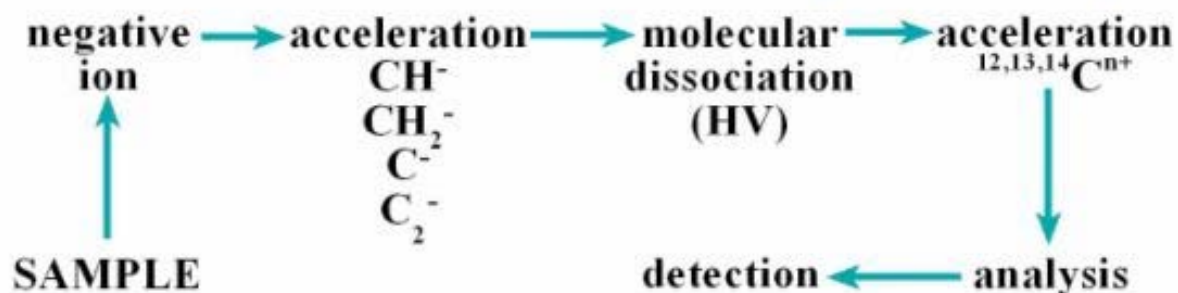
- In-vitro Blood Binding Study

- To investigate % binding and displacement of Drug X from RBCs
- Initial studies carried out at high concentrations (100 -10,000 ng/mL)
- Therapeutic exposures predicted to be much lower
- Further incubations carried out at 0.01 - 1 ng/mL (2.6 - 260 dpm/ml)
- Plasma, blood and diluted samples (10 and 100 - fold) assayed by AMS

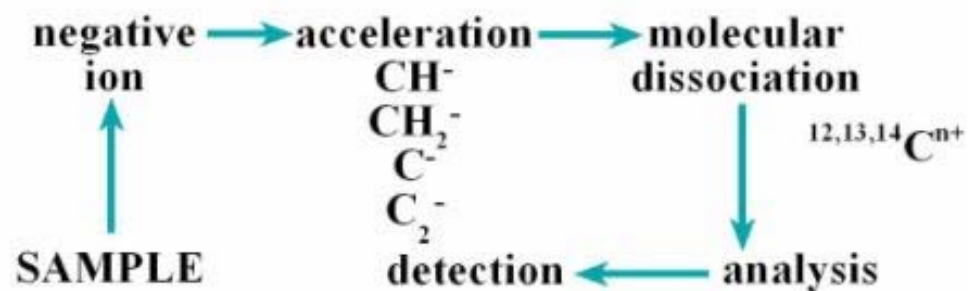
GSK's in-house AMS capability

Principle of Operation

Tandem Accelerator Mass Spectrometry



Single Stage Accelerator Mass Spectrometry



1998 :
Pilot Rat Excretion Balance study

Example study data

**1998 : Pilot rat excretion balance study
(Fluticasone Propionate - published work*)**

2 Groups of rats used (same total dose of FP for each group)

Group 1 : “High” dose group

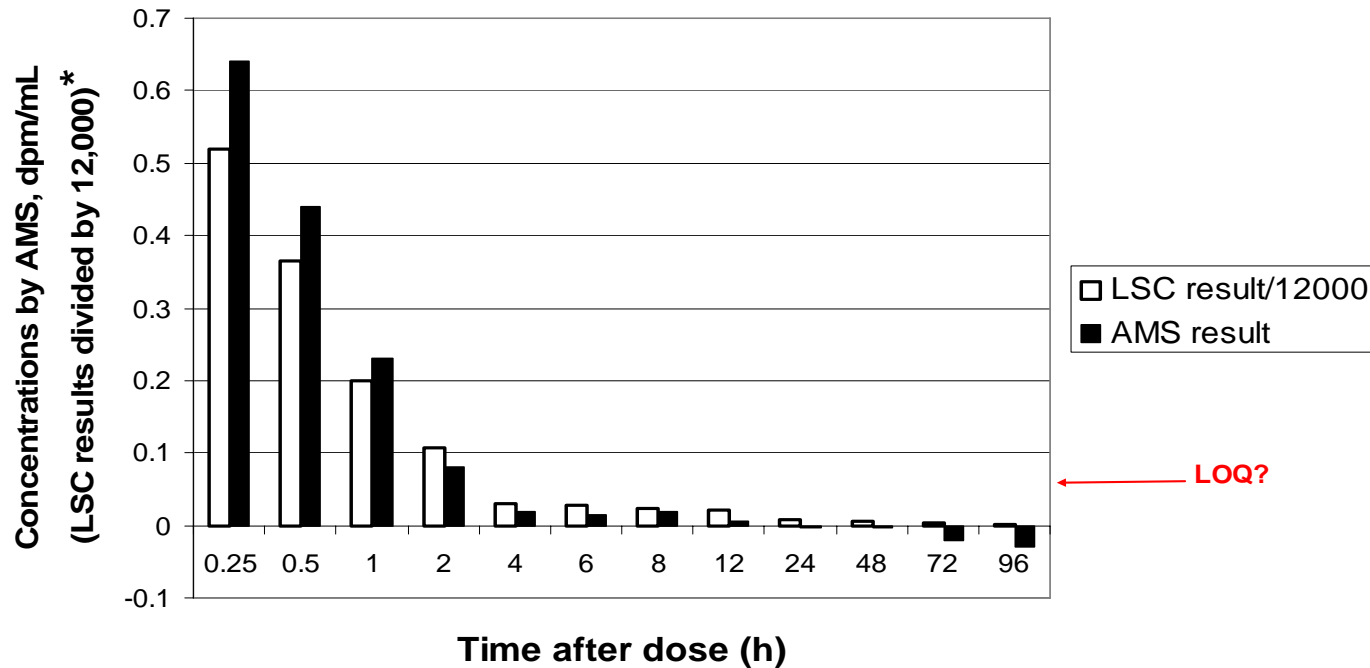
- 3 μ Ci administered to each rat
- samples analysed directly by LSC
- diluted (12,000-fold) for analysis by AMS
- comparison made between LSC and AMS data

* Garner *et al.*, JPBA, 2000, 24, p197-209

Example study data (ctd..)

1998 : Pilot rat excretion balance study – Group 1

Normalised comparison of the analysis of neat rat plasma samples by LSC with analysis of the same samples by AMS (following dilution by 12,000-fold)



Example study data (ctd..)

1998 : Pilot rat excretion balance study – Group 2

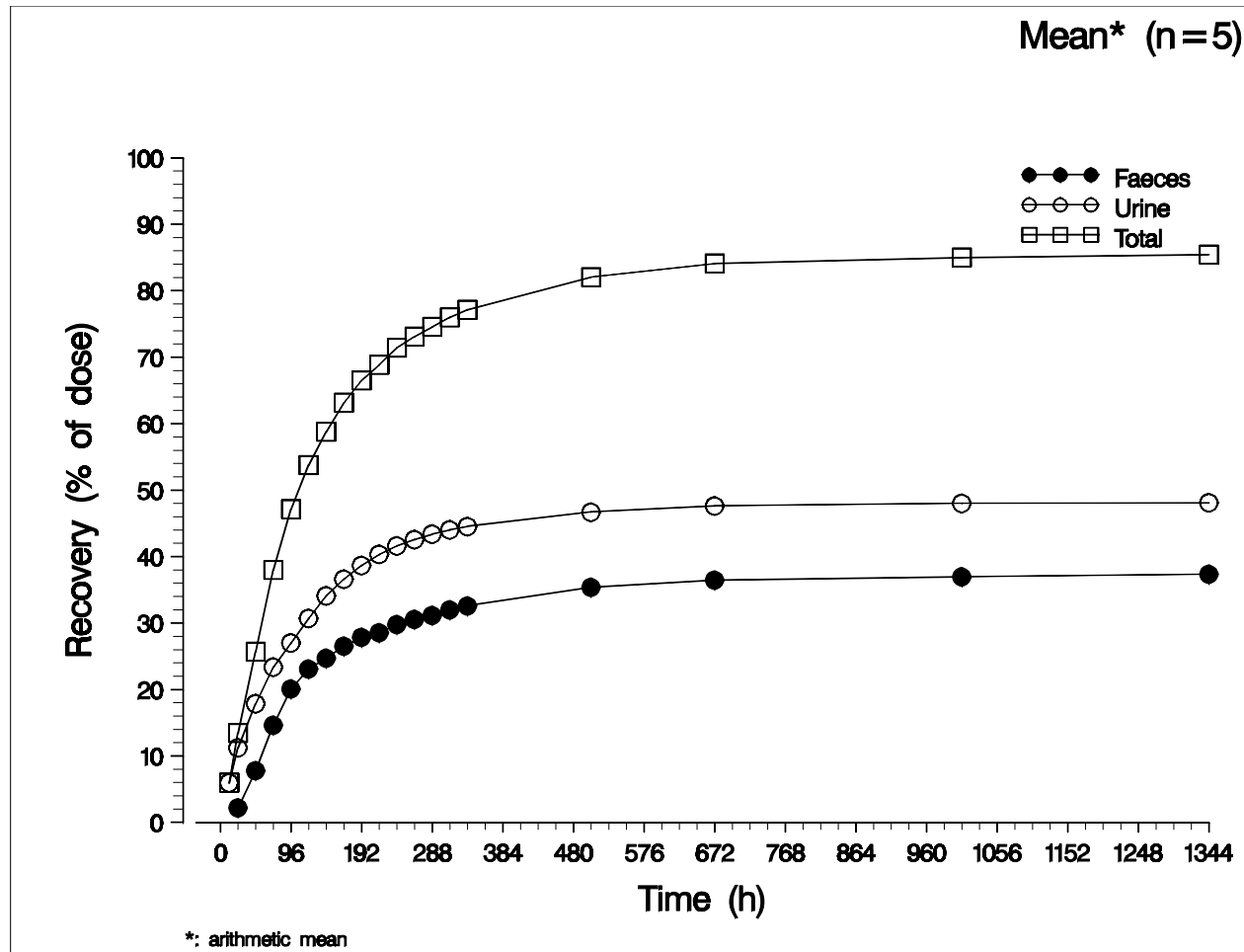
Group 2 : “Low” dose group

- 4nCi administered to each rat
[to simulate a human dose producing exposure of 0.9 μ Sv]
- analysis of undiluted samples by AMS
- excretion pattern (into urine and faeces) similar to that seen with Group 1 rats

Example study data (ctd..)

2004 : Low radioactive dose clinical study (ctd..)

Plots of cumulative excretion of radiocarbon with time



Note : AMS study showed protracted recovery in man!

Example study data (ctd..)

1999 / 2000 : Pilot low radioactive dose clinical study (ctd..)

Percent Recovery of ^{14}C -GI181771 related ^{14}C in human faeces

Collection time (h)	Volunteer number						Mean %
	61	62	63	64*	65	66	
0-24	11.8	<6.3	34	38	43.6	103	38.5
24-48	85.5	44.6	53.4	NS	15.8	<6.0	39.9
48-72	<1.2	19.2	8.7	NS	40.3	<5.8	13.6
72-96	<6.2	<7.4	<2.2	NS	<3.3	<6.4	<5.3
96-120	NS	<3.7	<3.2	<5.2	<6.4	<8.9	<5.8
Total	97.3	63.8	96.1	*38	99.7	103	92.0

* excluded from mean calculations