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

Graeme Young
DMPK,
GlaxoSmithKline Research and Development Ltd.

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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 $10^6 \times$ more sensitive than Liquid Scintillation Counter
  ➢ $\sim 1000 \times$ more mass sensitive than Mass Spec.
  ➢ $10^{10}$ more sensitive for $^{14}$C than for $^{13}$C
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”
The graphitisation process..... flames, furnaces and cryogenics
The chemistry of graphitisation

Combustion stage

Sample eg. plasma, urine, LC fraction is flame-sealed in an evacuated quartz tube.

Quartz tube placed in furnace:

$$\text{sample + CuO } \xrightarrow{900^\circ C \ 2\ hr} \text{CuO}_2/\text{Cu} + \text{CO}_2 + \text{N}_2$$

Reduction stage

- CO$_2$ is cryogenically transferred and sealed into a borosilicate tube.
- Borosilicate tube placed in furnace and reaction is carried out at 500$^\circ$C for 4 hrs and 550$^\circ$C for 6 hrs.
- Zn is present to become oxidised as is TiH$_2$ as a source of hydrogen.

- TiH$_2$ $\rightarrow$ 2H$_2$ + Ti
- CO $\rightarrow$ CO + H$_2$O
- CO + Zn $\rightarrow$ CO + ZnO
- C + H$_2$O

Cobalt catalyst

3 day process !! – batch size of 200 samples
Analyte isolation sample prep: LC followed by off-line analysis by AMS ["LC+AMS"]

- Human plasma sample or extract
- Fractions graphitised prior to analysis by AMS

Parent peak isolation by chromatography

Parent fraction collected from each sample
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 publication¹ in Bioanalysis, Vol. 3, Issue No. 4, Feb 2011
## Key differences between LC-MS & LC+AMS

<table>
<thead>
<tr>
<th>Consideration</th>
<th>LC-MS</th>
<th>LC+ AMS</th>
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</table>
| 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)”

<table>
<thead>
<tr>
<th>Study Type</th>
<th>Primary output</th>
<th>Decisions</th>
<th>Main Customer(s)</th>
</tr>
</thead>
</table>
| ¹⁴C 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 |
| Microdose (stand alone) [Cold or ¹⁴C] dose of ≤ 100µg via IV and/or PO route | | | |
| IV ¹⁴C tracer + Oral therapeutic (concomitantly) | - Absolute Bio. %  
- Metabolism info. | - Direct formulation effort?  
- Project progression? | - Project Team  
- Clinical PK/DMPK  
- Formulation scientists  
- Regulators |
Assay quality

- What is appropriate?
- What do we need?
- What is achievable?
## What kinds of assay quality do we achieve?

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Specific activity</th>
<th>Assay range (LLoQ-HLoQ)</th>
<th>QC acceptance criteria; actual</th>
<th>No. of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (DDI microdose)</td>
<td>[9.3kBq (250nCi)/50µg]x2</td>
<td>4.3423 - 1400 pg/mL (0.0548dpm/mL [CV = 14.5%] - 17.7dpm/mL)</td>
<td>+/- 30%; 70% of QCs in 3 assay batches within limits</td>
<td>10M Healthy</td>
</tr>
<tr>
<td>2 (early microtracer)</td>
<td>10kBq (270nCi)/100µg</td>
<td>0.882 pg/mL - 441 pg/mL (0.0045 to 2.25 dpm/mL)</td>
<td>+/- 25%; &gt;70% of QCs in 4 assay batches within limits</td>
<td>6 M Healthy</td>
</tr>
<tr>
<td>3 (early microtracer)</td>
<td>10kBq (270nCi)/100µg</td>
<td>12.3 - 1820 pg/mL (0.0684 to 10.1 dpm/mL)</td>
<td>+/- 20%; 90% of QCs in 2 assay batches within limits</td>
<td>8M Healthy</td>
</tr>
<tr>
<td>4 (early microtracer)</td>
<td>9.3kBq (250nCi)/100µg</td>
<td>30.2 - 1460 pg/mL (0.16 to 7.7 dpm/mL)</td>
<td>+/- 20%; 86% of QCs in 2 assay batches within limits</td>
<td>7F Healthy</td>
</tr>
<tr>
<td>5 (“regulatory” microtracer)</td>
<td>7.4kBq (200nCi)/50µg</td>
<td>1.8 - 470 pg/mL (0.0163 to 4.17dpm/mL)</td>
<td>+/- 20% at High and Mid; +/- 25% at Low; study ongoing</td>
<td>4 M/F Cancer Patients</td>
</tr>
<tr>
<td>6 (“regulatory” microtracer)</td>
<td>7.4kBq (200nCi)/5µg</td>
<td>1.1 - 104 pg/mL (0.1 to 9.1dpm/mL)</td>
<td>Study ongoing</td>
<td>6 (?) M/F Cancer Patients</td>
</tr>
</tbody>
</table>
AMS Technology development: Laser interface – gas analysis
Collaboration initiated October 2007
- Based upon MIT’s patented design for laser interface to generate CO$_2$ from dried HPLC/UPLC fractions
- 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)

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

1970’s: AMS Invented

1970-1980s AMS first used for carbon dating

1990’s: First use in biochemistry

1990s: First use in biochemistry – Mass Balance and Metabolite Profiling

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

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

2011: White Paper on assay validation processes being refined

Several studies ongoing…assay validation processes being refined

Several studies ongoing…assay validation procedures being refined

Several studies ongoing…assay validation procedures being refined

Several studies ongoing…assay validation procedures being refined
Summary

- No formal guidance exists for application of AMS in bioanalysis

- Use in determining intravenous PK in humans justifies that appropriate 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

The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents.
Novel clinical study design for PK
– IV Microtracer
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
Study design

- IV $^{14}$C-tracer dose administered concomittantly at $T_{\text{max}}$ 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 $\leq 1/200^{\text{th}}$ of oral dose @ $<270\text{nCi}^{14}\text{C}$

- Conventional dose of $^{14}$C is $>200x$ greater
IV Microtracer + Therapeutic Oral Dose

Conventional IV clinical dose approach:

1. Animal dosimetry
2. Intravenous tox.
3. IV tolerance
4. GMP Manufacture
5. Dosing

High effort level and ~12 months
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 $T_{\text{max}}$ 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)
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 $^{14}\text{C}$ 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 $^{14}\text{C}$ 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
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
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?
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