Capillary Plasma Microsampling – Letting the data speak for itself

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Outline

A technique refresher

Share the journey to implementation in the regulatory (GLP) setting

Challenges & opportunities

Summarise the data we have generated
The Technique
AZ approach to capillary plasma microsampling

Blood collected 32μL into a hematocrit capillary

Capillary centrifuged within 30 min

Capillary scored and cut using capillary cutter

Plasma is transferred to accurate 8 or 4μL capillary

8 or 4μL capillary transferred to labelled fluid X tube

Diluted plasma sample processed for analysis and transferred for frozen storage

Automated addition of diluent and sample preparation

Samples are handled using automated decapping device
Implementation
Opportunities - The benefits were compelling

**Ethical**
- Reduce the number of animals used in pre-clinical research
- Remove the need for satellite TK animals from toxicology studies
- Quicker and less stressful sampling for the animals
- Smaller blood volumes can be beneficial in the clinical setting

**Scientific**
- Relate toxic effects to exposure in the same animals
- Not previously possible due to total blood volume constraints
- Improved modelling of individual animal effects

**Cost**
- Less compound required (as less animals to dose)
- Particularly valuable in early project lifecycle
- Less animal husbandry and study handling

Capillary plasma microsampling
More challenging for the bioanalyst but we’re enabling animal ethics & improved interpretation
Our journey to implementation – was LONG

All TK sampling in rodent from main study animals for GLP and non-GLP studies

Agreed implementation with our pre-clinical preferred partner
Definitive In-vivo data generated to support move to main study animals in GLP rodent toxicology studies
MHRA reviewer visited AZ to observe technique

Cross functional implementation team finalised proposal
Safety and DMPK leadership teams approve capillary microsampling in main study animals for use in regulatory facing non-GLP rodent toxicology studies

Work pioneered by Ove Jonsson
Blood microsampling in routine use within Discovery functions
Transitioned from blood to plasma microsampling
Extensive evaluation across multiple AZ sites
Global bioanalysis business case developed in 2010
Implementation team - Challenges

- Defining best lab practices
- What studies, study design & MIST
- Addressing stakeholder scientific & regulatory concerns
Influencing stakeholders

- 2012 SARB
- Internal and external meeting presentation
- AZ Endorsement for GLP work in 2014
- David Jones MHRA visit
- Bespoke study data shared widely
- NC3Rs
- Impact on Clinical pathology seen as blocker
**Scope**

Rodent
Investigational toxicology
Safety pharmacology
Genetic toxicology
Reproductive toxicology
GLP & Non-GLP

* Inhalation and on a case by case basis other projects with expected low exposure levels and/or analytical sensitivity issues

**Study Design**

Non-GLP dose finding studies
Serial sampling
GLP repeat dose studies
Composite sampling
Accurate volume capillary method validation

Validation samples are prepared in bulk, and then transferred to 8 µL capillaries
Validation samples diluted on day of extraction
As preparation mimics study samples, the following additional validation experiments are required:

Stability (Low, High & Dil QC)
- Freeze Thaw – Undiluted (2 cycles), Diluted (3 cycles)
- Room Temperature – Undiluted (24 hours), Diluted (24 hours)
- Long term – Undiluted, Diluted

Assessment of 4 µL sample receipt (Low and High QC)
- Validation samples prepared in bulk and then transferred to 4 µL capillaries
- Diluted on day of extraction by adding ½ volume of diluent
- Analysed in one batch (n=6)

Validation
• Selectivity
• Accuracy
• Precision
• Sensitivity
• Matrix effects
• Stability
Range of the Assays

Concentration (µmol/L) vs. Analytical Method Number

- LLOQ
- ULOQ
Inter Assay Accuracy (8µL Capillary)

Analytical Method Number

Bias (%)

20%

15%

-15%

-20%
Inter Assay Precision (8 µL Capillary)

LOQ within 20%

CV(%)
4 µL Capillary intra assay Accuracy

Required evaluation of low QC data

- Multi-analyte method (2 metabolites passed, parent failed)
- 8µL from same bulk preparation passed with high bias
4 µL Capillary intra assay Accuracy

- Bulk re-preparation & analysis of 8 and 4 µL passed
- Conclusion: high bias on preparation compared to nominal
4 µL Capillary intra assay Precision

Required evaluation of low QC data
• 8 µL same bulk preparation passed
• No obvious reason for the failure
4 µL Capillary intra assay Precision

- 4 µL repeated and passed (CV of 2.8% and a bias of 1.0%)
- Conclusion: Potential issue with quantitative washout
- Highlights further investigation into 4 µL capillary procedures may be required
Long Term Stability – Plasma stored at -20°C in capillaries (approximately 1 month)
Long Term Stability – Diluted Plasma stored at -20°C in capillaries (approximately 1 month)
24 hour Room Temp Stability – Plasma stored in capillaries

Analytical Method Number

Bias (%)
24 hour Room Temp Stability – Diluted plasma in capillaries
Freeze Thaw Stability – Plasma stored at -20°C in capillaries

• Low QC -18%
• Diluted sample freeze/thaw result, -0.7%, derived from same sample
• Bias confirmed as analysis issue not stability
Freeze Thaw Stability – Diluted Plasma stored at -20°C in capillaries

Results are a combination of 2 or 3 cycles
Dependent on what was included in the validation

Analytical Method Number
Incurred Sample Reproducibility Data

1 Month Repeat Dose Toxicology Study
Opportunities - The benefits are compelling

- Ethical
- Scientific
- Cost

Performance is acceptable
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Any Questions?
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