Pushing the Boundaries of Microsampling

Realising and Understanding the Full Potential

EBF Symposium 2014

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The AstraZeneca Journey So Far....

Work pioneered by Ove Jonsson
Blood microsampling in routine use within Discovery functions
Transitioned from blood to plasma microsampling for toxicology evaluations

Definitive in-vivo data generated to support move to main study animals in GLP rodent toxicology studies

Safety and DMPK leadership teams approve capillary microsampling in main study animals for use in regulatory facing non-GLP rodent toxicology studies

TK sampling in rodents from main study animals for GLP and non-GLP studies
Recap on Benefits of Microsampling

**Macrosampling**

- Causes less distress to animals during sampling
- Only one technician needed for blood sampling
- Lower costs - Fewer animal required per study & less test item used
- More TK sampling time-points feasible
- TK samples taken from main group animals - improved data quality

**Microsampling**
AZ approach to capillary microsampling

Sample collection and processing

32 µL blood taken → Plasma separated → Accurate plasma volume taken → 8 µL plasma + 90 µL water

Haematocrit tube → Centrifugation → Tube scored and plasma transferred into capillary → Plasma CMS diluted with water* prior to extraction

4 µL plasma + 45 µL water

* Water used as default diluent, but stabilisation agents can be added if required
So how far can we push?

- Smaller blood volumes (< 32µL)?
- Smaller plasma volumes (< 8µL)?
- Different blood processing methods?

Parameters to evaluate....

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Pushing the boundaries – Evaluation

Analytes Used

- Midazolam (Basic)
- Diclofenac (Acidic)
- Compound X (Neutral)
- Compound Y (Acidic)

Tests carried out

- 20 µL vs 32 µL Blood
- 2x 4 µL vs 8 µL plasma
- Microsamples vs macrosamples
- 20, 40, 50 & 60% Haematocrit
- Blood/PBS vs plasma
Sample preparation methods used

Control blood  Spiked blood

- Analyte in MeCN
- Analyte in 50% MeOH\(_{(aq)}\)

Mix for 15 min but ≤30 min

Plasma microsample

- From 20µL Blood
  - 4µL plasma \(\times 3\)
- From 32µL Blood
  - 4µL plasma \(\times 3,\) Rep 1
  - 4µL plasma \(\times 3,\) Rep 2
  - 8µL plasma \(\times 3\)

Blood/ PBS

- 4µL blood/PBS \(\times 3\)
- 8µL blood/PBS \(\times 3\)

Plasma macrosample

- 4µL plasma \(\times 3\)
- 8µL plasma \(\times 3\)
Outcome of Evaluation...
Blood Volume & Sample Homogeneity

- Does blood volume have an impact on accuracy?
  None observed; a 20µL vs 32µL blood sample are equivalent

- Is analyte distribution homogeneous throughout plasma in capillary?
  Yes; 4µL and 8µL plasma microsamples are equivalent
Plasma volume

- Does plasma volume have an impact on quantification?
  *None observed:* With the exception of Diclofenac, a micro and macrosample appear to be equivalent.
Haematocrit Level

How does haematocrit level affect quantification in a plasma microsample?

Similar trends observed between micro & macro samples

Differences between haematocrit levels are more analyte-specific rather than technique related.
Blood Processing

- Are alternative blood processing methods comparable to plasma microsampling?
  **Yes;** there is potential to use blood dilution in place of plasma separation.
Conclusions

Blood volume
• Use of a 20µL blood sample to generate 4µL plasma is viable (assay sensitivity dependent)

Sample Homogeneity
• Is not affected by using 4 or 8µL capillary microsamples as opposed to traditional macrosamples

Plasma Volume
• No significant differences in quantification between micro and macrosamples

Haematocrit Level
• Similar trends seen between micro and macro plasma samples. Differences between haematocrit levels not sampling technique related

Blood Processing
• There is potential to use PBS for dilution of a blood microvolume, but analyte blood partitioning and haematocrit level to be considered

Going forward.....

• Perhaps carry out further tests on blood/ PBS and investigate the impact of haematocrit level as well as analyte blood/plasma partitioning properties on reproducibility?
Acknowledgments

Amanda Wilson
Sara Viney (University of Leeds)
Kirsty-Jackson Addie
Michael Spreadborough
Teresa Collins
Thank you for listening

Any questions?