Plasma Microsampling

Have we already reached the horizon?

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Content

- Benefits of plasma microsampling
- Predicted bioanalytical barriers
- What has occurred in reality
- Solutions for bioanalytical barriers
Benefits

- Ethical
  - 3Rs
    - Quicker sampling
    - Reduced blood loss
    - Reduced animal numbers

- Scientific
  - Data quality
    - Relate tox directly to exposure
    - TK samples from main study animals/reduced satellite groups

- Financial
  - Budgets
    - Fewer animals = less cost
Bioanalytical Barriers

- Method development
- Validation activities
- Capital investment
- Sample processing

8 μL Plasma Sample
In Reality

5 Drug projects

TK support for 9 studies

LLOQ (nmol/L)
2, 5, 10, 10, 20, 50, 100, 260, 500, 1200

10 Analytes

100% Of Data Delivered On Time

Rodent: DRF MTD Piv tox

2 Methods Validated

8 Bioanalytical methods (HPLC-MS/MS)

On column LLOQ (pmol/L)
10, 30, 160, 160, 250, 300 600, 950, 2100, 4100

Approx 12 fold lower vs. macrosample

100% Of Required LLOQ’s Achieved
Why?

Understanding the importance of ‘accurate volume’ by In-life technicians

- Small amount of additional method validation activities
- Standardisation of wet lab consumables

- Minor capital investment
- Minor additional method development time
Understanding the importance of accurate volume

In early studies many samples received from In-life facility with:
- 4 μL capillaries
- Unknown volumes (cannot be analysed)
- Incomplete profiles & additional sample processing time

In recent studies almost all samples received from In-life facility with:
- 8 μL capillaries
- Few 4 μL capillaries
- No unknown volumes
- Complete profiles & reduced sample processing time

Why?
- Importance of accurate volume samples communicated to and understood by In-life facility
- Repeated exposure to microsampling under ‘real’ study conditions produced a consistent sample quality. Up-front, under pressure training is valuable
Method development time

Plasma samples received from in-life facility as 8 µL capillaries
Require addition of water to create bulk sample for analysis/storage/re-analysis
90 µL water added to 8 µL plasma sample
1 in 12.25 dilution prior to sample preparation

Upfront planning to ensure most sensitive mass spectrometers available
Additional time optimising source conditions post chromatographic optimisation

PPT filter plates used for sample prep:
- Quick method development time
- Greater extract recovery vs. traditional PPT – maximising sensitivity
Method validation activities

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, only the following additional validation experiments are required:

Stability (at LowQC and DilQC levels)
- 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
- Validation samples prepared in bulk at LowQC and DilQC levels, and then transferred to 4 μL capillaries
- Diluted on day of extraction by adding ½ volume of diluent
- Analysed in one batch (n=6)
**Consumable Standardisation / Capital Investment**

- Must remove lids, add water & replace lids for large batches of samples/STD's/QC’s
  - Very time consuming process
  - Significantly increases analysis timelines

- Tube type used in wet lab and in-life facility standardised for all samples
  - Tube selected has 96 well format racks

- Hamilton de-capper purchased
  - Method written to add water to samples on Tecan Genesis RSP 150
  - Takes < 5 minutes to dilute samples
How the solutions effect time in the lab

**Method Development**
- Add Time - 3 Hours – Optimising MS source conditions

**Method Validation**
- Add Time - 30 Minutes – Preparing and extracting extra validation samples
- Sample Receipt
  - Add Time - 15 Minutes – QC prep using capillaries
  - Add Time - 10 Minutes – Identifying any 4 µl capillaries
    - Addition of water using automation

**Sample Preparation**
- No additional time

**Sample Dilution**

**Sample Acquisition**

**Data Report**

Total Add Time Pre Sample Receipt = Approx 3 hr 45 mins

Total Add Time Per Batch = Approx 10 mins
Summary

A small amount of additional steps

Can overcome bioanalytical barriers

The benefits of plasma micro sampling can be easily achieved