Issues with Tissues

Strategies for developing fit-for-purpose PK immunoassays in tissue

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AGENDA

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1. Approaching PK tissue assays
2. Why can't the ICH M10 be applied to tissue assays
3. Development considerations
    1. Analyte Extraction
    2. Deciding tissue concentration
    3. Testing Analyte recovery

Approaching PK tissue assays

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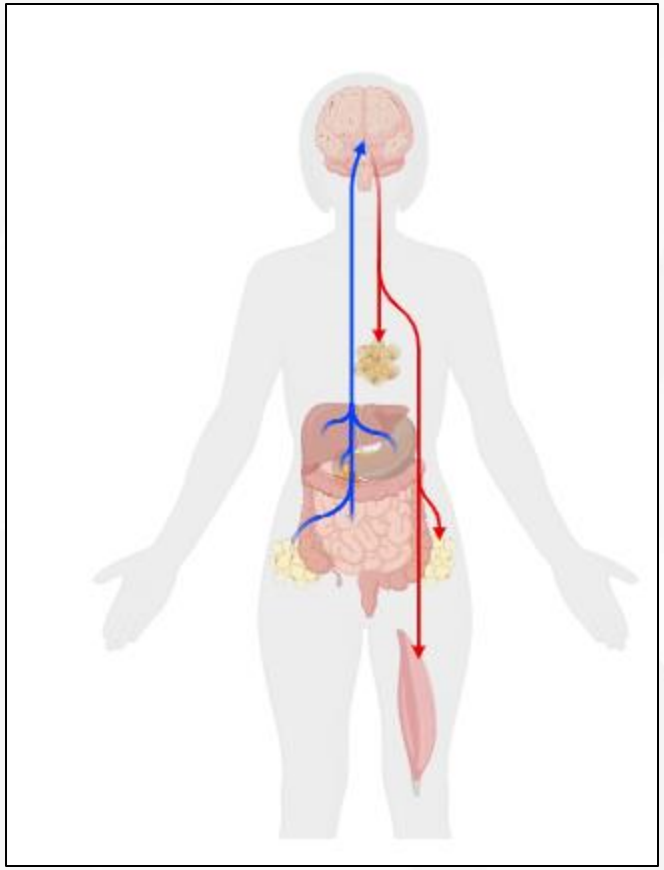
- Tissue PK assays can be employed to determine **distribution of drug to target tissues**
  - Neurology – ability of drug to cross blood brain barrier (BBB)
  - Oncology – ability of drug to penetrate tumour tissue

- Typically **outside the scope of the ICH M10**
  - Usually not the primary matrix
  - Practically very difficult to perform many parameters in ICH M10

- Tissue assays can be important for internal decision making so **appropriate validation** should be performed
  - Eg/ For Neurology, lead candidate selection may be based on ability of the drug to penetrate BBB

- Employing a **fit-for-purpose approach** based on **context of use** is often most appropriate



Why can't ICH M10 be applied to tissue assays

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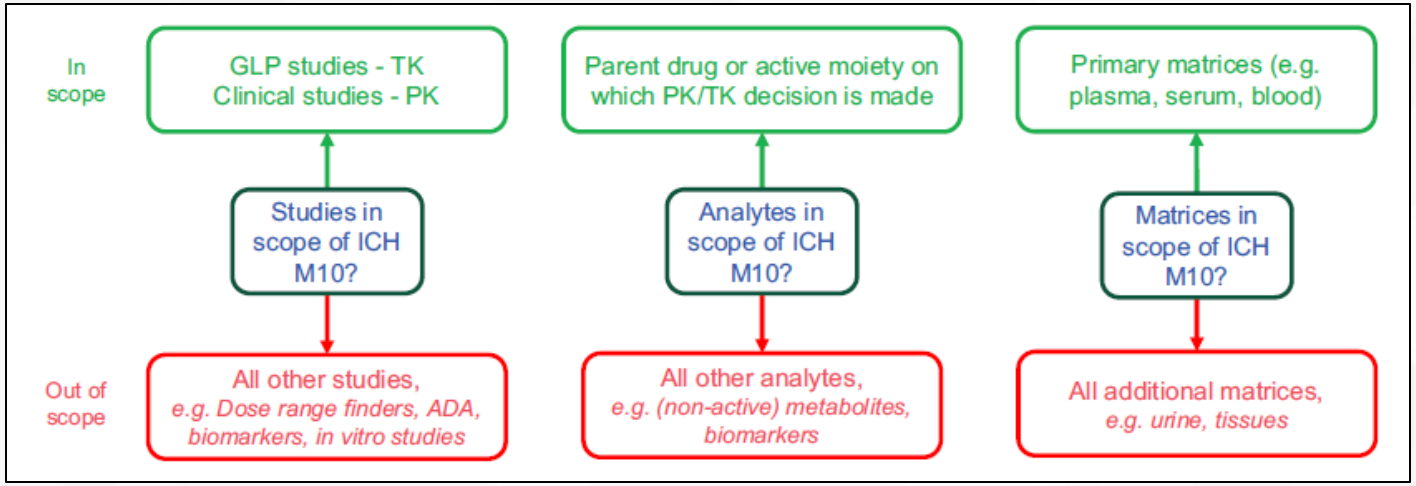
- The main limitation of tissue-based PK assays is the **inability to spike directly into the tissue**
  - Cannot fully assess **recovery, stability or selectivity** from tissue samples

- A CoU based **scientific validation** may be more suitable for tissue assays

- 2025 EBF paper **provides guidance on validation level** however suggested validation parameters are focused on chromatographic assays:

European Bioanalysis Forum recommendation on embracing a context-of-use-driven scientific validation for chromatographic assays in the light of ICH M10



Why can't ICH M10 be applied to tissue assays

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- **Some validation parameters require alternate approaches, or cannot be tested**

Parameter	Limitation	Alternate approach
Selectivity	Cannot spike directly into samples	Spike into homogenate, incubate to replicate *in vivo* conditions Parallelism of incurred samples
Analyte Recovery / consistency		Spike into homogenate, incubate to replicate *in vivo* conditions
Stability in tissue		Perform stability of homogenate and/or supernatant
Matrix	Limited volume of true matrix	Establish surrogate matrix Demonstrate suitability by P&A and selectivity of real matrix vs. surrogate matrix curve

- These approaches need to be applied throughout development to ensure method is suitable for use

Development of LBAs for tissues

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- Development of PK tissues assays require several additional steps:

- Homogenisation method
- Lysis buffer
- Protease inhibitors

- How much tissue is present in homogenate?
- High tissue conc. = higher sensitivity but more matrix effects

- How to ensure consistent recovery of analyte from tissue?

- Optimise format, range, MRD, etc.
- Assessment for a surrogate matrix
- Ensure assay meets context of use

Stage 1

Optimise analyte extraction

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STEP 1: Optimising the physical homogenisation of tissue

- There are many variables to optimise to ensure maximum analyte extraction:

Parameter	Considerations
Homogenisation method	Bead based, Rotor-stator, blender etc.
Bead size and type	Optimise based on type of tissue (soft / hard)
Homogenisation programme	Optimise shaking speed, time and cooling based on type of tissue
Lysis buffer	Optimise based on where analyte is located (nucleus, cytoplasm, membrane bound etc.)
Protease inhibitors	Required to prevent degradation of analyte

Stage 1

Optimise analyte extraction

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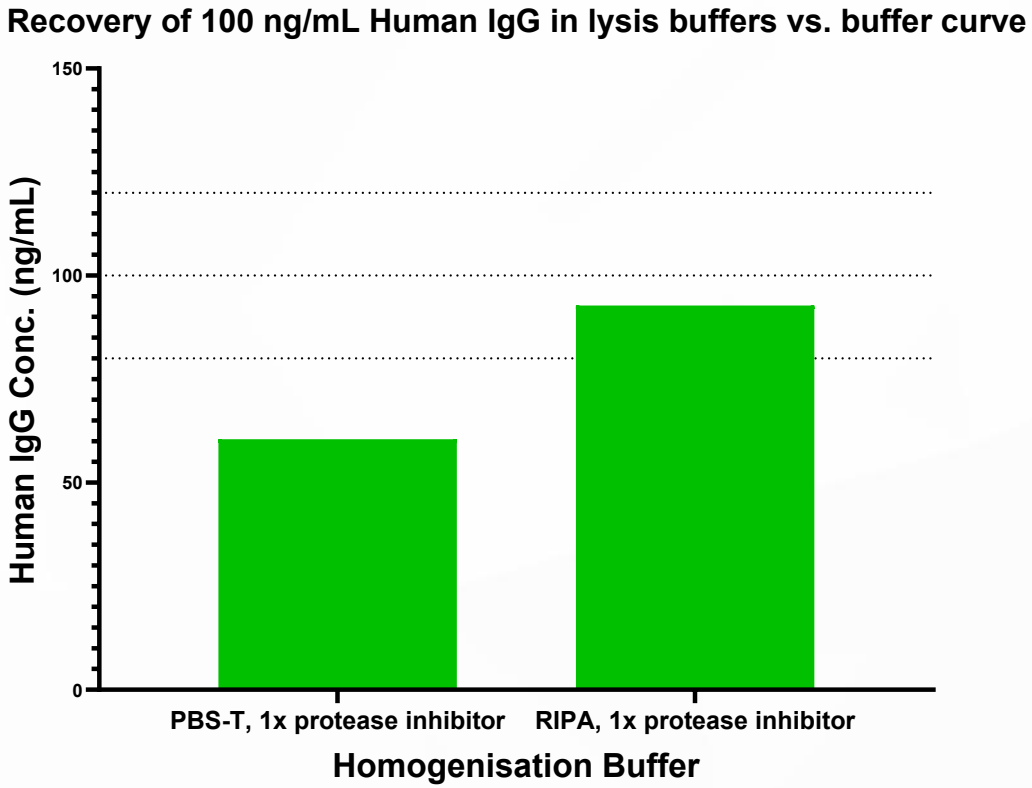

Why is this important?

- **Homogenate recovery** of spiked analyte from rodent tissue homogenised using:
  - 0.05% PBS-T vs RIPA buffer
- **Homogenate spiked** and **incubated at 37°C for 1 hour** prior to processing and analysis
- Progressed with RIPA buffer with 1x protease inhibitor
- Use of suitable lysis buffer is critical for analyte recovery

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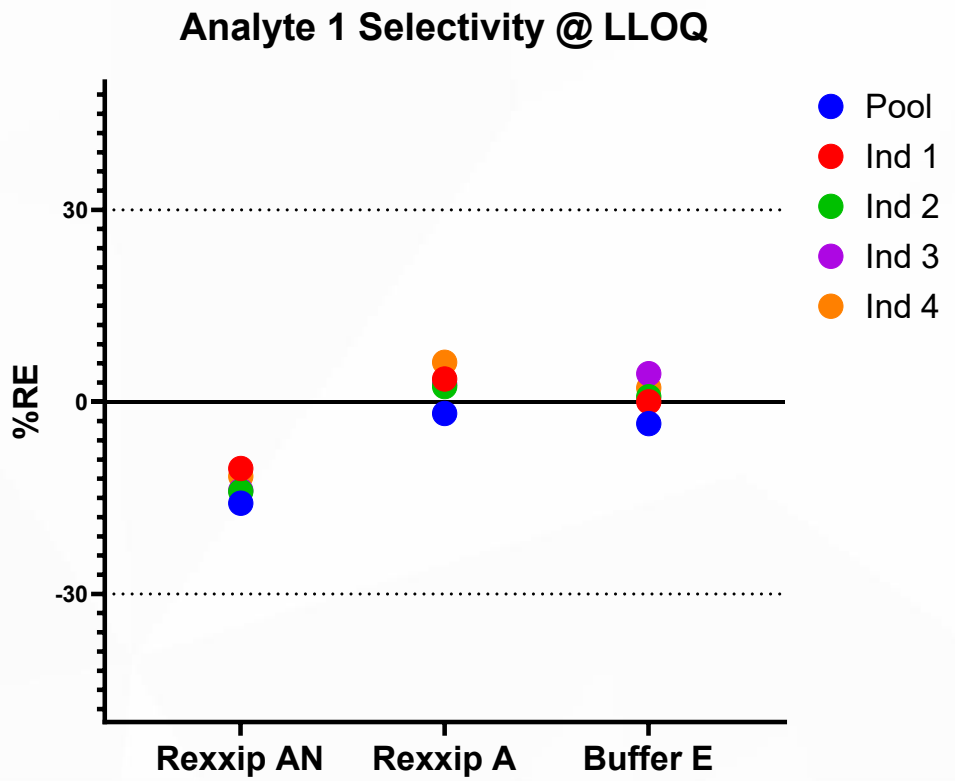
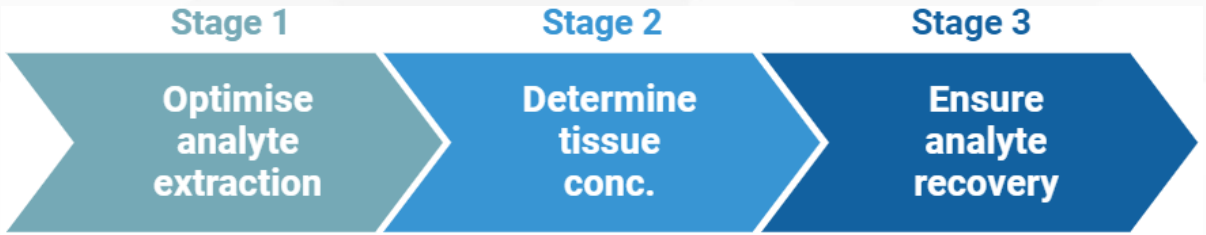
STEP 2: Determine tissue concentration based on desired LLOQ

Determining tissue conc. post homogenisation is a balance between **reducing matrix effects** and having an achievable **assay LLOQ**:

- Low tissue conc. (more lysis buffer) = lower analyte conc. but lower endogenous interference
- High tissue conc. (less lysis buffer) = higher analyte conc. but higher endogenous interference
- Confirm through selectivity spiked into homogenate





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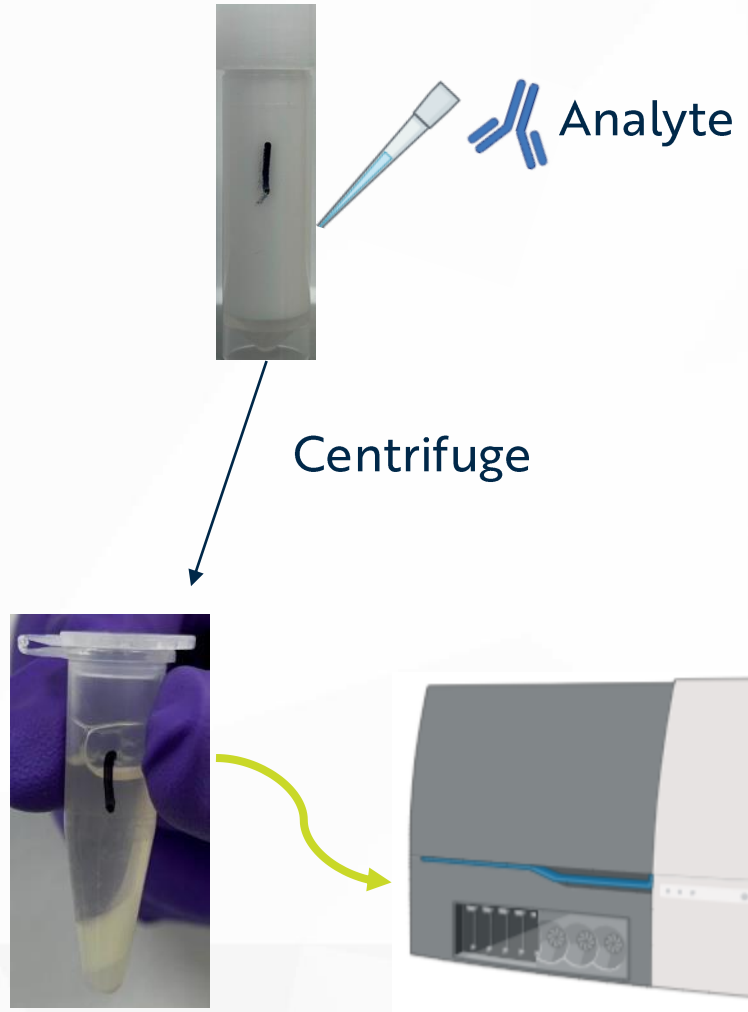
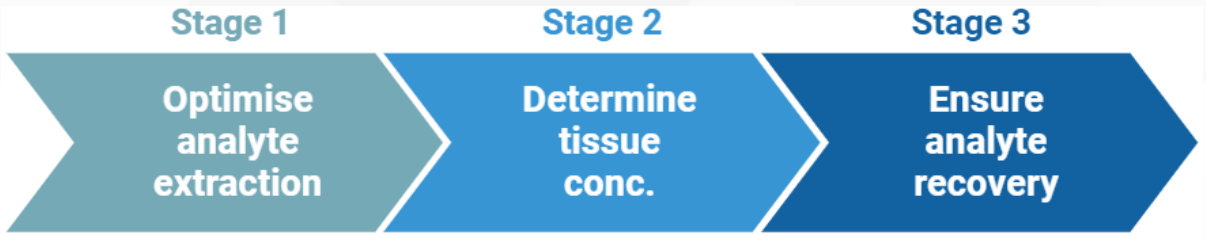
STEP 3: Ensuring analyte recovery

- It is **impossible to truly test** analyte recovery as we are **unable to spike directly into the tissue**

- As a surrogate, we can ensure analyte is not lost when cell debris is removed from homogenate via centrifugation
  - Spike homogenate with analyte
  - Incubate at 37°C for 1 hour to replicate *in vivo* conditions (allow any target/analyte interactions to occur)
  - Centrifuge and remove clean supernatant
  - Analyse vs. a spiked clean supernatant curve





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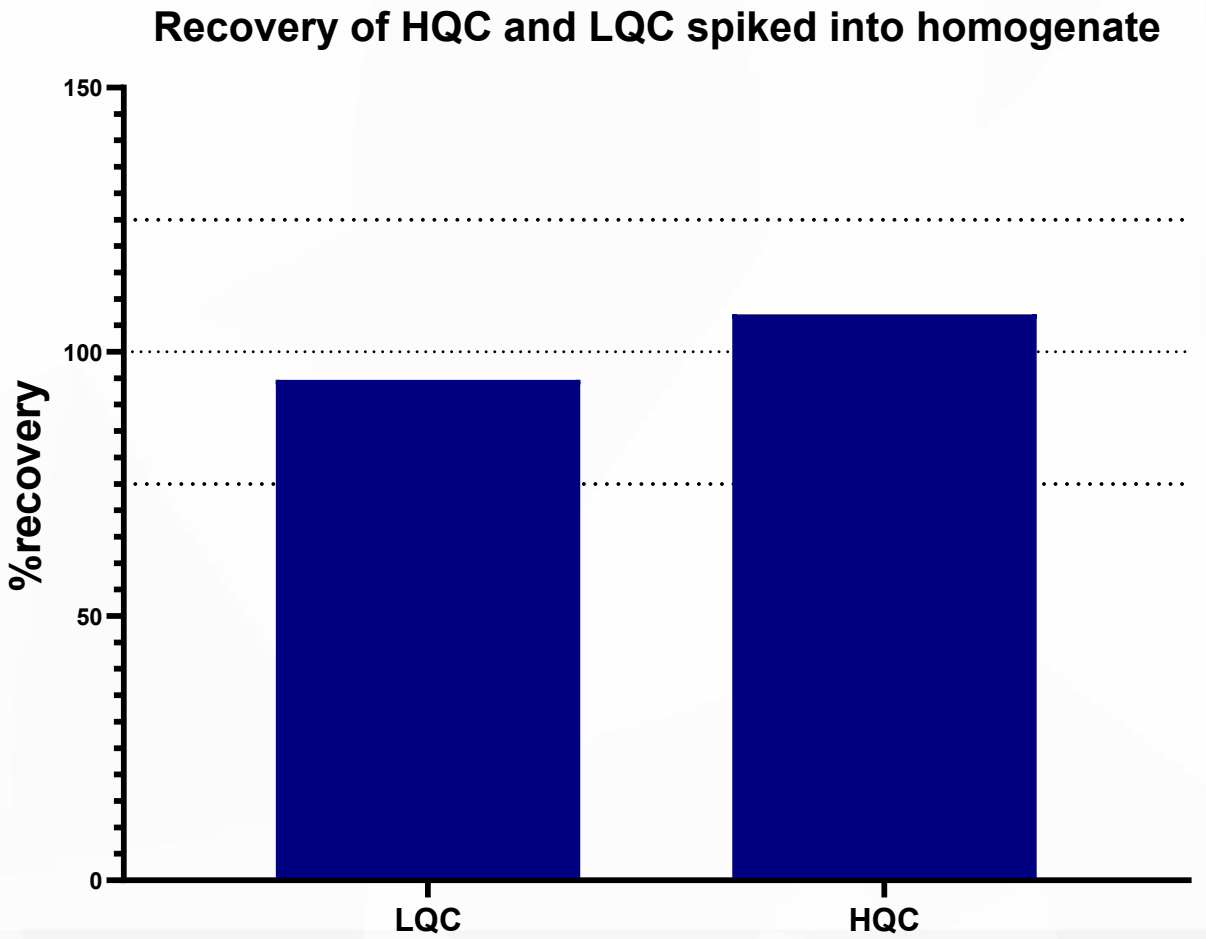
Spiked homogenate shows good recovery against spiked supernatant curve

Analyte is not being lost during sample processing



Summary

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- There are **limitations** when developing and validating a tissue-based LBA due to the **inability to truly replicate samples**

- A **CoU driven scientific validation is recommended** for tissue assays

- Optimisation of tissue homogenisation is important for optimal performance in an LBA
  - Selection of appropriate homogenisation conditions for maximum recovery
  - Appropriate tissue concentration
  - Must ensure analyte is being recovered and not lost during the sample processing steps

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Thanks for listening