Considerations on *ex vivo* Conversion of Prodrugs during Bioanalysis

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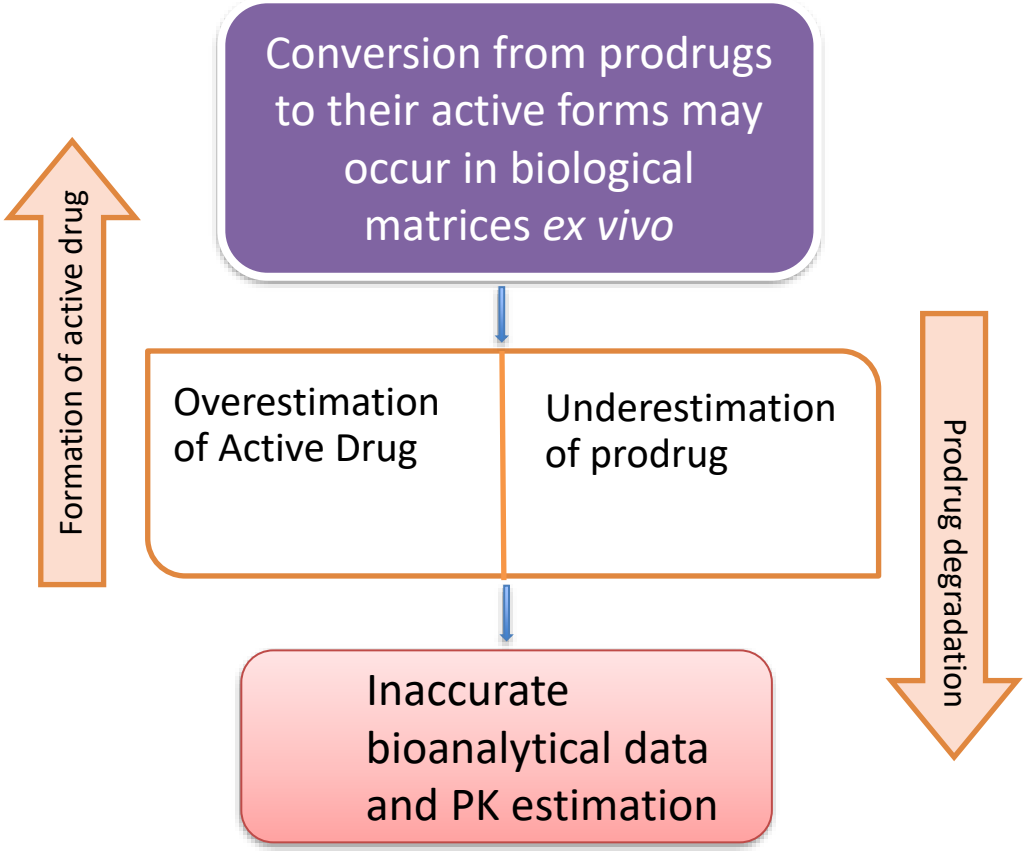
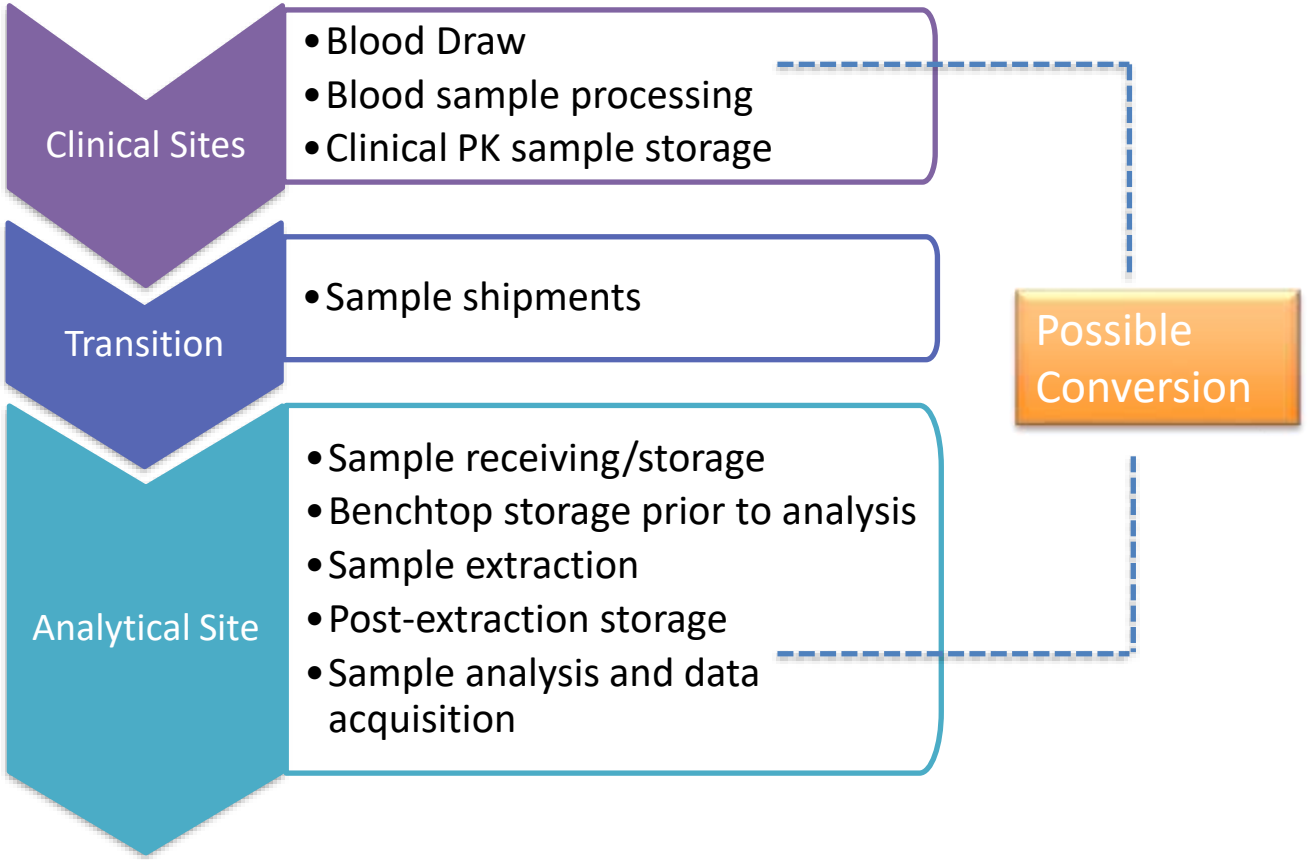
Prodrugs and Common Types

- Prodrugs are designed to transform to active drug moieties *in vivo*
  - Address limitations of the active form in ADMET
  - Through a chemical/enzymatic process or combination
- Common prodrug classes:
  - Ester prodrugs – e.g. Fesoterodine
  - Phosphates prodrugs – e.g. Tedizolid phosphate
  - Amides prodrugs – e.g. Tenofovir alafenamide
  - Carbamate prodrugs – e.g. Loratadine

Prodrugs in Biological Matrices



What happen to Study Samples *ex vivo*?



Considerations during Method Development

- Understand the chemical and/or enzymatic pathways leading to the conversion
  - Different stability profiles in varied species and matrices
- Rigorous stability testing
  - In applicable biological matrices
  - Impact from sample extractions
    - Processing temperature
    - Type of extractions: PPT, LLE, SPE, etc
    - Extraction solutions
  - Different storage conditions: benchtop, frozen, F/T cycles, post-extraction
- Possible in-source fragmentation of the prodrug when using an LC-MS/MS method

Considerations during Method Development

- If possible, monitoring the changes for both the prodrug and active drug under stressed conditions
  - Provides information on the rate and level of conversion
- Consider the range of concentration ratio between the prodrug and the active drug in the in vivo study
  - Critical information to decide the possible data impact for the in vivo studies
  - For example, 10% *ex vivo* conversion has different impact on concentration change for the active drug when:
    - Sample with prodrug/active drug molar ratio at 1:1
    - Sample with prodrug/active drug molar ratio at 10:1

Things to Consider during Method Development

- When needed, test different approaches to stabilize prodrugs in biological samples ex vivo. Common approaches include:
  - Temperature control
  - pH control
  - Enzyme inhibitors
  - Vacutainer tubes containing inhibitors
  - Other effective approach

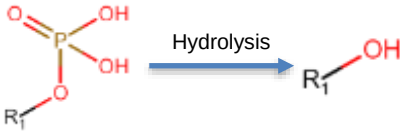
Consideration during Method Validation and Sample Collection

- Method Validation: Parameters validated according to the latest BMV guidance. Valuable information gained from
  - Specificity test
  - Stability tests for the prodrug
  - Stability tests for the active drug in the presence of prodrug
- Clinical Samples Handling
  - Sample collection procedure to implement stabilization method if needed
  - Cover the conditions of sample collection and storage

Consideration during Sample Analysis

- Well controlled and consistent sample analysis procedure
- Analysis within the validated storage conditions
  - Cumulative Benchtop and long term storage
  - F/T cycles
  - Post-extraction storage
- Incurred Sample Reanalysis (ISR)
  - Verify whether the method and stabilization procedure (if applicable) is suitable for a PK study

Case Example 1



Phosphate Ester Prodrug Active drug

Spiked Concentration of Prodrug /Active drug	% Converted Active Drug	
	Heparin-treated human blood	EDTA-treated human blood
5000/0 ng/mL (ice bath for 1 hour)	25.80%	0.61%
5000/0 ng/mL (ambient temperature for 1 hour)	24.80%	2.88%

Final Method includes:

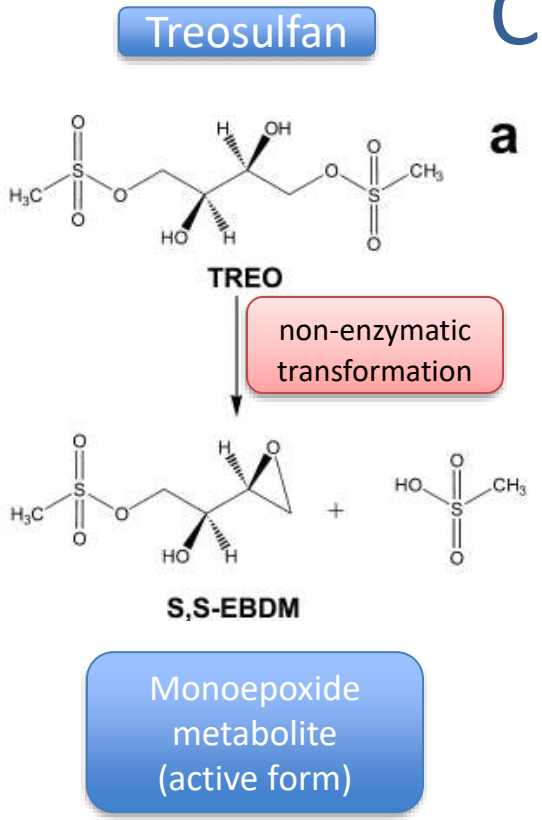
- K_3EDTA as the anticoagulant
- Sample processing at ambient temperature

- Method Validation: QC (1:1 of prodrug/active drug) stability evaluated in plasma, whole blood, and post-extraction solvent

- Sample Analysis:
  - Prodrug/active drug concentration ratio for all study samples < 1:2

  - ISR results
    - Prodrug: 88% passing
    - Active drug: 85% passing

Case Example 2



- Temperature and pH are of crucial importance for stability of the prodrug
- Study Sample drug concentration: Treosulfan: S,S-EBDM at 100-fold
  - 1% ex vivo conversion of treosulfan leads to approximate 100% increase of the SS-EBDM level
- Approach to stabilize treosulfan:
  - Treat human blood samples with 1M citric acid (pH below 5)
  - Minimize clinical sample processing time
  - Sample processing during bioanalysis at 5 C
  - Plasma sample stored at -80 C

https://doi.org/10.1016/j.jpba.2018.02.049
Romański, Michał & Główka, Franciszek. (2018). Journal of Pharmaceutical and Biomedical Analysis. 153. 10.1016/j.jpba.2018.02.049.

Summary

- Ex vivo conversion of prodrugs to active drugs brings challenges in bioanalysis
  - Assay specificity
  - *Ex vivo* degradation of the prodrugs

- Carefully designed method development and validation tests
  - Addressing key assay challenges early on
  - Avoiding pitfalls caused by ex vivo conversion during sample analysis
  - Details are important to capture issues and evaluate impact

Challenge Question

1. For bioanalysis, the *ex vivo* conversion from a prodrug to its active drug may occur in sample collection, shipment and storage, but not during sample extraction.

True or False?

False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