



Alturas Analytics, Inc.

The LC-MS Experts



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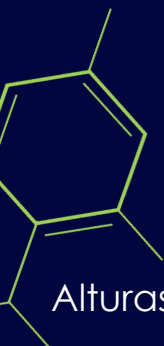
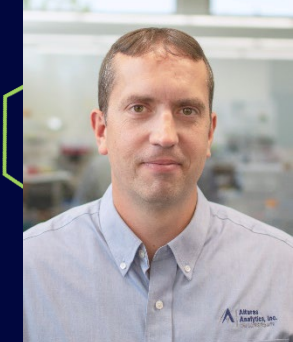
Ready, willing, and stable? Advice from the LC-MS experts on analysis of unstable compounds and prodrugs



Chad Christianson
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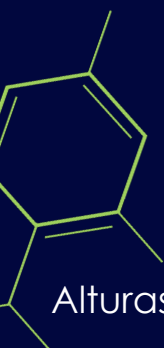
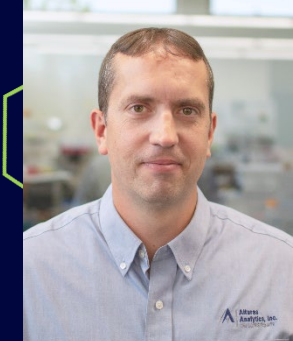
Prodrug background

- Pharmacologically inactive compounds that are converted to their active form by metabolism
 - Chemical or enzymatic
- Rapidly converted *in vivo* to biologically active metabolites
 - Often ester or amide linkage hydrolysis
- Allow drug development to proceed for compounds that would otherwise be considered unsuitable using traditional ADME criteria



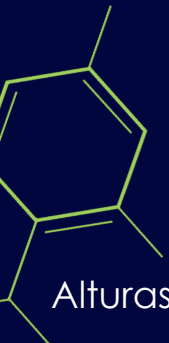
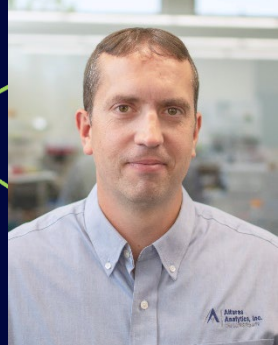
Stability Concerns

- While instability is beneficial for drug development, it creates challenges for the bioanalytical scientist
- Analytical method must be developed to analyze samples for the prodrug
- Stability throughout the entire sample lifecycle must be considered
 - Collection
 - Plasma or whole blood?
 - Storage
 - Extraction
- Each prodrug is unique, there is not a “one size fits all” approach



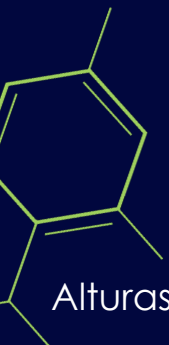
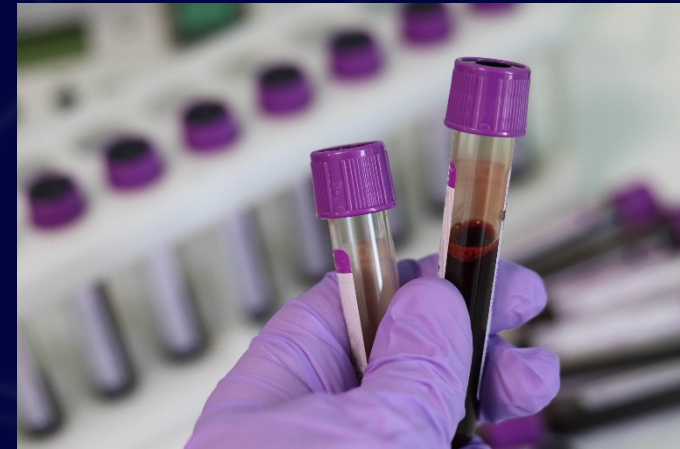
Stabilization Approaches

- Chemical or enzymatic?
 - Alternative anticoagulants
 - Enzyme inhibitors
 - pH adjustment
 - Temperature control
 - Combination of above

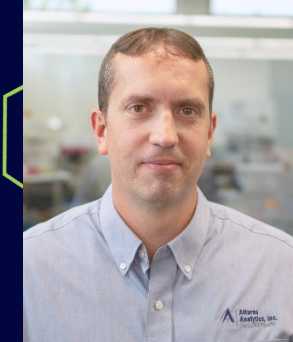


Alternative Anticoagulants

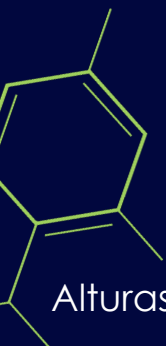
- Off the shelf collection tubes
- Enzyme inhibitors
 - Sodium fluoride/Potassium oxalate
 - Sodium fluoride/EDTA
 - Sodium fluoride/heparin
 - Sodium fluoride/citrate
- Chemical inhibitors
 - Chelating Agent
 - EDTA
 - pH adjustment
 - Citrate
 - Acid citrate dextrose



Enzyme Inhibitors

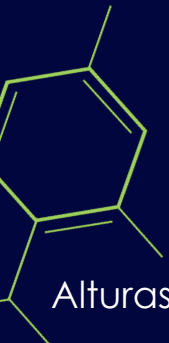


- Develop a screening procedure
 - Dichlorvos
 - Sodium fluoride
- Test whole blood stability with multiple esterase inhibitors
- If multiple species for same drug, repeat screening in each species
- Assay developed that combined pH adjustment with esterase inhibitors
 - Successfully validated in 4 species
 - Required use of different esterase inhibitors for different species
 - Successfully analyzed > 20,000 samples



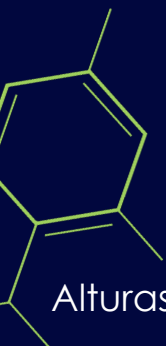
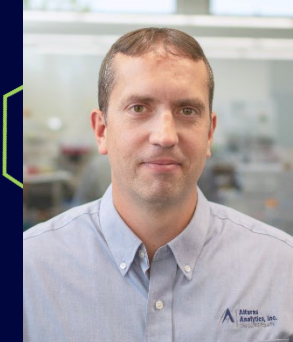
pH Adjustment

- pH < 5 may cause gelling or coagulation
- Formic Acid
- Phosphoric Acid
- Citric Acid
- TCA



Temperature Control

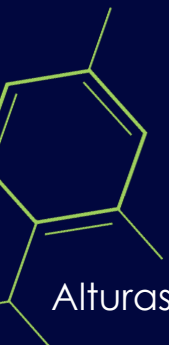
- Temperature controlled centrifuge
- Flash freezing
- -70°C storage
- Dry ice shipping



Implementation of Stabilization Techniques

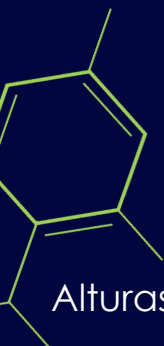
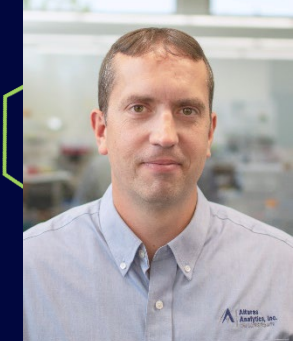


- Ensure sample collection procedures reflect validated method
- Coordinate so that any critical reagents are provided to collection site(s)
- Minimize sample handling after collection
- Freeze-thaw
- BENCHTOP stability
- Long term stability



Conclusions

- Important to review compound structure when working on a stabilization technique
- Species differences may exist
- Work closely with the sponsor to ensure collection techniques mimic validated method



Alturas Analytics – Celebrating 20 Years!

- CRO focused on LC-MS/MS and GC-MS/MS bioanalysis
- GLP bioanalytical analysis
 - Support non-clinical and clinical studies
 - SOPs are written to current industry guidelines
 - Multiple Sciex LC-MS/MS systems and Thermo GC-MS/MS systems
- Pharmacokinetic/toxicokinetic analysis and reporting
- Method development and method validation
 - Blood, Plasma, Serum, Urine, CSF, Tissues and and samples collected using microsampling techniques
 - Small and large molecule quantitation including ADC's, mAB, peptides and biomarkers
 - Plasma protein binding determinations
- NonGLP bioanalytical analysis
- SEND compliant datasets



Acknowledgments

- Jennifer Zimmer
- Jason Watts
- Cody Hawkins

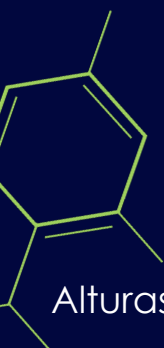


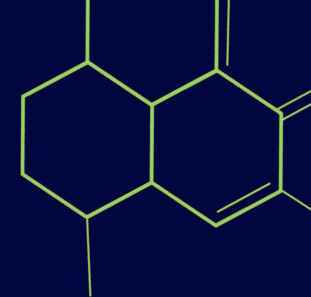
Contact Information

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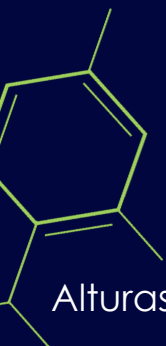
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