Bioanalysis of Unstable Analytes in Pharmacokinetic Bioequivalence Studies Submitted in Abbreviated New Drug Applications (ANDAs)

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SBIA Regulated Bioanalysis Workshop
June 30, 2020

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Learning Objectives

- Explain regulatory requirements for stability
- Identify and resolve stability issues

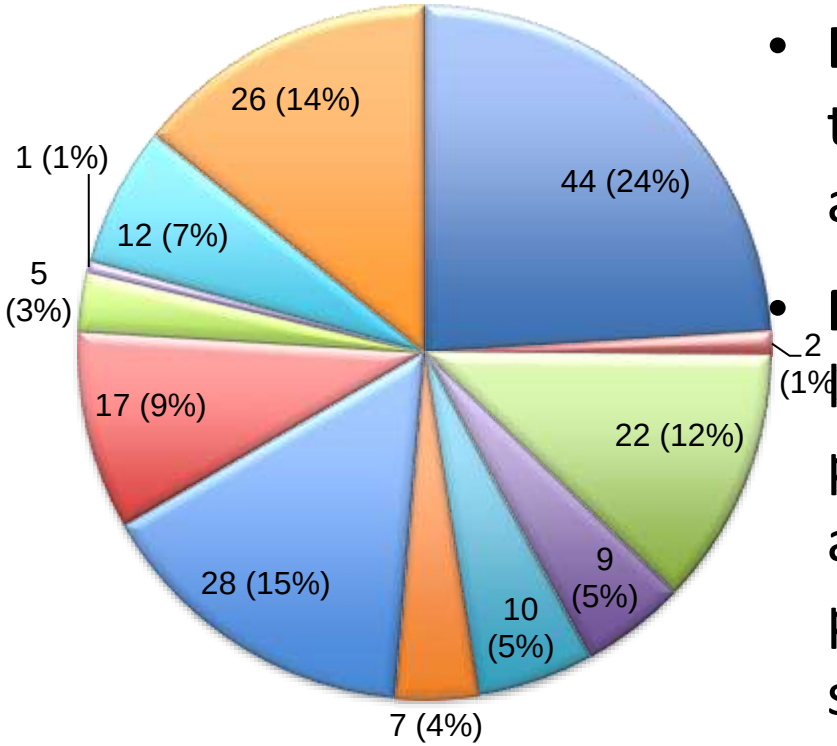
Analyte Stability

- During method development, the applicant should determine the chemical stability of the analyte in a given matrix
- During method validation, stability evaluations should cover the expected sample conditions from sampling collection to the last sample analysis
  - Bench-top Stability
  - Autosampler Stability
  - Extraction (or processed sample) Stability
  - Freeze-thaw Stability
  - Long-term Stability
  - Stock Solution Stability
  - Whole Blood Stability

Examples of Analytes with Stability Issues in ANDAs

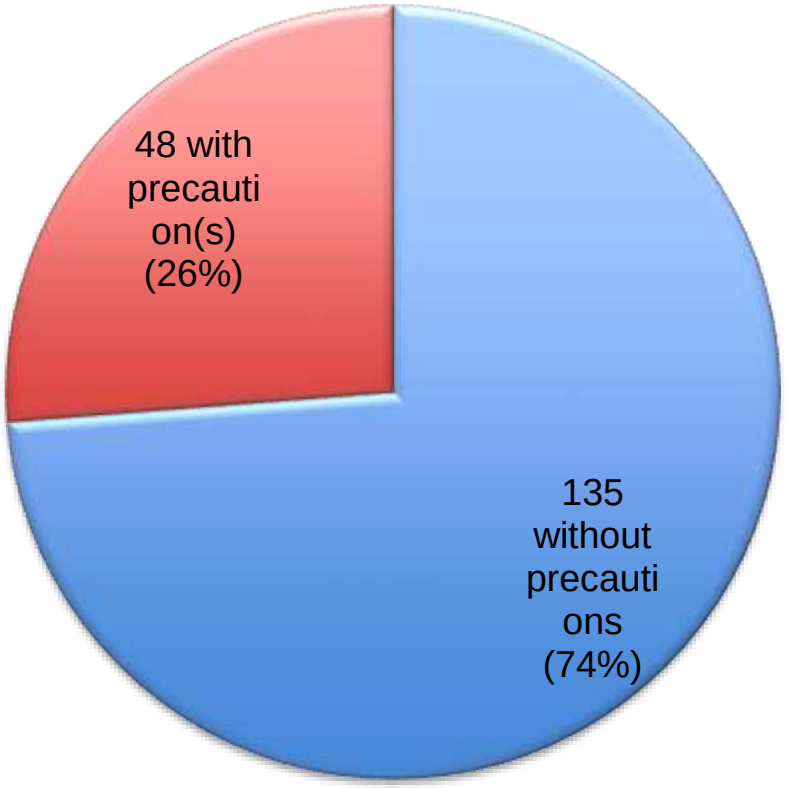
Categories	Mechanism
Acyl-glucuronide	Hydrolysis to parent drug
Alkyl Ester	Conversion into active drug by cleavage of ester bonds by esterases
Benzimidazole	Acid transformaiton
Carbamate	Hydrolysis
Dihydropyridine	Light sensitive
Lactones	Reversible hydrolysis to hydroxy acid
Thiols	Dimerization
Alkylating Agents	Hydrolysis to acid
Cis/trans Isomers	Interconversion
Enantiomers	Racemization
Oxidizable compounds	Oxidative degradation
Other mechanisms	Possible hydrolysis from glucuronide metabolite and then oxidized to parent drug
	Etherification under methanolic condition

Categories of 183 Surveyed ANDAs with Potential Analyte Stability Issues



- **Leading cause**: back-conversion of the unstable metabolite to its parent analyte
- **Most commonly used precautions:** low temperatures for sample processing, control of sample pH, addition of enzyme inhibitor, light protection, change of extraction solvent, or the combination of the above methods

Survey criteria: 1) ANDA submitted between 2007 and 2016; 2) potential analyte stability issues; 3) oral dosage form; and 4) analyte instability directly affecting the measurement of the analyte concentration for the bioequivalence (BE) determination



26% ANDA submissions did not take precaution(s) to stabilize the analyte in 183 surveyed ANDAs with potential analyte stability issues

Evaluation of Analyte Stability

- Is the analyte stable?
  - Chemical structure, reference listed drug (RLD) label, literature, incurred sample reanalysis (ISR), etc.
- If the analyte is not stable,
  - Is the precaution taken to stabilize the analyte (or its metabolite)?
  - Is the precaution sufficient to stabilize the analyte (or its metabolite)?
  - Is the stabilizer added to quality controls (QCs) and calibration standards (CCs)?
- Conduct additional studies to evaluate the analyte stability under the study conditions.

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Case Studies

- Case Study #1: Analyte is Stable without Precaution
- Case Study #2: Analyte is Unstable without Precaution
- Case Study #3: Analyte is Unstable in the Extraction Process

Case Study #1: Analyte is Stable without Precaution

Background

- The BE determination is based on the measured parent analyte concentration
- Potential back-conversion from acyl-glucuronide conjugated metabolite to parent analyte

Original ANDA Submission and BE Assessment

- No precaution to prevent back-conversion
- Only included the parent analyte in the method validation
- Deficiency: Evaluate back-conversion

Case Study #1: Analyte is Stable without Precaution

Two Rounds of BE Amendments

- Samples containing both parent analyte and acyl-glucuronide metabolite
- Complete set of stability data without precautions which covers the study conditions from blood sample collection, sample storage to sample assay

BE Assessment

- There is no stability issue (back-conversion) without precautions in the bioanalysis process

Case Study #2: Analyte is Unstable without Precaution

Background

- The BE determination is based on the measured parent analyte concentration
- Potential back-conversion from lactone conjugated metabolite to parent analyte

Original ANDA Submission and BE Assessment

- No precaution to prevent back-conversion
- Only included the parent analyte in the method validation
- Deficiency: Evaluate back-conversion

Case Study #2: Analyte is Unstable without Precaution

Three Rounds of BE Amendments and One Post-Complete Response Meeting

- Samples containing both parent analyte and lactone metabolite
- Complete set of stability data which covers the study conditions from blood sample collection, sample storage to sample assay
- Bench-top stability and long-term stability failed to meet acceptance criteria

Case Study #2: Analyte is Unstable without Precaution

BE Assessment

- The measured parent analyte concentration did not reliably reflect the true systemic parent analyte concentrations at the time of sample collection

4th BE Amendment and BE Assessment

- The applicant repeated its in vivo BE studies by adding stabilizer to prevent back-conversion
- The new in vivo BE studies were adequate

Case Study #3: Analyte is Unstable in the Extraction Process

Background

- The BE determination is based on the measured parent analyte concentration
- Potential back-conversion from carboxylic acid metabolite to parent drug by reaction with methanol in the mobile phase in the extraction process

Original ANDA Submission and BE Assessment

- Methanol used in the mobile phase
- Failed incurred sample reanalysis
- Higher sample concentrations than other in house ANDAs

Case Study #3: Analyte is Unstable in the Extraction Process

BE Deficiencies: Two Options

1) Re-assay samples for parent drug; or
2) Repeat in vivo BE studies and analyze samples with a method without back-conversion issues

BE Amendment and BE Assessment

- Repeated in vivo BE studies
- Changed methanol to acetonitrile in the extraction process
- Analyzed study samples using validated new analytical method
- The new in vivo BE studies were adequate

Summary

- Appropriate precautions should be exercised during the bioanalysis of unstable analytes.
- Lack of precautions for unstable analytes in the bioanalysis process is a common issue in ANDA BE studies and increases the number of assessment cycles.
- Conduct thorough method validation in the presence of a precaution (e.g., stabilizer) covering the study conditions from blood sample collection, sample storage, to sample assay.
- Provide adequate justification if a precaution is not used.

Challenge Question # 1

When parent drug is measured, which of the following analyte(s) spiked in the medium can be used to demonstrate that 1) there is no significant back-conversion from metabolite A to parent drug, and 2) parent drug is stable?

A. Parent drug (HQC, MQC, LQC)

B. Metabolite A (HQC, MQC, LQC)

Measure parent drug

C. Parent drug (HQC, MQC, LQC, blank) + metabolite A (HQC)

D. Parent drug (HQC) + metabolite A (HQC, MQC, LQC)

Challenge Question # 2

Which one of the following stability tests is needed to cover the analyte stability right after sample collection from subjects and prior to preparation for storage if the matrix used is plasma in the bioanalysis?

A. Bench-top Stability

B. Autosampler Stability

C. Extraction (or processed sample) Stability

D. Whole Blood Stability

Acknowledgement

Rong Wang, Ph.D., Pharm.D. (DBI/OB/OGD)
Utpal Munshi, Ph.D. (DBI/OB/OGD)
Nilufer Tampal, Ph.D. (IO/OB/OGD)
Ethan Stier, Ph.D. (IO/OB/OGD)
Bing V. Li, Ph.D. (IO/OB/OGD)
Tian Ma, Ph.D. (DBI/OB/OGD)
Ke Ren, Ph.D. (DBIII/OB/OGD)

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Questions?

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