



# Detection of Drug Residues: Analytical Methods

FDA Center for Veterinary  
Medicine

# Overview

- Background
- Requirements of a method
- Validation of methods
- Updating old methods
  - Bridging studies

# What Are Drug Residues?

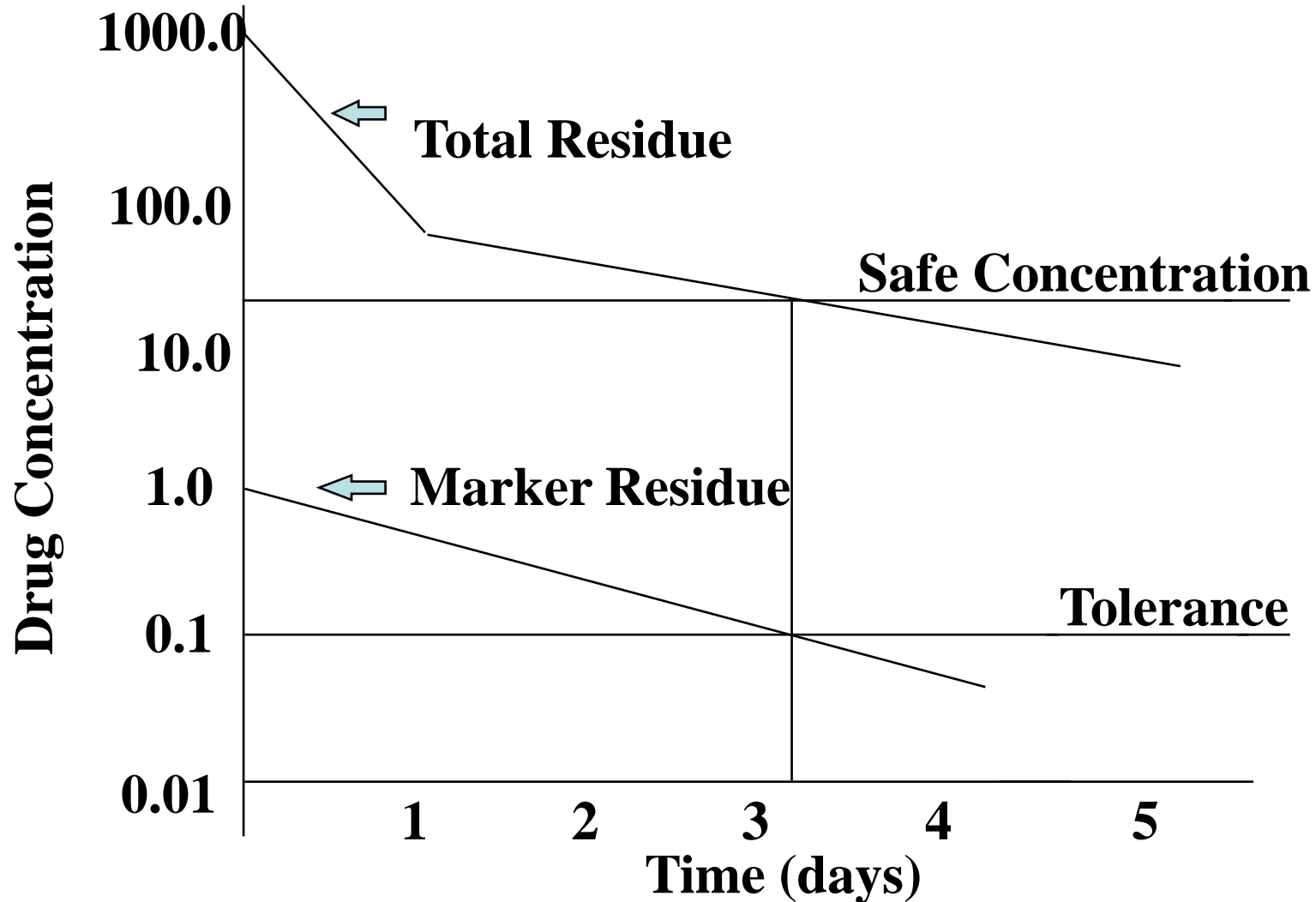
- Any compound present in edible tissue of the target animal which result from the use of the sponsored compound
  - Parent Drugs
  - Metabolites
  - Bound Residues

# Residue Chemistry

## Total Residue and Metabolism Study

- ◆ Determines the **MARKER RESIDUE**
  - ◆ the residue that monitors the depletion of total residues in a tissue
- ◆ Determines the **TARGET TISSUE**
  - ◆ generally, the edible tissue from which residues deplete most slowly
  - ◆ often, liver or kidney
  - ◆ rarely, muscle or fat

# Selecting a Tolerance



# Requirements for a Method

- Analytical methods for residues

“Applications shall include a **description of practicable methods for determining the quantity**, if any, of the new animal drug in or on food, and any substance formed in or on food because of its use, and the proposed tolerance or withdrawal period or other use restrictions **to ensure that the proposed use of this drug will be safe**. When data or other adequate information establish that it is not reasonable to expect the new animal drug to become a component of food at concentrations considered unsafe, a regulatory method is not required.”

*21 CFR 514.1 (b) 7*

# Regulatory Methods

- Methods are used by USDA and FDA to monitor food supply for veterinary drug residues
- Testing for veterinary drugs provides information on level of compliance

# What Must a Method Do?

- Provide information on amount of residue present (Quantitation)
  - Determinative procedure
  
- Positive identification of drug (Specificity)
  - Confirmatory procedure

# Method Attributes

- Method must be practicable
  - Access to reagents and standards
  - Commercially available equipment
  - Reasonable sample throughput
  - Can be done by a competent analyst
  - Capable of being used in a government laboratory

# Method Validation

- Three types of samples
  - Control target tissue from untreated animals
  - Fortified target tissue containing known concentrations of MR
  - Dosed target tissue from animals that have been treated with the drug

# Method Trial

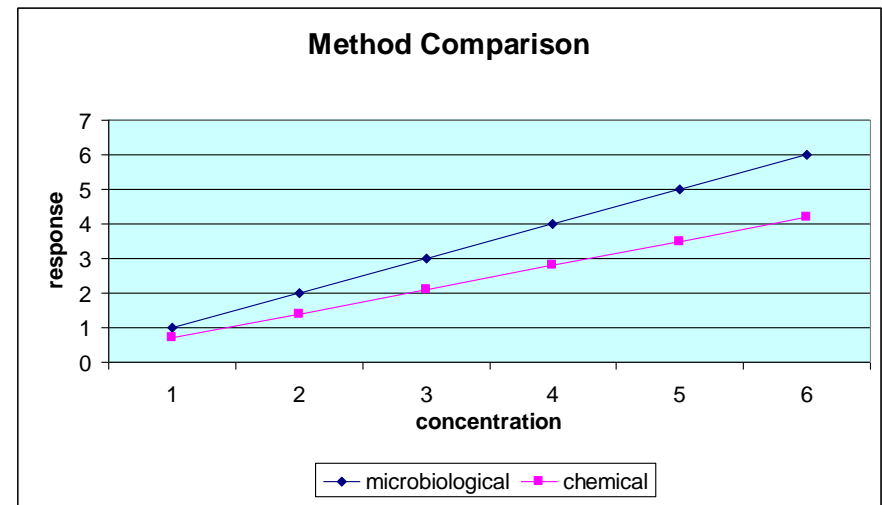
- Independent evaluation of method
  - Three or more laboratories
    - Include one government laboratory
    - Evaluate written procedure
  - Demonstrates transferability of technology
    - Address ambiguities in written procedure
    - Method is capable of being used “off the shelf”

# Updating Old Methods

- Some methods are functionally obsolete
  - Need to develop model for updating methods
- Tolerance is based on validated regulatory method
  - Need to determine equivalence of proposed new method to official regulatory method  
“Bridge Method”

# Bridging Study

- A parallel analysis from a common sample should be undertaken. The common sample should be carried through a common homogenization step before proceeding to the assay specific extraction steps and procedures



# Test Case for Bridging Study: Penicillin

- Quantification and confirmation use an NADA method that is based on outdated or difficult-to-implement/-maintain technology
- Known to be problematic in the National Residue Program (NRP)
- Using these criteria, discussions within CVM and with FSIS determined that penicillin was the ideal candidate

# Penicillin Official Method Characteristics

- Bioassay
- Final confirmation (including a reported concentration) is time-consuming
- Penicillin residues represent a significant portion of the violations
- Ambiguous results: residues that remain putative penicillin violations precluding final regulatory action

# New Penicillin Determinative Procedure

0.2 g Kidney, 0.2 g Liver, or 0.5 g Muscle

Prepare Working Standards  
Fortify QC Samples, add IS

Add phosphate buffer  
Homogenize with polytron  
Add ACN vortex and centrifuge

Combine supernatants  
Evaporate ACN under N<sub>2</sub>

Add phosphate buffer

Centrifuge

Strata X, 60 mg, 3mL SPE  
Condition with MeOH, EDTA, and buffer  
Load and wash with buffer 2 x 2 mL  
Elute with 3 mL ACN

Add 2 mL water  
Evaporate ACN under N<sub>2</sub>  
Prepare calibration standards in water

LC-MS/MS

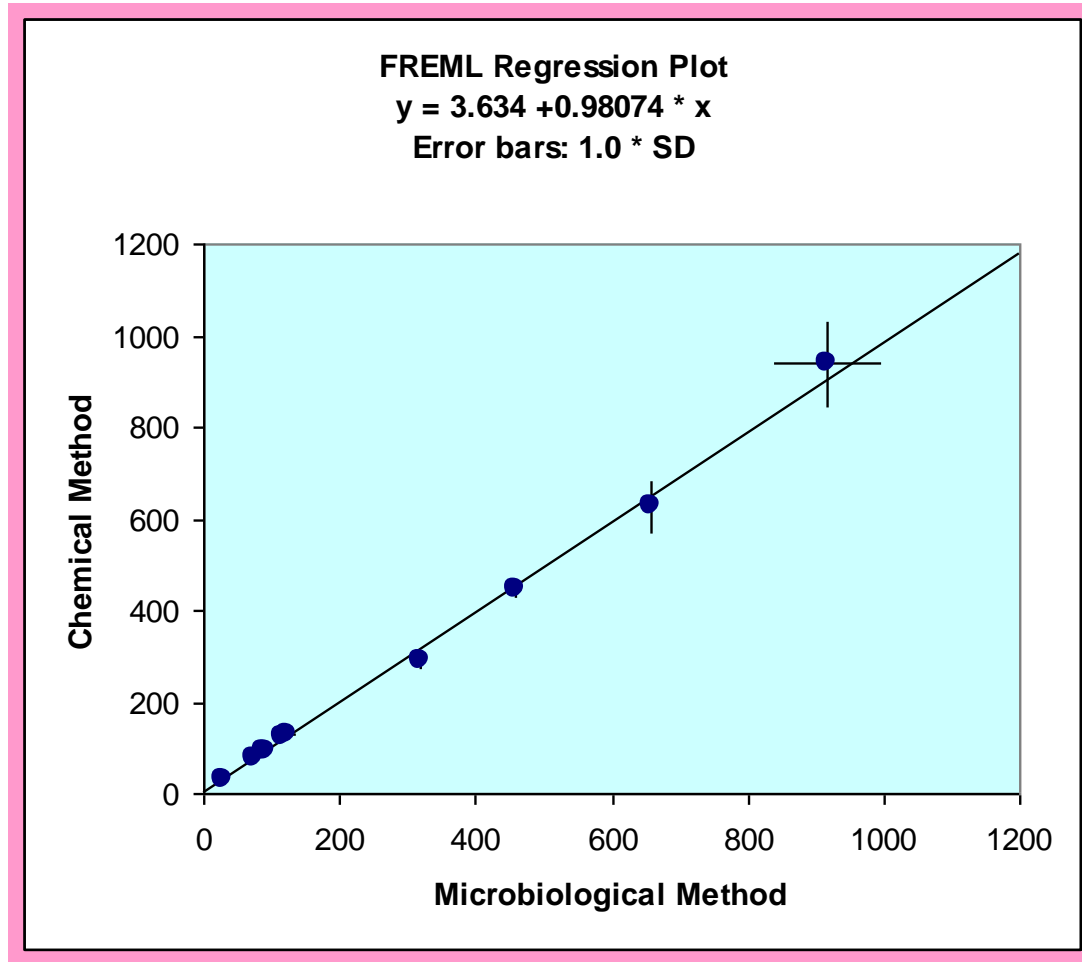


LC-MS/MS: UPLC- Micromass Quattro Micro  
MS/MS Transitions: 335.10 → 160.10, 176.15

# Bridging Study

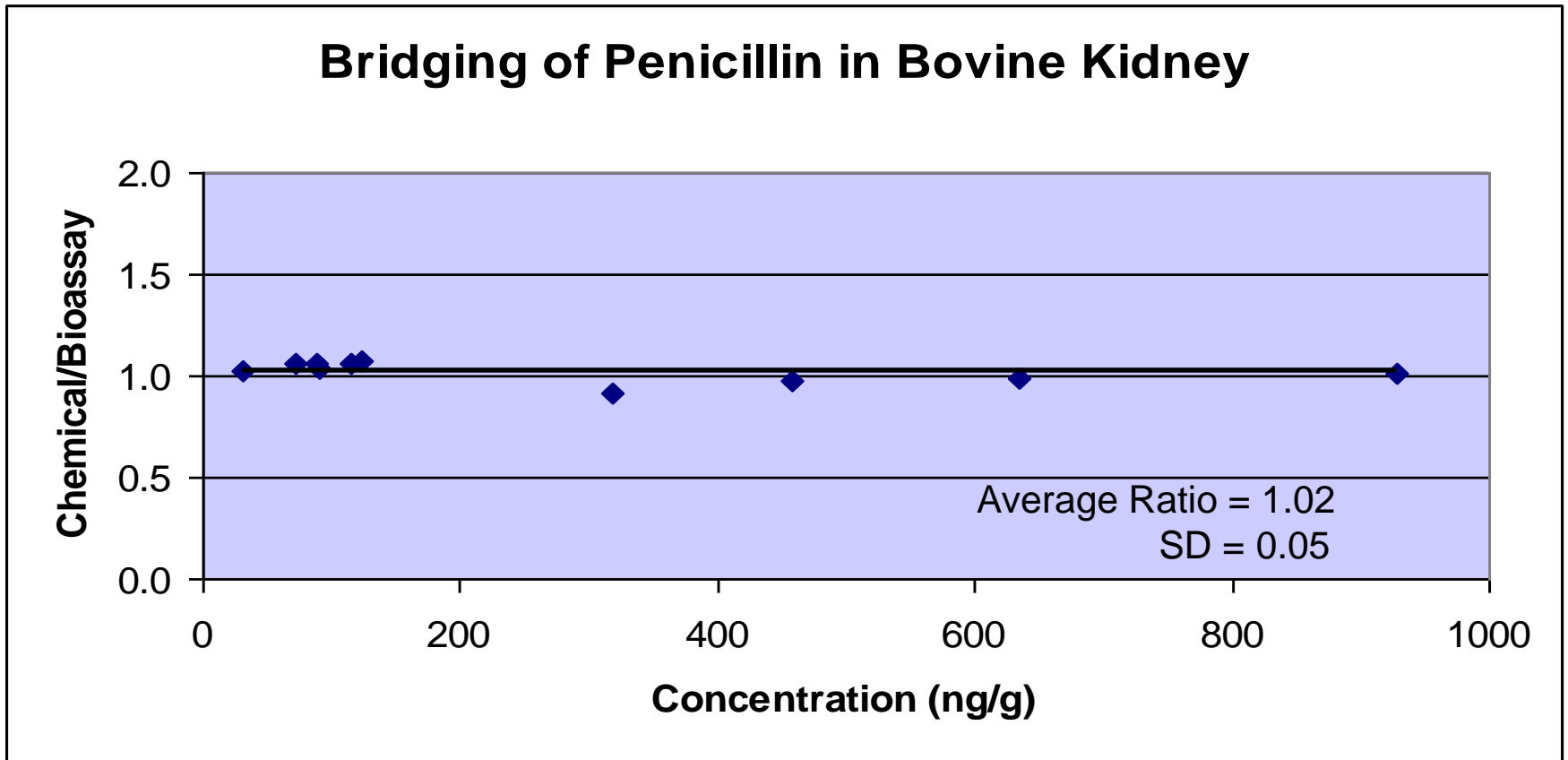
- Done with incurred residues
- Concurrently test tissues with all methods
- Multiple concentrations of drug
- Test tissues immediately after harvesting
  - Allow modifications if needed to dosing

# Bridging Results





# Bridging Results





# Outcomes

- Demonstrated penicillin method to FSIS
- FSIS will have an FDA accepted method for penicillin based on LC-MS/MS



# Current Efforts for Updating Methods

- Bridging tetracycline drugs
  - Currently quantitation based on the microbiological regulatory method



# Summary

- Requirements of a method
- Updating old methods
  - Bridging studies



# Thank you

