Bioanalysis of Endogenous Compounds in Pharmacokinetic Bioequivalence Studies Submitted in Abbreviated New Drug Applications (ANDAs)

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Learning Objectives

- Identify challenges in establishing bioequivalence (BE) of drug products containing endogenous compounds
- Explain regulatory requirements and approaches for bioanalysis and establishing BE of drug products containing endogenous compound
- Discuss two case studies

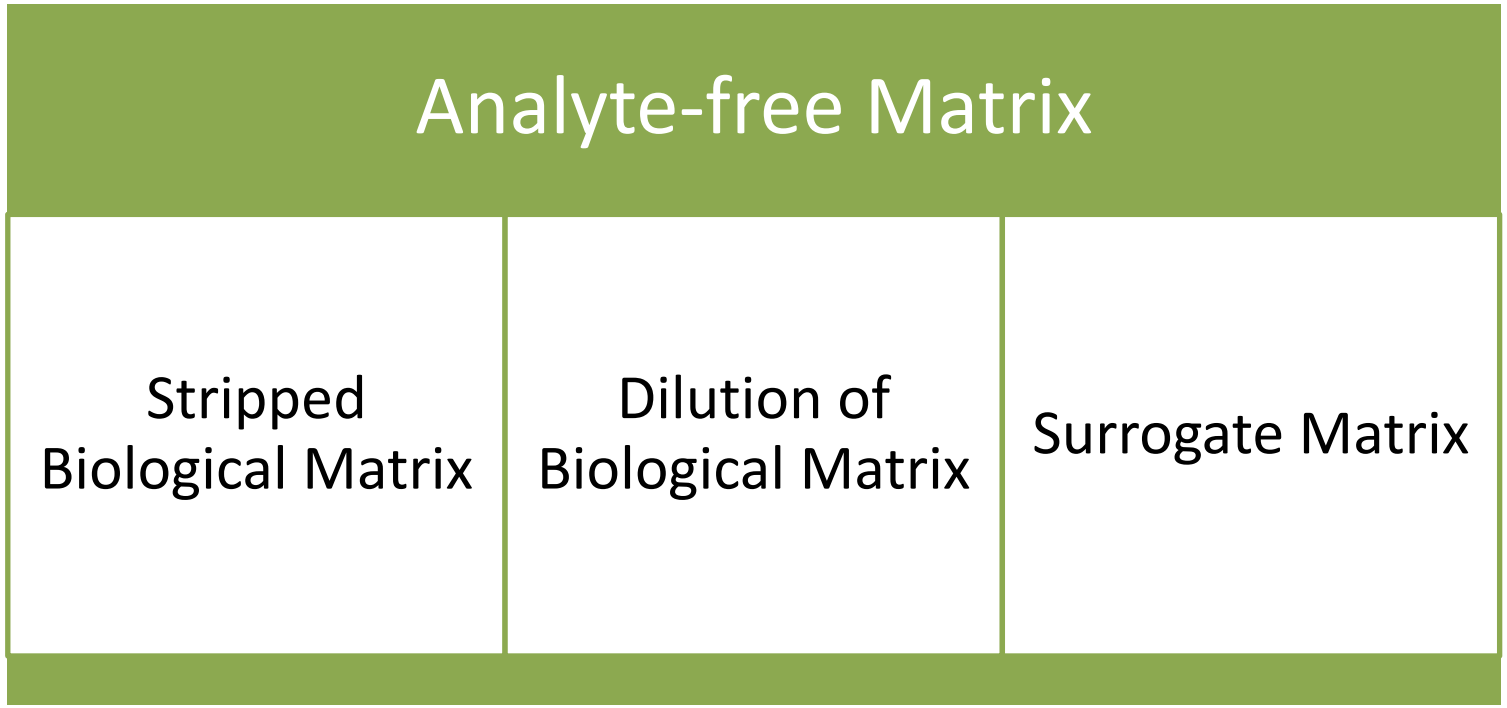
Examples of Endogenous Compounds

- Thyroid Hormones (e.g., levothyroxine)
- Sex Hormones (e.g., estrogens, progesterone)
- Vitamins (e.g., D2, D3, K)
- Bile Acid (e.g., ursodiol)
- Omega-3 Fatty Acid
- Potassium
- Iron

Challenges in Establishing Bioequivalence of Endogenous Compounds

- Lack of analyte-free blank matrix to prepare calibration standard (CS) samples
- Determination of endogenous levels in biological matrix which are used for quality control (QC) sample preparation
- Baseline levels are impacted by circadian rhythm, dietary intake, homeostasis
- Release of the drug from the dosage form and endogenous production contribute to the systemic levels of compound

Preparation of CS Samples for Endogenous Compounds



Surrogate Matrix for Preparation of CS Samples for Endogenous Compounds

- Justification for using surrogate matrix
- Comparable recovery data between QCs in surrogate matrix and QCs in authentic matrix
- Comparable matrix effect
- Stability in both surrogate matrix and authentic matrix
- Parallelism test

Preparation of QC Samples for Endogenous Compounds

- The QC should be prepared by spiking quantities of analyte(s) in the same biological matrix as the study samples

Biological Matrix used for Calibration Standards		
	Method Validation	Study Sample Analysis
Matrix used to prepare Quality Controls	Biological Matrix	Biological Matrix
Stripped Biological Matrix used for Calibration Standards		
	Method Validation	Study Sample Analysis
Matrix used to prepare Quality Controls	Stripped Biological Matrix and Unstripped Biological Matrix	Unstripped Biological Matrix
Surrogate Matrix used for Calibration Standards		
	Method Validation	Study Sample Analysis
Matrix used to prepare Quality Controls	Surrogate Matrix and Authentic Biological Matrix	Authentic Biological Matrix

Measuring Endogenous Levels when Matrices without Interference are not Available

- Standard Addition Approach
- Background Subtraction Approach
- Surrogate Matrix Approach
- Surrogate Analyte Approach

Case Studies

- Case Study #1: Inadequate method validation for surrogate matrix method
- Case Study #2: An example of background subtraction method

Case Study # 1: Inadequate Method Validation for Surrogate Matrix Method

- Endogenous compound
- Analytical Method: HPLC/MS/MS with Liquid-liquid extraction
- Surrogate Matrix: Phosphate-buffered saline (PBS) + 2% Bovine serum albumin (BSA)
- Deficiency: Lack of justification for the use of surrogate matrix

CS and QC Preparation and Insufficient Recovery Study

- Surrogate Matrix was used to prepare CS and QC samples for method validation and study sample analysis
- Human serum was used to prepare QC samples for method validation and study sample analysis
- Recovery data in the method validation report is from surrogate matrix only
- **Deficiency**: Lack of recovery data in authentic matrix (human serum)

Parallelism Study

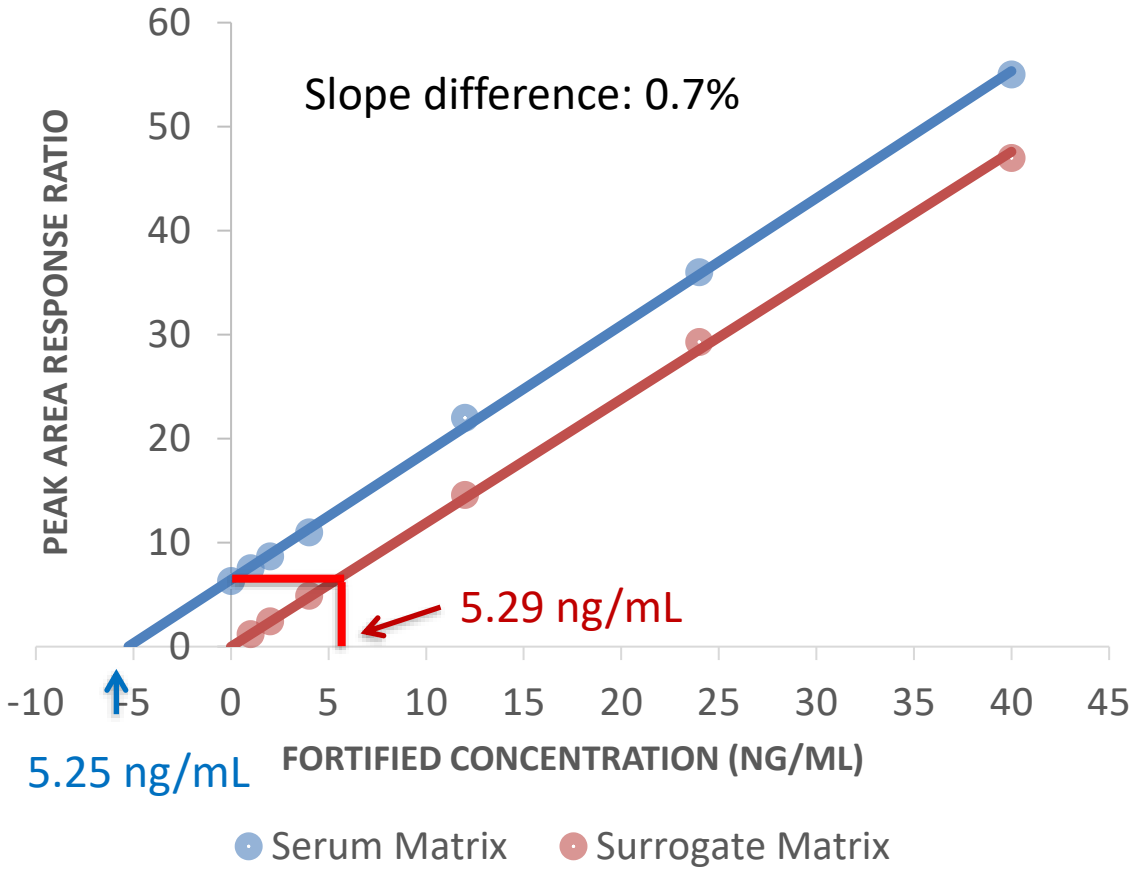
- CS samples were prepared from both surrogate and authentic matrices

	Concentration (ng/mL)
Replicate 1	5.03
Replicate 2	5.24
Replicate 3	4.99
Replicate 4	5.17
Replicate 5	5.32
Replicate 6	5.10
Mean	5.14
S.D.	0.13
CV%	2.45

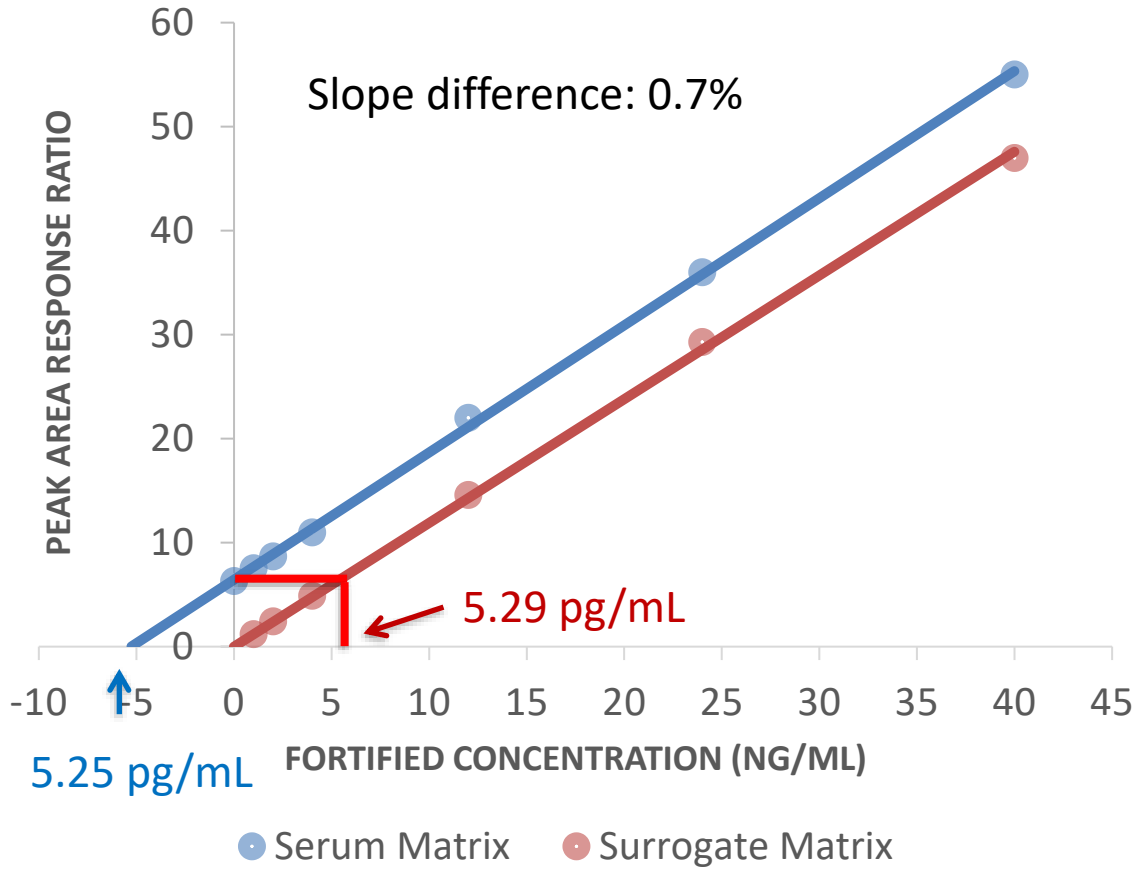
Calibration Standard	PBS + 2% BSA (ng/mL)	Pooled Human Serum (ng/mL)*
CS1	1	6.14
CS2	2	7.14
CS3	4	9.14
CS4	12	17.14
CS5	24	29.14
CS6	40	45.14
CS7	64	69.14
CS8	80	85.14

*Theoretical concentrations for CSs in human serum = baseline endogenous level + fortified concentration

Parallelism Study – Cont'd



Parallelism Study – Cont'd



- There is only one run each from surrogate matrix and human serum
  - **Deficiency**: At least 3 sets of parallelism data
- Linear regression was used in the parallelism study while quadratic regression was used in the pivotal BE study
  - **Deficiency**: Use same regression and weighting factor

Summary for the Use of Surrogate Matrix

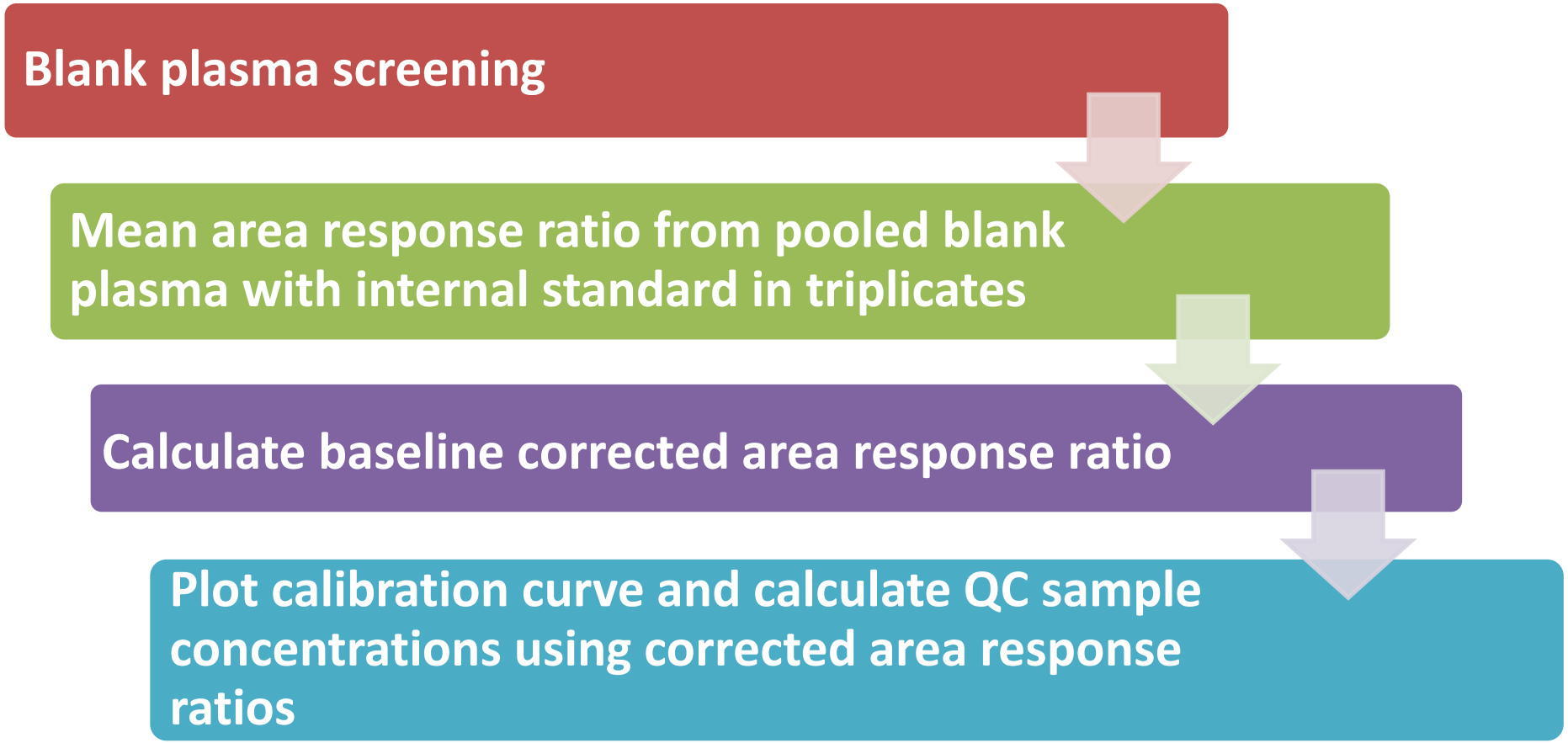
- Justify the use of surrogate matrix
- Comparable recovery data between QCs in surrogate and authentic matrices
- Parallelism Study
  - At least three sets of parallelism data
  - Comparable slopes of calibration curves (CC) in surrogate and authentic matrices
  - Endogenous level in blank matrix back calculated versus CC in surrogate matrix comparable to the negative x-intercept extrapolated from the CC in authentic matrix
  - Provide data from parallelism study using same regression and weighting factor as used in the pivotal BE study

Case Study # 2: Background Subtraction Method

- Endogenous compound
- Analytical method: HPLC-MS/MS with solid phase extraction
- **Baseline Corrected Peak Area of the Analyte**
  - Recovery, Specificity, Selectivity, Matrix factor
  - Example: Recover Study
    - Baseline: Mean area of analyte of three blank samples processed during the recovery experiment
    - Recovery = Extracted Samples/Post-extracted Spiked Samples after baseline correction
    - Results: Comparable recovery at all QC levels

Case Study # 2: Background Subtraction Method

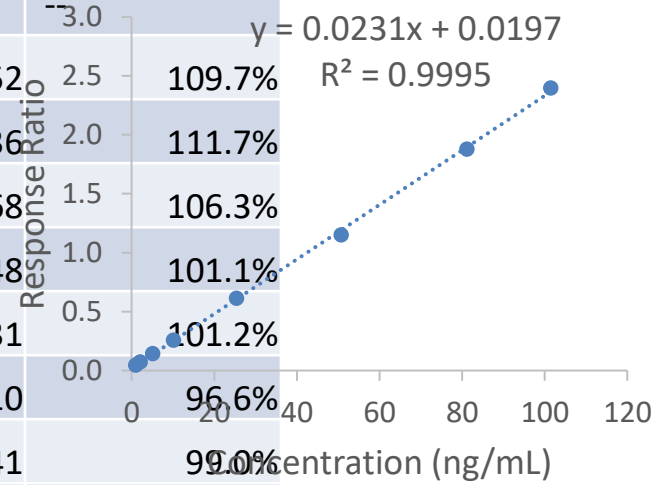
- **Baseline Corrected Area Response Ratio (Analyte/Internal Standard)**



Background Subtraction Using Baseline Corrected Response Ratio

Samples	Conc(ng /mL)	Area Response Ratio (Analyte/IS)	Corrected Area Response Ratio (Analyte/IS)	Back – calculated Conc (pg/mL)	%Accuracy
Blank+IS	unknown	0.3454	---	---	--
Blank+IS	unknown	0.3370	---	---	--
Blank+IS	unknown	0.3357	---	---	--
CS1	1.01	0.3846	0.0452	1.1052	109.7%
CS2	2.08	0.4128	0.0734	2.3236	111.7%
CS3	5.07	0.4838	0.1444	5.3968	106.3%
CS4	10.15	0.5962	0.2568	10.2648	101.1%
CS5	25.37	0.9521	0.6127	25.6731	101.2%
CS6	50.75	1.4915	1.1521	49.0210	96.6%
CS7	81.20	2.2169	1.8775	80.4241	99.0%
CS8	101.50	2.7361	2.3967	102.9006	101.4%
LQC	3.03	0.4294	0.0900	3.0433	100.4%
MQC	42.03	1.4054	1.0660	45.2962	107.8%
HQC	80.05	2.2307	1.8913	81.0233	101.2%

- The area response ratios of blank+IS samples in each run for all study subjects are consistent between 0.03-0.05



- **The background subtraction method is considered acceptable.**

Summary

- Challenges in establishing BE for drug products which contain endogenous compounds
- Interference from endogenous analytes in blank matrix
- Step-wise procedure for the preparation of CCs and QCs
- Step-wise procedure for the measurement of endogenous analyte concentrations from blank matrix
- Adequate justification and complete cross-validation data when surrogate matrix is used

Challenge Question # 1

Which one of the following statements is NOT true for the parallelism study to support the use of surrogate matrix?

A. At least 3 sets of parallelism data

B. Quality controls used in the parallelism study should be representative of the measured subject concentrations

C. Same sets of quality controls should be used to parallelism and pivotal BE studies

D. Same regression and weighting factor should be used to parallelism and pivotal BE studies

Challenge Question # 2

Which one of the following statements is true for the quality control (QC) preparation of endogenous compounds?

A. The QCs should be prepared by spiking known quantities of the analyte in the same biological matrix as the calibration standards for study sample analysis

B. The endogenous concentrations of the analyte in the biological matrix should be evaluated after QC preparation (e.g., by replicate analysis)

C. The concentrations for QCs should account for the endogenous concentrations in the biological matrix (i.e., additive)

D. QC concentrations calculated by background subtraction are actual QC concentrations (endogenous + spiked concentration)

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Questions?

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