Bioanalysis of Endogenous Drugs Using Surrogate Matrix Approach in Abbreviated New Drug Applications

Yang Lu, Ph.D.

Division of Bioequivalence III
Office of Bioequivalence
Office of Generic Drugs
CDER, US FDA

[SBIA Generic Drug Forum] – April 23, 2026

Disclaimer

This presentation reflects the views of the presenter and should not be construed to represent FDA's views or policies

Learning objectives

- Describe the bioanalytical challenges of endogenous drugs in ANDAs
- Summarize different approaches recommended in the ICH M10 guidance for endogenous drug bioanalysis
- Explain the surrogate matrix approach
- Evaluate analytical data acceptability in case studies using the surrogate matrix approach, implementing ICH M10 criteria and FDA regulatory assessment

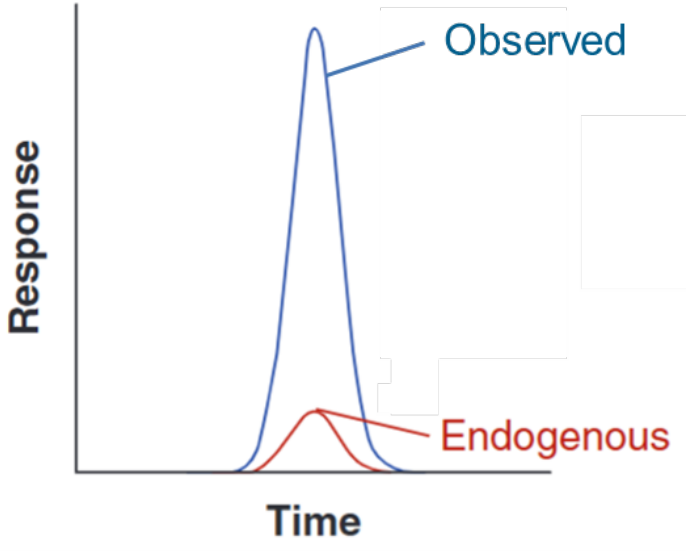
Endogenous Drugs

- Endogenous molecules play important physiological and pharmacological roles
  - Examples: sex hormones, thyroid hormones, fatty acid, elemental iron, vitamins, etc.
- Endogenous molecules as the active pharmaceutical ingredients (APIs) in ANDAs
- Post-dose endogenous drug concentrations are the fundamental data for pharmacokinetic (PK) assessment to demonstrate bioequivalence

Endogenous Drug Bioanalysis

Bioanalysis of endogenous drug concentrations is challenging!

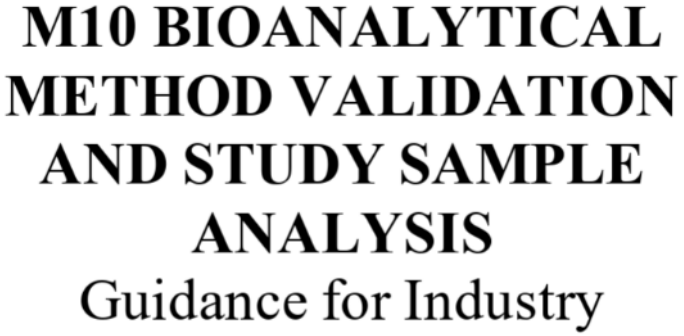
"*Interference*" of the endogenous level



Yu, C. et al., Bioanalysis, 2024 Feb;16(3):171-184

ICH M10 Recommendations

- Section 7.1 - Methods for analytes that are also endogenous molecules
- Recommended approaches
  - Surrogate matrix
  - Surrogate analyte
  - Background subtraction
  - Standard addition



ICH M10, Nov 2022

Surrogate matrix

- Benefits of the surrogate matrix approach
  - Analyte-free
  - Robustness
  - Promising sensitivity
- Interference-free authentic matrix (same matrix type as BE study samples) is acceptable

Case study 1 – method sensitivity

- Endogenous drug A
- Matrix type: human plasma
- Human plasma endogenous concentration of A: 0.2 - 3 ng/mL (applicant's literature search)
- Lower limit of quantitation (LLOQ): 2 ng/mL

Note: all case studies in current presentation include mock data based on representative ANDAs

Case study 1 – method sensitivity

Applicant's method design and rationale

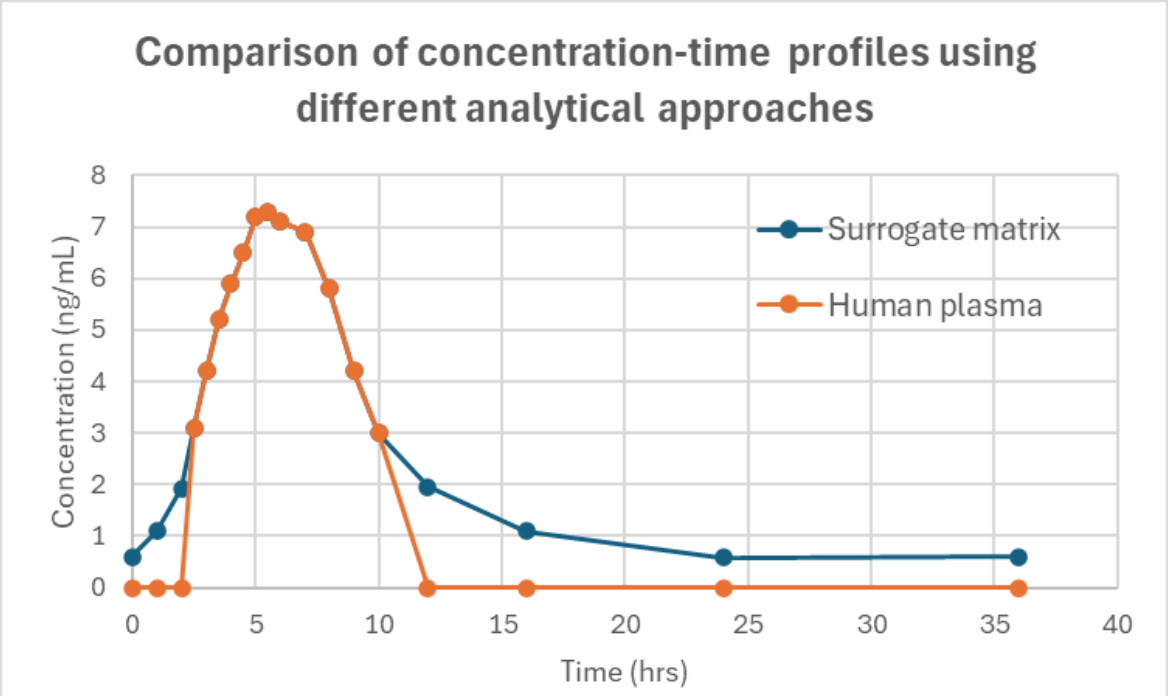
- Authentic (human plasma) matrix was used (all calibration standards, quality controls, etc.)
- Human plasma was 'interference-free' (i.e., <20% LLOQ)
- Method was validated and used to support BE study as a non-endogenous method

Case study 1 – method sensitivity

Concerns about the applicant's approach:

- All pre-dose samples were below quantitation limit (BQL)
- No baseline correction was performed (baseline correction is recommended by product specific guidance)
- Insufficient measurable concentrations in the elimination phase in multiple subjects
  - E.g., Cmax = 7 ng/mL, concentration after 2 x $t_{1/2}$ = 1.75 ng/mL (not measurable due to BQL)

Case study 1 – method sensitivity



Selection of surrogate matrix

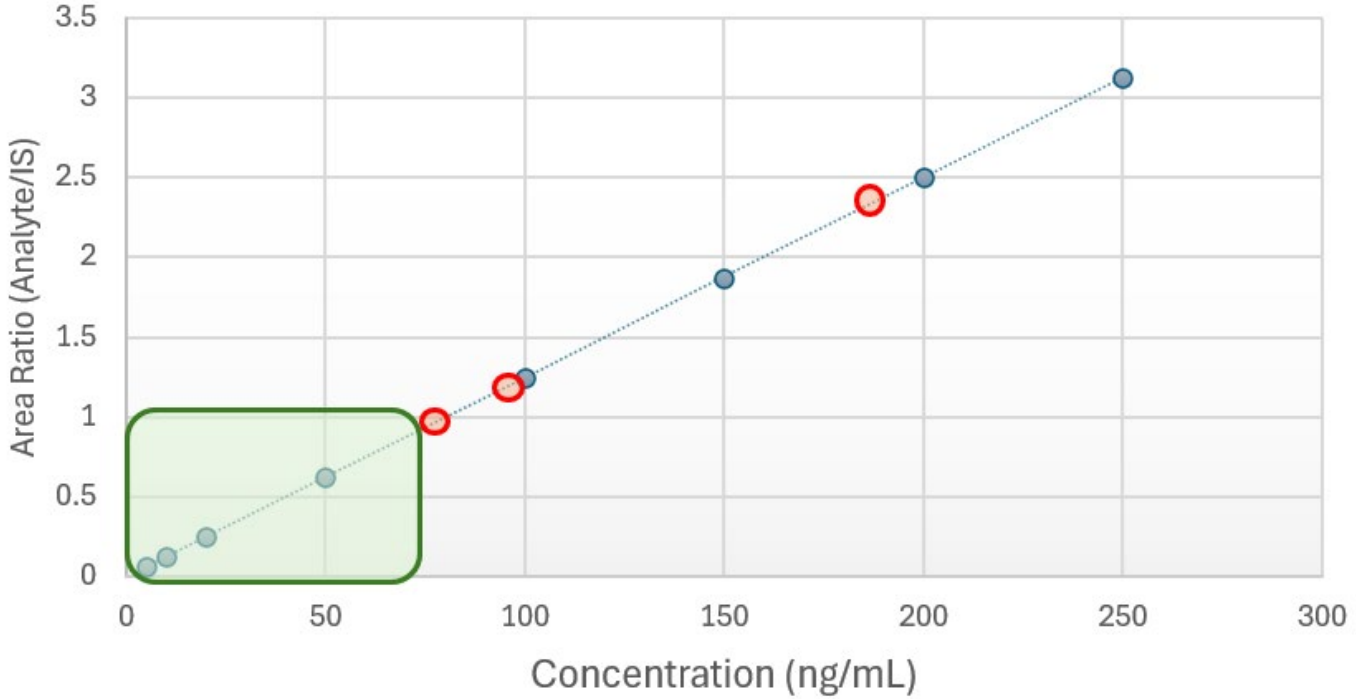
General recommendations

- Surrogate matrix may vary widely in complexity
  - buffer, depleted biological matrix, matrix from different species, etc.
- Quality control (QC) preparation
  - Method validation: QC in surrogate and authentic matrices
  - Subject sample analysis: QC in authentic matrix (at least)

Case study 2 – endogenous concentration in blank authentic matrix for QC preparation

- Endogenous drug B
- Calibration range: 5 – 250 ng/mL (LLOQ = 5 ng/mL)
- Blank plasma contains 60 ng/mL endogenous concentration (used in both validation and subject sample analysis)
- Authentic Low QC: nominal concentration is 75 ng/mL

Case study 2 – endogenous concentration in blank authentic matrix for QC preparation



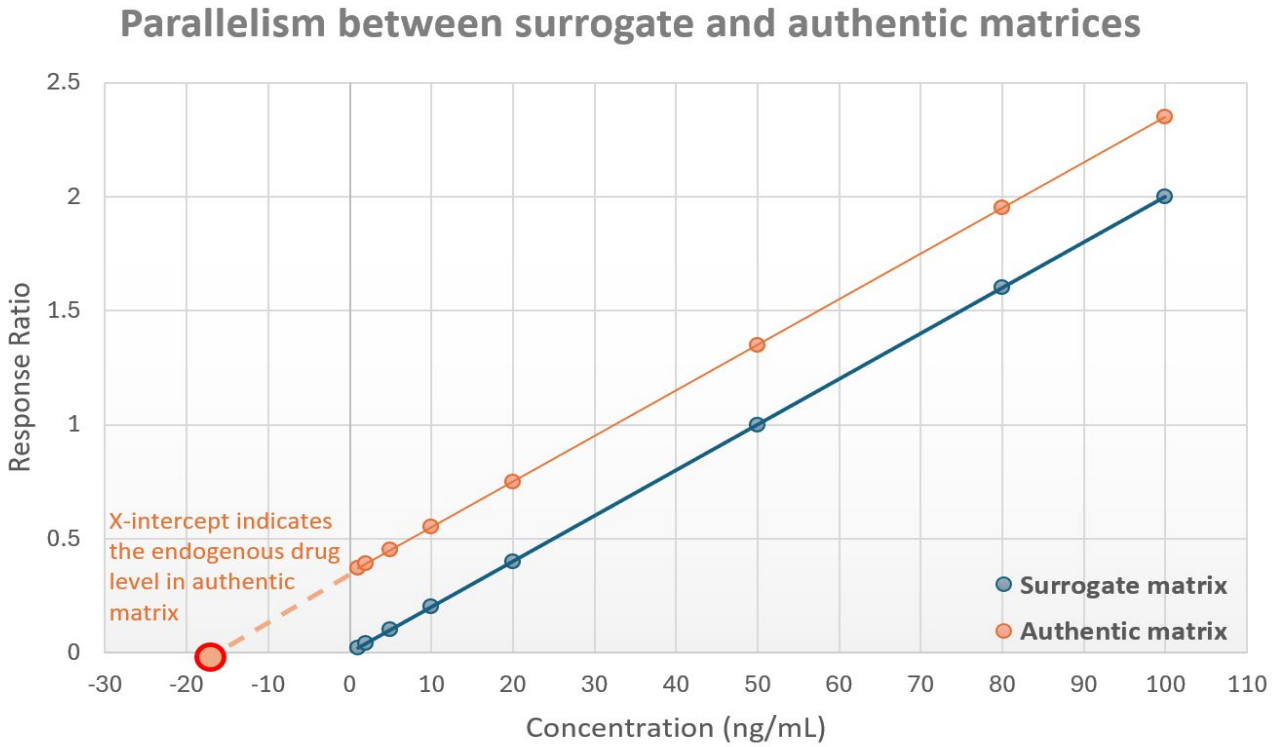
Case study 2 – endogenous concentration in blank authentic matrix for QC preparation

- Concern: insufficient data supporting the accuracy of lower concentrations in BE study
- Proposed justification: re-conduct a partial validation using authentic QCs (Low) prepared in blank plasma with lower endogenous level (e.g., 10 + 15 = 25 ng/mL)
- Recommended approach: screen additional authentic matrix lots for lower endogenous concentration before QC preparation

Parallelism: surrogate and authentic matrices

- Parallelism is expected in both chromatographic and ligand binding assay (LBA) methods – ICH M10 Guidance, Section 7.1.3
- Missing parallelism data may cause inadequate bioanalytical validation
- Determination of endogenous concentration in blank authentic matrix

Parallelism: surrogate and authentic matrices



Matrix effect assessment

- Surrogate matrix may present different matrix effect than authentic matrix
- Sample extraction should be optimized to mitigate potential impact of matrix effect difference
- Stable isotope labeled internal standard (SIL-IS) provides benefits
- **Matrix effect must not impact quantitation accuracy**
- **Consideration beyond ‘acceptable’ QC accuracy data**

Case study 3: variable matrix effect and its impact

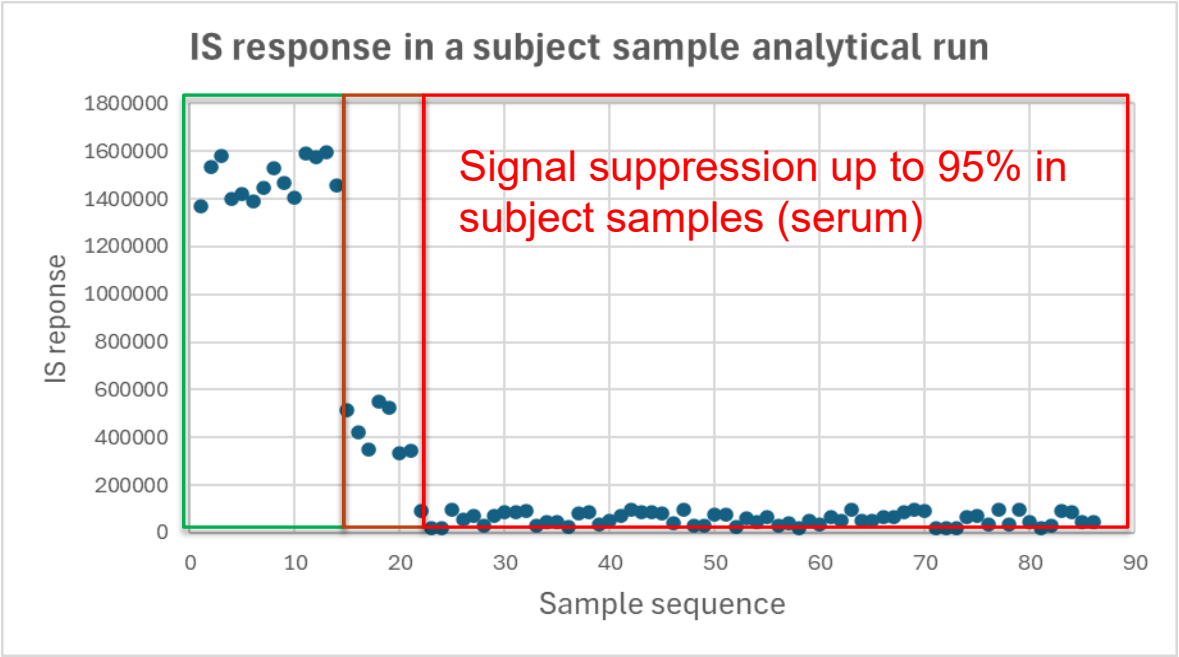
Method validation

Sample Name	Matrix type	Accuracy	IS response
STD1	BSA	101%	**1520000**
STD3		103%	**1720000**
STD6		99%	**1480000**
STD8		97%	**1550000**
BSA QC Low	BSA	95%	**1440000**
Serum QC Low	Serum	101%	**580000**
BSA QC Low	BSA	98%	**1610000**
Serum QC Low	Serum	96%	**600000**
BSA QC Low	BSA	97%	**1390000**
Serum QC Low	Serum	100%	**550000**

Case study 3: variable matrix effect and its impact

Subject sample analysis in BE study

- Standards and BSA QCs
- Serum QCs
- Subject samples in BE study



Case study 3 – variable matrix effect and its impact

- Concern: significantly elevated matrix effect (signal suppression) may impact the accuracy of concentration data in the BE study
- Proposed justification: conduct an investigation by demonstrating the accuracy of QCs prepared using pre-dose samples in the BE study
- Recommended approach: efforts to mitigate matrix effect during method development and validation are recommended

Challenge Question #1

Which of the following is considered a surrogate matrix if the subject samples of a BE study are in human plasma?

A. BSA buffer

B. Depleted human plasma

C. Rat plasma

D. All of the above

Challenge Question #2

An endogenous drug analytical method using the surrogate matrix approach may be considered inadequate even if all QCs, prepared in surrogate matrix and authentic matrix, meet the acceptance criteria.

A. True

B. False

Summary

- The surrogate matrix approach offers benefits but introduces challenges
- Additional experiments (parameters) and data in method validation are essential
- ‘Matrix cleanness’ and ‘matrix sameness’ should be demonstrated in addition to QC accuracy
- Potential impact of using surrogate matrix on bioanalytical data reliability may require scientific justification

Acknowledgement

- Dr. Patha Roy, OB Director
- DBIII Management: Drs. April C. Braddy (DBIII Director), Ke Ren (DBIII Deputy Director), Yi Zhang (DBIII Associate Director)
- SBIA committee