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REGULATORY PERSPECTIVE OF CONSIDERATIONS IN ENDOGENOUS THERAPEUTIC ANALYTE BIOANALYSIS

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PURPOSE

- Endogenous substances are compounds that are naturally present in the body either because the body produces them, or they are present in the normal diet [1].
- Examples of endogenous therapeutics include hormones, neurotransmitters, vitamins, fatty acids, inorganic elements, and others.
- The accuracy of analyte measurement following the administration of an endogenous therapeutic agent poses a challenge as the exogenous therapeutic analyte and its endogenous counterpart cannot be distinguished.
- This presentation will highlight the challenges encountered in endogenous therapeutic analyte bioanalysis and share the regulatory perspective of important considerations when developing bioanalytical methods and conducting bioanalysis for endogenous therapeutic analytes during drug development.

METHODS

- Real case examples encountered during developing and validating bioanalytical methods for endogenous therapeutic analytes used when conducting clinical studies including bioavailability (BA) and bioequivalence (BE) studies during drug development in support of new drug applications (NDAs), biologics license applications (BLAs), or abbreviated new drug applications (ANDAs) were surveyed.
- Practical lessons learned were summarized and practical tips, strategies to consider from a regulatory perspective were provided.

RESULTS

Common Issues Found

1. While surrogate matrix was used to prepare calibration standards (CSs) and quality controls (QCs), the endogenous concentration of the analyte was not accounted for in the incurred study sample analysis.

Case 1:

- Blank plasma obtained from females was used to prepare calibration standards (CSs) and quality controls (QCs), the male hormone concentrations (i.e., analyte of interest) in CSs and QCs were not adjusted to account for the endogenous hormone concentration in the blank matrix used to prepare them. Therefore, the accuracy of the male hormone concentrations in the incurred samples from study subjects could not be assured.
- The hormone concentrations for the CSs, QCs, and incurred study samples had to be recalculated by adding the endogenous hormone concentration that was derived by employing the standard addition approach.

2. Absence of cross-validation data supporting the accuracy and precision of the analyte measurements addressing the potential matrix effect (e.g., absence of parallelism test) and differences in recovery when a surrogate matrix was employed instead of the authentic matrix (e.g., human serum).

Case 2:

- The CSs in a clinical study were prepared in artificial matrix (i.e., 4% bovine serum albumin [BSA] in 0.9% saline) instead of authentic matrix (i.e., human serum).
- An investigation comparing the responses of CSs for analytes (i.e., hormones) prepared in artificial matrix vs. in authentic matrix had to be carried out. The performance of CSs prepared in both matrices was parallel and showed linear correlation regression slopes of the CSs near to unity between responses from both matrices for all analytes of interest indicating no matrix effects. The precision and accuracy using QCs prepared in both matrices were comparable.

Case 3:

- The bioanalytical method was developed using a surrogate matrix (i.e., methanol) but incurred samples in human serum were analyzed for the pivotal BE study.
- Recovery data for only the QCs in human serum were reported (but not for QCs in methanol).
- Accuracy and precision were assessed during validation using low QCs in methanol but medium and high QCs in human serum, using a standard curve with CSs prepared in methanol.
- Concentrations of QCs in methanol and human serum were not comparable and did not represent the concentration range of study samples.
- A parallelism test (i.e., Parallelism demonstrates that the serially diluted incurred sample response curve is parallel to the calibration curve. Parallelism is a performance characteristic that can detect potential matrix effects.) is warranted to detect potential matrix effects.

Case 4:

- CSs in a surrogate matrix (i.e., phosphate buffered saline [PBS] with 2% BSA) were used to construct the standard curve.
- During method validation, the recovery data were collected for QCs in buffer only but not for those with the authentic matrix (i.e., human serum).
- Recovery data of both surrogate and authentic matrices should be provided to ensure that there is no significant matrix effect.
- Only 1 run each from the surrogate matrix (i.e., PBS with 2% BSA) and authentic matrix (i.e., human serum was performed → At least 3 sets of parallelism data comparing both matrices should be obtained.
- Linear regression was used in the parallelism study while quadratic regression in the incurred study sample analysis → The same regression model and weighting factor should be used in both the parallelism study and study sample analysis.
- The concentrations of QCs in human serum for both method validation and study sample analysis were not representative of study sample concentrations.

3. The stability of the analyte during sample collection and handling was not adequately demonstrated during bioanalytical method development and validation. Deviation for standard procedures of sample handling and processing led to unexpectedly high analyte concentrations (i.e., false positive).

Case 5:

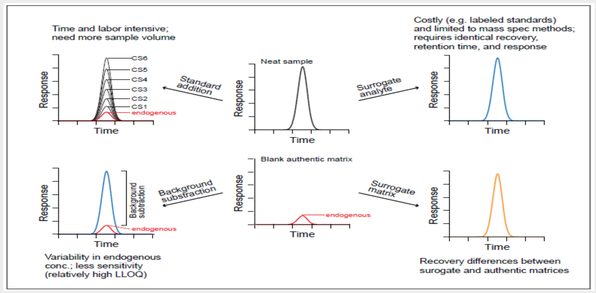
- Measurements of drug concentrations from prodrugs may be confounded if there is *ex vivo* conversion of the prodrug to the drug during blood sample collection and processing.
- Testosterone undecanoate (TU) is a prodrug of testosterone (T) formed by esterification of a hydroxyl group. The average T concentration is directly related to the primary efficacy endpoint for testosterone replacement therapy (TRT).
- Deviation from standard procedures of sample handling and processing may lead to unexpectedly higher T concentration from plasma compared to serum prepared from blood when collected at the same timepoint from the same subject) → There can be a significant consequence as it can cause false positives (e.g., higher T concentration than the actual T concentration *in vivo*).
- The stability of the analyte in the sample should be demonstrated beginning from the blood drawn into a collection tube through the separation of the plasma or serum from the red blood cells and other blood components, as a part of the method development and validation process.
- The following factors were found to contribute to the TU to T *ex vivo* conversion that affects the concentration measurements in both serum and plasma [2, 3]:
  - Post-collection incubation temperature: Lowering the temperature reduces conversion.
  - Post-collection incubation time: TU to T *ex vivo* conversion occurs most rapidly during the first 30 minutes post-collection. Reducing the incubation time will help reducing the TU to T *ex vivo* conversion.
  - TU concentration: The TU to T *ex vivo* conversion is TU concentration-dependent.
  - Presence of esterase inhibitor in test tubes: The presence of esterase inhibitor (e.g., NaF in NaF/EDTA tubes) further reduces the TU to T *ex vivo* conversion.

4. Incurred study samples went through a different sample preparation method compared to the CSs and QCs resulting in an uncertainty of the accuracy and precision of the incurred study samples.

Case 6:

- The assay's recovery of free hormone analog from human serum exhibited high variability due to significant matrix effects. While the regular human hormone including the analogs are minimally protein bound, only about 50-60% of hormone analogs were accounted for as free with this methodology.
- Incurred study samples went through a different sample preparation process compared to the CSs and QCs (in precipitated serum) resulting in uncertainty of the accuracy and precision of the incurred study samples.

Some Strategic Approaches [4]



CONCLUSIONS

- This presentation highlights the common challenges encountered, issues identified, and lessons learned related to bioanalysis of endogenous therapeutic analytes and provides practical tips and strategic approaches (as shown in the Figure above) to consider from a regulatory perspective.
- Reliable, reproducible, and robust bioanalysis of endogenous therapeutic analytes is pivotal for the success of drug development when they are the primary analyte of interest.

REFERENCES

1. U.S. FDA Draft Guidance for Industry: Bioequivalence Studies With Pharmacokinetic Endpoints for Drugs Submitted Under an ANDA , August 2021 (https://www.fda.gov/media/87219/download)
2. Lachance S, Dhingra O, Bernstein J, Gagnon S, Savad C, Pelletier N, Boudreau N and Levesque A. Importance of measuring testosterone in enzyme-inhibited plasma for oral testosterone undecanoate androgen replacement therapy clinical trials. *Future Science* 1(4) (2015).
3. NDA 213953 Clinical Pharmacology Review, US FDA, June 6, 2022 (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2022/213953Orig1s000MultidisciplineR.pdf)
4. ICH Guidance for Industry: M10 Bioanalytical Method Validation and Study Sample Analysis, November 2022 (https://www.fda.gov/media/128343/download)

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