Evaluation of Internal Standard Responses During Chromatographic Bioanalysis: Questions and Answers

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

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Contains Nonbinding Recommendations

Evaluation of Internal Standard Responses during Chromatographic Bioanalysis: Questions and Answers Guidance for Industry[1]

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I. INTRODUCTION

This guidance provides recommendations to sponsors, applicants, and contract research organizations regarding internal standard (IS) response [2] variability in chromatographic analytical data submitted in investigational new drug applications, new drug applications, abbreviated new drug applications, biologics license applications, and supplements. Chromatographic analytical methods are commonly used to quantitate analyte concentrations in samples from nonclinical and clinical studies to support regulatory submissions. Depending upon its source, IS response variability may impact the accuracy of analyte concentration measurements. This question and answer (Q&A) document provides the Agency's current thinking on IS response variability and its potential impact on the accuracy of analyte concentration measurements. This Q&A also suggests approaches to determine whether observed IS response variability is likely to impact the accuracy of the data such that further investigation into the root cause(s) is warranted.

In general, FDA's guidance documents do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

[1] This guidance has been prepared by the Office of Study Integrity and Surveillance, the Office of Generic Drugs, and the Office of Clinical Pharmacology in the Center for Drug Evaluation and Research at the Food and Drug Administration. You may submit comments on this guidance at any time. Submit comments to Docket No. FDA-2017-D-6821 (available at https://www.regulations.gov/docket?D=FDA-2017-D-6821). See the instructions in that docket for submitting comments on this and other Level 2 guidances.

[2] An IS response is a measurement of the signal from the IS, which is typically generated during liquid chromatography-mass spectrometry bioanalysis.

II. QUESTIONS AND ANSWERS

Q1: What are internal standards in analytical chemistry, and why are they used in bioanalysis?

A1: In analytical chemistry, ISs are usually a structural analog or stable isotope of an analyte of interest and are commonly used in chromatographic analytical methods to correct for variability in sample processing and analysis. In chromatographic bioanalysis, an IS is added to all samples, including calibration standards (Cals), quality controls (QCs), and subject samples prior to extraction. The specific IS selected for a method should have similar physicochemical properties to the analyte of interest so that it behaves in a similar fashion to the analyte and reflects any changes to the analyte measurement that may occur during sample processing and analysis.

Q2: What are sources of IS response variability?

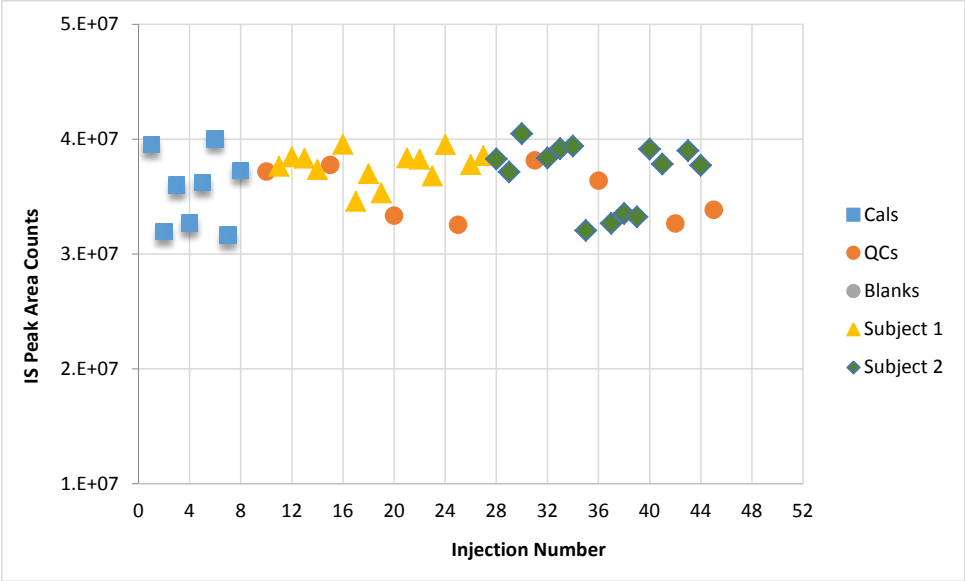
A2: By design, the intention is to add the same amount of IS to all samples. However, variability in IS responses among samples is occasionally observed, even among samples analyzed in the same run. Potential sources of IS response variability include human errors made during sample preparation or processing, instrumental issues that may occur during analysis, and matrix effects.

Q3: When is IS response variability not likely to impact the accuracy of the data?

A3: IS response variability is not likely to impact the accuracy of the data when the range of IS responses for subject samples is similar to the range of IS responses for Cals/QCs in the same analytical run. For example, variability in the IS responses for subject samples is not likely to impact the accuracy of the data under the following circumstances:

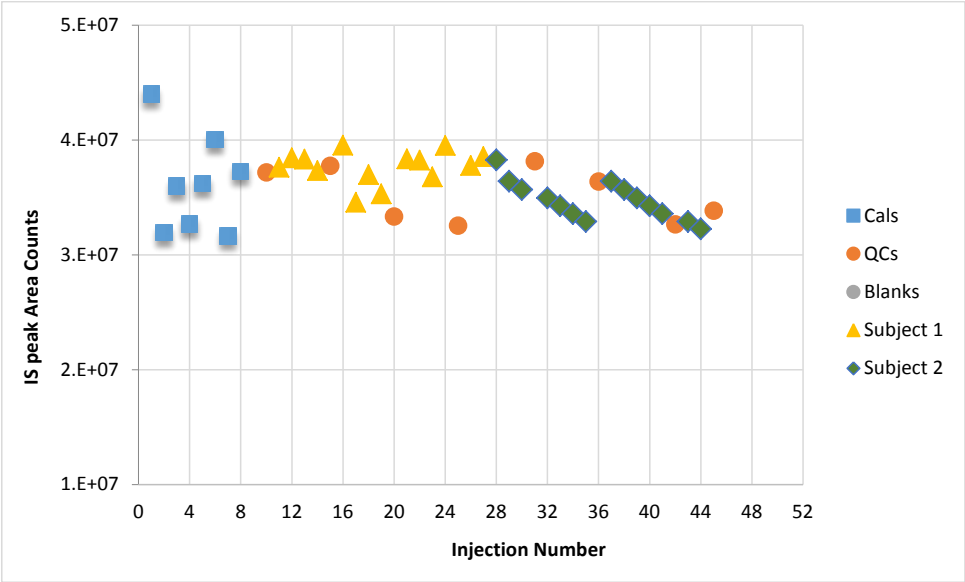
Example 1: IS responses for subject samples are similar to IS responses observed for Cals/QCs in the same analytical run (see Figure 1).

Figure 1. Plot of IS responses in an analytical run, exemplifying IS responses for subject samples that are similar to IS responses observed for Cals/QCs



Example 2: IS responses for subject samples demonstrate a gradual drift or repeating pattern, but the range of IS responses for subject samples is similar to the range of IS responses for Cals/QCs in the same run (see Figure 2).

Figure 2. Plot of IS responses in an analytical run, exemplifying IS responses for subject samples demonstrating a gradual drift or repeating pattern similar to Cals/QCs

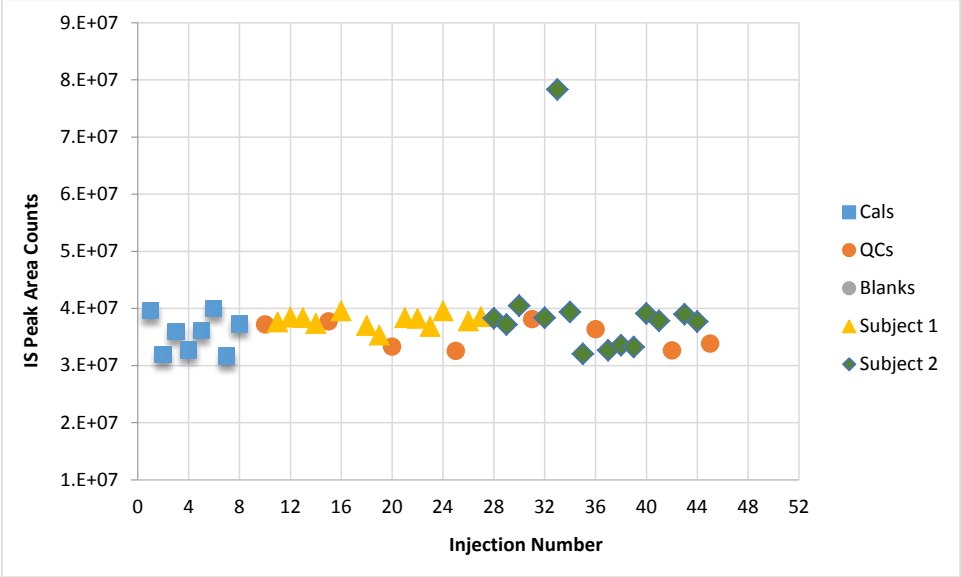


Q4: When can IS response variability impact the accuracy of the data?

A4: IS response variability may impact the accuracy of the data when the range of the IS responses for subject samples is different than the range of IS responses for Cals/QCs in the same run. For example, IS response variability for subject samples may impact the accuracy of the data under the following circumstances:

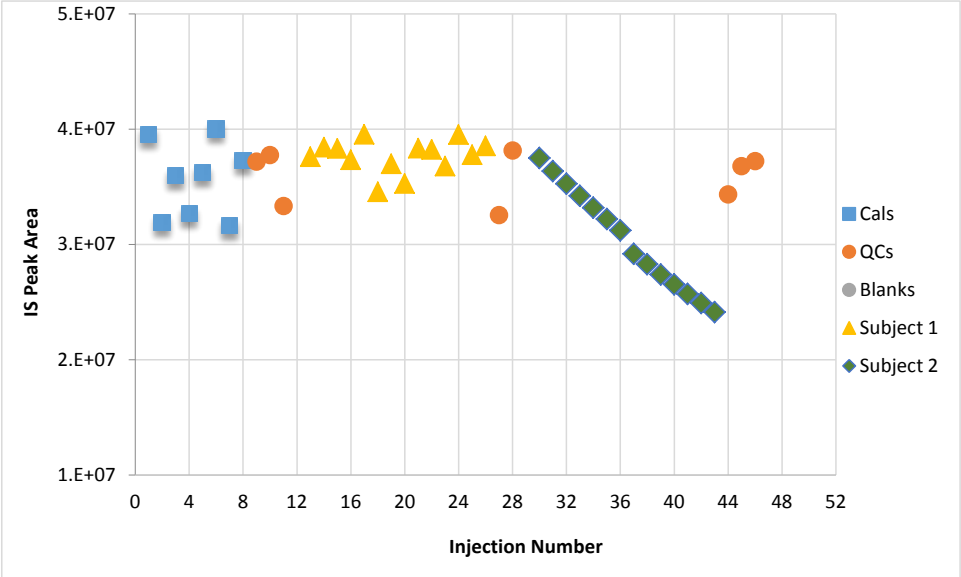
Example 1: IS responses for one or more subject samples are substantially different from the IS responses for the majority of the other subject samples, and the IS responses for Cals/QCs do not demonstrate a similar variability pattern (see Figure 3).

Figure 3. Plot of IS responses in an analytical run, exemplifying an IS response for a subject sample that is substantially different from the responses for the majority of the other subject samples and Cals/QCs



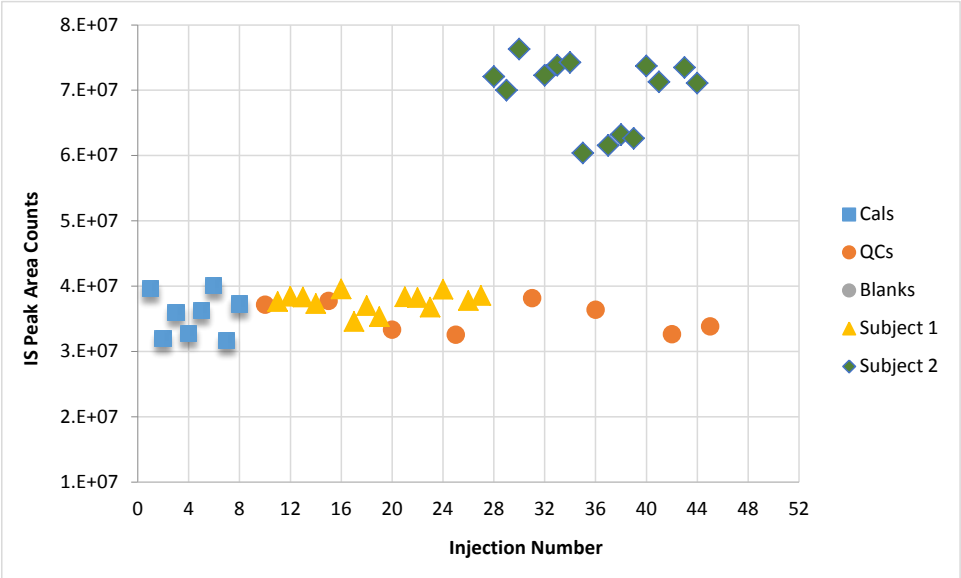
Example 2: There is a gradual increase or decrease extended beyond the range of the IS responses for subject samples, and there are no IS responses for QCs interspersed among the impacted segment of the run (see Figure 4).

Figure 4. Plot of IS responses in an analytical run, exemplifying a gradual decrease in IS responses for subject 2 samples without IS responses for QCs interspersed among the impacted segment of the run



Example 3: IS responses for subject samples are consistently lower or higher than IS responses for Cals/QCs (see Figure 5).

Figure 5. Plot of IS responses in an analytical run, exemplifying IS responses for subject 2 samples that are consistently higher than IS responses for Cals/QCs



Reviewing the scatter plot of IS responses for all samples in the run might help identify variability trends that may or may not impact the accuracy of the data.

Q5: How can sponsors address concerns about IS response variability?

A5: When IS responses for subject samples are outside of the ranges observed for Cals/QCs that meet the run acceptance criteria, the subject sample concentration measurements might not be accurate. Therefore, sponsors should determine whether this observed difference impacts the accuracy of the data for subject samples.

For subject samples with IS responses significantly different from the responses for Cals/QCs (as pre-defined in a standard operating procedure (SOP)) in a run (see Figure 3), pre-established criteria for repeat analysis should be followed. The repeat analysis criteria should be justified and the protocol for the number of replicates reanalyzed for each sample and final values reported should be established in an SOP before initiation of the study.

For runs where a gradual drift or pattern in IS responses is observed between subject samples and there are no IS responses for Cals/QCs within the impacted segment of the run, subject samples displaying the aberrant IS responses (see Figure 4) should be reanalyzed. If the IS responses for subject samples in the repeat analysis are similar to those of Cals/QCs, and if the analyte concentrations in a repeat run are within 20% of the original assay results of the individual samples, generally no further investigation into the cause of the initial variability is necessary. Sample values to be reported should follow a pre-established procedure. However, if the IS responses for subject samples in the repeat analysis are similar to those of Cals/QCs, but the analyte concentrations in the repeat analysis are not within 20% of the original assay results of the individual samples, then sponsors should report analyte concentrations from the repeat analysis.

When IS responses for subject samples are consistently lower or higher than IS responses for Cals/QCs (see Figure 5), a subset of subject samples should be reanalyzed. If IS responses of subject samples in the repeat analysis are again not within the range for those of Cals/QCs (i.e., differences in IS responses in the original run are reproducible), and repeat analyte concentrations are still comparable to the original values, additional data might be needed to demonstrate that the differences in sample matrices (i.e., matrices for subject samples vs. matrices for Cals/QCs) do not impact the accuracy of the analyte concentration measurements. The approach(es) for investigation might include, but are not limited to, an analysis of QCs prepared in pre-dose matrix of individual impacted subjects, serial dilution of impacted subject samples using the matrices used to prepare Cals/QCs, or any other scientifically justified approach.