Commentary
SPECIAL FOCUS ISSUE: Stability Issues in Bioanalysis

Bioanalysis

Validating stability and selectivity in the presence of co-administered compounds

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Contract research organizations (CROs), pharmaceutical companies and regulators have extensively discussed co-administered stability since it was brought up in 2010 at the Fourth Calibration and Validation Group Workshop on Recent Issues in Regulated Bioanalysis [1]. The questions are whether or not co-administered drugs impact the stability of an analyte, and what regulatory requirements should exist for cases of co-administered drugs. As of 2014, no cases of co-stability issues were reported and no scientific rationale seemed to exist on why co-administered drugs would impact stability. However, no consensus was reached among regulators [2].

In 2018, the US FDA reached a conclusion that is included in the 2018 Bioanalytical Method Validation Guidance for Industry. The FDA requirement is that sponsors assess the stability of an analyte in the presence of other drugs that may be present in the matrix when the drugs are administered as fixed combinations or as part of a specific drug regimen. In addition, stability of the analyte should be considered in the presence of other known co-medications. Selectivity and specificity should be demonstrated in the presence of potentially interfering compounds such as co-administered drugs or metabolites [3]. The draft ICH M10 guideline for bioanalytical method validation contains similar recommendations [4].

It is known in the bioanalytical industry that study samples can never be perfectly mimicked with prepared quality control (QC) samples, although we do the best we can. In the case of co-administered drugs, performing stability and interference testing on every possible concentration, combination and storage temperature would simply be impractical and cost prohibitive. We are left with the task of creating standard operating procedures (SOPs) that cover most possible situations most of the time. In this case, we must determine exactly how to prepare QCs for testing co-administered drug stability and interference, and exactly which experiments to perform on them.

Our company is a CRO specializing in LC–MS/MS and GC–MS/MS methods. We interpret and implement these recommendations in the following ways for these types of assays.

Scope of the guidance

First, the scope of the guidance must be considered. If a series of drugs are administered in combination, is it important to assess the stability of each drug in the mixture in the presence of the other elements of the combination? This would be the most exhaustive and thorough approach. However, it may be an excessive amount of experimentation that yields little useful information. For example, often novel drug candidates of interest are co-administered with well-established medications that are known CYP enzyme inhibitors. These studies aim, as a primary end point, to assess the impacts of this class of drugs on the pharmacokinetic (PK) profile of the drug of interest. Plasma concentrations of the CYP inhibitor are often monitored to verify that the study subjects have reached a steady state by analyzing only a few samples. But since the PK profile of the CYP inhibitor is already well established, it is not assessed as a part of the study. In this case, we would suggest that it is not necessary to test

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the stability of the CYP inhibitor (or other drug that is co-administered in a similar study design) in combination with the novel drug candidate. We would agree with an often stated industry perspective that co-administration stability testing should only be required when the PK profile of the drug that is assessed is a primary end point of the study being performed.

Co-administered compounds versus metabolites

Second, we make a distinction between co-administered parent compounds and their known metabolites. This is because it seems that the 2018 FDA guidance only requires stability experiments be performed in the presence of co-administered compounds, not necessarily the metabolites. However, selectivity and specificity experiments may need to be performed for all potentially interfering compounds: co-administered drugs, known metabolites and endogenous substances. The guidance calls for scientific judgment to be used when determining the need for interference testing [3].

For example, a study may dose patients with proprietary xenobiotic analyte 'X', and co-administer a prodrug such as capecitabine. As a prodrug, capecitabine has many known metabolites which eventually convert to the therapeutic agent 5-fluorouracil. Should stability experiments be performed for X in the presence of capecitabine and all metabolites, or perhaps just 5-fluorouracil, or some other combination? Since the guidance only requires stability be performed in the presence of co-administered compounds, at our company we would simplify the possibilities by only requiring stability be performed for X in the presence of capecitabine. However, selectivity and specificity must be performed for X in the presence of capecitabine and its metabolites – at least the ones that can be purchased for analytical purposes.

The case may also occur where analyte X has metabolites of its own. For reasons beyond the scope of this commentary, the sponsor may or may not choose to include a metabolite (M) in the validated bioanalytical method. If X and M are both validated, then of course stability of X and M must be determined in the presence of the prodrug, capecitabine (to continue the example). Specificity and selectivity of X and M would need to be determined in the presence of capecitabine and metabolites.

However, if only X is validated, then stability need only be verified for X in the presence of capecitabine. Specificity and selectivity would need to be determined for X in the presence of everything – M, capecitabine and capecitabine metabolites.

Concentration of the co-administered compound

Third, we need to determine the concentration of the co-administered drug(s) expected in the samples. Usually we prepare low, mid and high QC levels of analyte X in a matrix prepared with the co-administered drug(s) at a concentration near C_{max}. These we call 'co-ad QCs' at our company. We reason that if analyte X is stable in the presence of co-administered drug at C_{max}, it should be stable if less co-administered drug is present.

However, it is not uncommon for C_{max} of the co-administered drug to be unknown in the validated method's matrix. A co-administered compound may have a well-known C_{max} in plasma, but little to no data may exist for the concentration in other matrixes such as urine or cerebrospinal fluid. So, we must estimate. For example, if we know the plasma C_{max} of the co-administered drug, and we want to know the C_{max} in urine, we might take the plasma C_{max} and multiply by ten. We would then use this concentration to prepare the matrix for our co-ad QCs. In other situations, C_{max} for our matrix of interest may be available from a different species than our validated method. We have also used this estimate to prepare co-ad QCs. Of course, any estimate is imperfect and requires sponsor approval.

Suggested stability experiments

Fourth, we must determine which stability experiments to perform. At Alturas we have extensively discussed and considered: should the stability experiments performed on the co-ad QCs match every stability experiment performed in the validation, or should we pick and choose a handful of stability experiments to perform on the co-ad QCs? It would be easy to dive down a 'rabbit hole' here. We are assisted by the 2012 the Global CRO Council for Bioanalysis recommendation that only benchtop and freeze–thaw stability are necessary in the presence of co-administered compounds. Only if these failed should long-term freezer stability be evaluated [5]. Per these recommendations, Alturas has opted to perform these benchtop and freeze–thaw stability tests on the co-ad QCs. Additionally, for the sake of thoroughness, we have also opted to include one long-term stability evaluation at one storage temperature (-80°C) for every co-ad QC that is undergoing stability testing.

Incurred sample reanalysis

The final test of assay robustness and repeatability is incurred sample reanalysis (ISR). If multiple combinations of co-administered drugs are present in a study, it is prudent to take ISR samples from each combination, if possible. For ongoing studies initiated before the May 2018 FDA guidance was issued, if ISR is passing, it does not seem necessary to go back and validate co-ad QC stability.

Large molecules

Tandem MS assays are known to be highly specific and selective, especially when a stable label internal standard is used. Therefore, at our company, we perform interference testing for all co-administered small molecule drugs, but not large molecule therapeutics. This is because the large molecules are often removed as part of the extraction procedure. If remnants did remain in solution, the molecular weights of the small and large therapeutics are so different that the chance for interference in tandem MS is practically zero.

Summary

We have been performing co-administered stability and selectivity in some capacity for almost 10 years. As a CRO, we are inherently risk-adverse; thus, we have taken a conservative approach to implementing the FDA guidance in our SOPs and have standardized our method of evaluating the impact of co-administered compounds on the above strategy for the past several years. That said, over the past 10 years, we have performed validation experiments on over 50 combinations of co-administered compounds and proprietary novel drug candidates and to date, we have not seen any impact from the co-administered compounds on the ability to accurately quantify the drug candidate or any change to the stability of the candidate. While we agree that it does make scientific sense to prove the lack of interference between the co-administered compound(s) and the proprietary drug, we believe that our data along with the data that has been presented by other companies provides a strong argument that the requirement for co-administered compound stability should be reconsidered during the drafting of any upcoming guidance documents.

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All authors are paid employees of Alturas Analytics, Inc. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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