Cleaning Memo for March 2017
Establishing Clearance for Degraded Protein Actives

Last month we covered a possible approach for setting "health-based" limits for biotech manufacture based on the "fact" that the biotech active protein would be degraded and inactivated during cleaning. There is an important difference between "inactivation" (or deactivation) and "degradation". Inactivation means that the protein loses specific biological activity based on a change in the protein structure. Degradation usually means that the protein is broken down to smaller molecular weight fragments. In many cases, inactivation and degradation go hand in hand. However, it may be possible to inactivate a protein active without having significant reduction in the protein molecular weight. It may also be possible to have degradation without loss of biological activity.

Why am I bringing this up if the issue here is *establishing clearance*? The reason is that biotech firms also expect that in *bulk drug active manufacture*, degraded proteins which may be residues of previous cleaning processes are removed by the various purification processes used in the downstream bulk active manufacture. This is one basis for trying to limit shared surface areas in carryover calculations to only the equipment *after* the last purification process. This approach is based on the following ICH Q7 principle (Section 12.70):

> "In general, cleaning validation should be directed to situations or process steps where contamination or carryover of materials poses the greatest risk to API quality. For example, in early production it may be unnecessary to validate equipment cleaning procedures where residues are removed by subsequent purification steps."

Purification processes in biotech may involve filtration (for example, where the protein active is retained by the filter based on size or molecular weight), or it may involve chromatography, where the protein active is retained on a column resin (and other components are not), followed by removal or elution of the protein active from the column resin. My preference in establishing that the cleaning process residues are cleared by my downstream processing is to show multiple different ways in which clearance occurs; this is not unlike the approach for viral clearance in showing *orthogonal* processes for virus removal.

Last year the May 2016 Cleaning Memo addressed clearance in small molecule API manufacture. In small molecule API organic synthesis, the main clearance mechanisms are filtration and recrystallization, and the focus of that Cleaning Memo was both on possible theoretical considerations and on possible studies to demonstrate clearance. This Cleaning Memo will attempt the same for demonstrating clearance of biotech cleaning residues. In addressing the biotech situation, we must remember that the purification processes are primarily designed to remove "impurities" from the manufacturing process of a given protein active. Clearance of those impurities is generally addressed in process development and process validation. Clearance of residues left from prior cleaning processes is a different matter, particularly if those residues are from a *different* protein

active, and even more so if different purification steps are used for the two products (the cleaned product leaving the residues and the next product which could be potentially contaminated). A critical element for residues is not necessarily whether they are removed by the purification steps of the same product (as likely in a biotech campaign of the same product), but whether they are removed by the purification steps of the next product (as likely in any changeover from one product to another).

So, the first step might be taking a looking at "theoretical" concerns (theoretical in that these are likely to occur based on sound scientific principles). For example, for filtration (such as TFF) it might be possible to determine that the active protein being manufactured is retained by a filter based on a molecular weight cutoff of X kDa (kiloDaltons). The molecular weight of the active is >X kDa, so it is retained. I then use data from my degradation study (for example, using SDS-PAGE) on the estimated molecular weight ranges of the degraded fragments from the prior manufactured protein. If the molecular weights of the degraded fragments of the prior manufactured product was found to be < 0.5X kDa (I have chosen that 0.5 factor somewhat arbitrarily), then I might conclude that that the prior cleaning residues would be cleared from the process stream by that purification step.

The question then becomes "what percentage clearance occurs with that step?" This can be estimated if I know the maximum volume of liquid (or suspension) passed through the filter, and the volume of "liquid" retained by (that is, not passed through) the filter. This assumes that there is no interaction between the protein active and the degraded fragments. One of the functions of this type of filtration, in addition to removing of impurities, is to concentrate the protein. Therefore, you can expect significant clearance of smaller fragments that pass through the filter.

This assessment of clearance may be easily done by this type of "paper" exercise. Actually measuring residue by a technique such as TOC is probably not feasible (too many other sources of TOC). That said, it *might* be possible to make an assessment of clearance by performing a "before and after" SDS-PAGE to show that the presence of small molecular weight fragments in the "before" filtration suspension and in the filtrate, and the relative lack of such low molecular weight fragments for the retained product. If done on the lab scale, this could also be done using just a preparation of "degraded protein" (without the addition of the next native protein that might be present). This allows not only for SDS-PAGE analysis, but also for analysis by TOC and/or total protein.

What about showing clearance in a chromatographic purification process? Because of the variety of chromatographic options (ion-exchange, affinity, size exclusion, etc.), it's a little more difficult to make suggestions here. Let's assume that the mechanism is passing a suspension through a column, retention of the protein active by the column (with various other impurities, including impurities from the prior cleaning process residues, flowing through and exiting the column), and final elution of the protein from the column using a suitable buffer. A paper exercise will be much more difficult, in that predicting retention by the resin may be more difficult.

In such a situation, it may be best to perform a lab study, where the degraded protein alone is passed through the column (simulating actual production conditions). A comparison of the initial degraded protein and the exiting solution by SDS-PAGE, TOC and total protein may assist in determining clearance by the chromatographic procedure.

Note that in evaluating clearance by filtration and clearance by chromatography, the degraded protein preparation used for lab studies may be different in cases where filtration precedes chromatographic purification.

Finally, the purpose of this Cleaning Memo is not to say we should change our limits for cleaning validation in biotech bulk drug substance manufacture. The purpose is to help establish clearance and thus help provide an even better rationale for the acceptability of current limits.