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Sampling Recovery for Biotech

The focus of this Cleaning Memo is the issue of what residue to spike onto coupons for sampling recovery studies (whether they be swab sampling recoveries or rinse sampling recoveries) for biotechnology manufacture. The ideal is to spike the residue that will be sampled in the cleaning validation protocol. With small molecule drugs, for example, the drug active itself is spiked onto coupons to determine recovery of the active. This is done even though that residue may typically be associated with other residues on the manufacturing equipment surfaces, including residues of any excipients or cleaning agents. With biotech, what is done? Well, first of all, on the bulk side of biotech, what is cleaned is different in the upstream (fermentation, harvesting) processes as compared to the downstream (purification) processes. These earlier stages typically involve many more materials (such as cellular debris) other than the active protein. While it theoretically is possible to do recovery studies on the various "products" at each stage of biotech manufacture, what is actually done is to utilize materials which are representative of the different stages of manufacture. For example, I might utilize a sample of the harvest material to represent the upstream processes and a sample of the purified bulk to represent downstream processes. Note that the results for the purified bulk might also be applied to recovery for the final drug product (the finished drug product).

While this is typically done, it should be recognized that spiking with the harvest material or the bulk active might represent a worst case situation. Why is that? The reason is that the residues that are actually sampled in a cleaning validation protocol are those biotech residues that exist after the cleaning process. Since cleaning is generally performed in biotech with hot aqueous alkaline and acidic solutions, the protein actives are degraded by the cleaning process to smaller fragments. Those fragments are typically lower molecular weight than the native protein and therefore more likely to be more water soluble than the native protein. Because of this, recoveries are likely to be either the same or a higher with the degraded fragments as compared to the native protein. This argument can also be applied to the other organic constituents, particularly in the early processes (fermentation and harvesting).

What this means for cleaning validation in a biotech setting is that it may be more indicative of the actual recovery in a cleaning validation protocol if the laboratory recovery were performed on degraded product, not on the undegraded product (as is typically done). Admittedly, there may be problems in performing such a recovery. If the degradation is due to NaOH, then preparation of the degraded material should be done by exposing the material to NaOH (at the concentration of the cleaning solution) for the time and temperature of the cleaning process, followed by quenching the temperature and neutralizing the pH. The resultant solution should be used within a reasonable time frame so as to minimize any subsequent hydrolysis that might occur under neutral pH conditions. One concern with this is that the material that is spiked will also contain added salt (sodium phosphate if phosphoric acid is used to neutralize the caustic). It is unclear whether the added salt will affect the percent recovery. This effect can be minimized by use of dialysis to remove/reduce the salt concentration in the degraded material. If a formulated detergent is used for cleaning, then either that formulated detergent can be used for the degradation study, or else just the caustic portion of the formulated detergent can be used. If the formulated detergent is used for degradation, unless the surfactant can be removed (such as by dialysis), it may possibly cause higher recoveries because of its wetting properties.

Note that while the use of degraded materials may be more indicative of recovery in actual cleaning validation protocols, the use of undegraded materials for recovery studies is perfectly acceptable because undegraded materials represent a worst case (that is, they may result in lower recoveries as compared to recoveries performed with the degraded materials).

A caution is in order here. I have never seen as side-by-side study done comparing native versus degraded protein. But, good science tells us (I think) that degraded fragments should be more water soluble. However, it may be the case that a swab recovery study is done comparing degraded versus native protein, and the result shows that the recovery with the native protein is 75% and the recovery with the degraded protein is only 70%. Does this disprove my assertion? Probably not. The answer is that this slight difference (really an insignificant difference) in recoveries is probably due to variabilities in the swabbing process itself, not due to a difference between the degraded and native materials. On the other hand, if the difference was between 90% (for the native protein) and 60% (for the degraded protein), then this would suggest that either this particular protein is an exception to the rule, or that there is some factor that I have missed in claiming that degraded proteins will have either the same or higher recoveries as compared to the native protein. Of course, if it is the case that the degraded protein results in a lower recovery, then it makes sense to do the recovery study with that degraded material.

The purpose of this Cleaning Memo is not to specify how to perform recovery studies for biotech, but rather to discuss the degradation of the biotech materials as it relates to sampling recovery studies.