Cleaning Validation for biotechnological substances : What acceptance criteria ?

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EMA Guideline published in 2014(1) and PDA TR49(2) clarify some cleaning validation aspects for therapeutic macromolecules. But they don't bring a clear position on acceptance criteria definition. Based on scientific rational arguments, biotechnological Process is considered as a "self cleaned" process because many purification steps are performed to remove process impurities as well as degradation products generated by the cleaning process. According to this principle, ICH Q7(3), defining GMP requirements for cleaning validation for API, states that no validation is requested for the early steps of the process if the effectiveness of purification is demonstrated. However, no identification of degradation products following cleaning is generally performed to confirm that these specific impurities are removed during the downstream process. In these conditions, it is not possible to definitively conclude on the level of acceptable limit in residues on the surface of manufacturing equipment after cleaning. Reasonable justifications must be provided if different acceptance criteria are applied for equipment used for the upstream and downstream phases of the manufacturing process and establish the clearance of degradation impurities in the drug substance.

Unlike chemicals, it cannot be considered that for a train of equipment there are 100% of the impurities that are transferred in the final product because purification steps of the manufacturing process contribute to their removal. Currently, companies apply different standard acceptance criteria for upstream and downstream equipment. This approach is based on the premise that degradation by the cleaning agent leads only to amino acids which are totally removed during the purification steps. Founded on that justification, acceptance criterion is defined for equipment used after the last purification step (usually a TFF) and a fixed multiplication factor of 5 to 10 is generally adopted for equipment used for early steps of the manufacturing process as described in PDA TR 49. The document reports that "typical values established a acceptance criteria by manufacturers are 1-2 ppm TOC for downstream process and 5-10 ppm TOC

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for upstream process" but in fact, no scientific rationale is established to justify these values.

As specified in GMP annex 15[4] for macromolecules and peptides, it is well known that biological products are sensitive to hydrolysis at extreme pH[5], [6], [7] and/or high temperature. The cleaning operations are carried out generally with sodium hydroxide and/or acid solutions at high temperature. Because chemical stability of peptide bonds is very high, hydrolysis is not complete, the chemical treatment leads to amino acids, oligomers and fragments. Capacity of the cleaning process to degrade proteins is related to process parameters, temperature, contact time and concentration of cleaning agent. According to validation principles these parameters are studied and adjusted during development of cleaning process but impurities produced by hydrolysis are rarely characterized. Even if denaturation or degradation of biological active proteins lead generally to inactive fragments, the lack of identification of impurities does not guarantee the complete safety of the cleaning residues. In the Design Phase, laboratory studies may be conducted to assess hydrolysis capacity in cleaning conditions.

The scientific methodology, in line with the approach described in Part II of the EU cGMP (ICH Q7), is to consider separately the contribution of each process step to remove impurities and to apply a specific acceptance criterion for equipment used between these steps. On the basis on the more restrictive acceptance criteria applicable for equipment used for formulating pharmaceuticals (all contaminants present on the surface of the equipment can be transferred to the following product), acceptance criteria are adjusted in function of reduction ratio of each process segment.

Two types of reduction of cleaning residues should be considered. First, the fragmentation of the product stream that consist in a physical separation of large and small fragments, soluble and insoluble residues. It is the case for precipitation, centrifugation or depth filtration, for example, that contributes to the removal of a part of impurities because only a portion of initial material is engaged in the following step of the process. The criterion applied to these steps assumes that only a part of the protein residues is found in the subsequent process. Without characterisation and quantification of impurities in the product before and after purification it is not possible to specify the ratio of removal.

The second way of reduction of cleaning residues is by the purifications steps of the manufacturing process. They limit the transfer of contaminants to the next step. The efficiency of removal and the nature of the impurities eliminated depend mainly on the purification method. For TFF, the expected product is retained by the membrane and degraded fragments under the molecular weight cutoff are removed. Because cleaning conditions have an important impact on the degradation of protein, efficiency of TFF for their elimination may be variable. The percentage clearance during that phase remains theoretical but if analysis of cleaning residues is performed during or after development it is possible to define approximatively a ratio for removal. The contribution of affinity chromatography for the removal of cleaning residues is certainly important but more difficult to estimate because it depends on the nature of denatured proteins. Product of interest is bound on the column However, the fragments generated by the cleansing process which possess the ligand binding epitopes can also interact with the medium. Most part of residues is removed by the washing of the resin, other remains in the final elution. Similarly, ion exchange chromatography will make it possible to eliminate degradation impurities generated by hydrolysis of the main product that are generally more polar and more soluble in water. As for affinity chromatography, without studies, it is not possible to clearly specify the level of clearance.

In order to identify major degradation residues, characterization studies should be performed. Laboratory experiments in cleaning conditions (concentration of cleaning agent, temperature, contact time) make it possible to obtain the degradation residues which are characterized by LC-MS or by simpler methods such as SDS-PAGE. These studies are crucial for assessing the risk of their presence in the finished product and justify its safety. The nature of the impurities identified by the characterization studies will determine the mode of sampling as well as the analytical methods. It is important to note that if sampling is carried out on the last rinsing water, recovery studies should be done as in the case of swabs. In the same way for analytical methods, if the identified tracer exhibits well-defined physicochemical or structural characteristics or if the concentration of the major component is very much superior to the other residues, a specific assay method for its quantification may be preferred to the TOC during validation.

Following identification of cleaning residues, down scale model studies should be performed to justify the percentage clearance applied for calculation of acceptance criterion for each part of the manufacturing process. At this stage, a weighting factor can be used to account for the variability for a best control the removal of the impurities and to ensure that the expected acceptance criterion is reached. The weighting factor remains empirical because it is not based on scientific data.

The benefits of this new approach are certain. It makes it possible to provide all the scientific rationales necessary for the justification of the criteria adopted and to check that the assumption that the manufacturing processes are "self-cleaned. Through the characterization of cleaning residues, it will also be possible to provide evidence of their non-toxicity. In addition to the scientific value of this approach, the methodology offers economic advantages. Indeed, small-scale, low-cost studies can be quickly implemented and ensure reliability of cleaning processes on an industrial scale.

Abbreviations

API : Active Pharmaceutical Ingredient
LC-MS: Liquid Chromatography Mass Spectrometry
MAC : Maximum Allowable Carryover
SDS-PAGE: Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
TFF : Tangential Flow Filtration
TOC: Total Organic Carbon

References

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