VAC_13 - Determining identity, purity and activity of Benzonase® endonuclease applied on the Recombinant Covid-19 Vaccine active pharmaceutical ingredient production

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Introduction: For Recombinant Covid-19 Vaccine active pharmaceutical ingredient (API) production, a restriction enzyme is employed as a raw material on downstream processing. Benzonase® is an endonuclease genetically engineered consisting of a 30kDa dimeric protein that degrades all forms of DNA and RNA, being widely applied on the purification of viral vaccines and viral vectors. To meet regulatory requirements, the Quality Control Department (DEQUA/ Bio-Manguinhos) developed three tests for routine analysis of raw material release.

Objective: This work aims to set up the identity, purity and activity suitable assays as tolls to assure the quality of Benzonase®, in compliance with the National Regulatoria Agency (ANVISA) statement.

Methodology: In this work the Laboratory of Microbiological Control (LACOM) established a denaturing polyacrylamide gel electrophoresis (SDS-PAGE) procedure to address the identity and purity of Benzonase®. In parallel, the Physical Chemistry Laboratory (LAFIQ) developed a spectrophotometric method to address the endonuclease activity and a semi-quantitative Induced Coupled Plasma Optical Emission Spectroscopy (ICP-OES) method for trace metal impurities (Al, Co, Cr, Cu, Fe, Mn, Mo, Ni and Zn). To meet regulatory requirements, the Laboratory of Metrology and Validation (LAMEV) established protocols that were followed to access performance parameters to demonstrate that all assays are suitable for their intended purpose.

Results: The results have shown that the SDS-PAGE method demonstrated a robust capability of resolving proteins and unequivocally differentiate between bovine serum albumin, ovalbumin and Benzonase®. The purity assessment by densitometric analysis of the SDS-PAGE gel was able to detect the presence of putative contaminant proteins in amounts as low as 0.6 µg. The enzymatic activity by UV-Visible method proved to be precise and robust for variations in the incubation time on ice and wavelength of reading. The validation of ICP-OES method for the elemental impurities complied with selectivity acceptance criteria and showed an acceptable sensibility degree.

Conclusion: Our results providing sufficient evidences to demonstrate that all assays are suitable for its intended purpose. This multidisciplinary work is now part of the routine of analysis required before Benzonase® utilization in the production of the Recombinant Covid-19 Vaccine API by Bio-Manguinhos/Fiocruz.

Keywords: Vaccine Production; Raw Material; Analytical Development; Quality Control