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7 SUMMARY, CONCLUSIONS AND PERSPECTIVES

7.1 SUMMARY

In this thesis the development and implementation of new analytical methods for the analysis of viruses and viral proteins in vaccine products are described. The research in this thesis aimed to expand the analytical toolbox for the characterization of vaccine products to overcome issues typically observed for traditional methods, such as low throughput, limited sensitivity and matrix incompatibility.

Chapter 2 described the development of a capillary gel electrophoresis (CGE) method for the quantification of the influenza proteins in influenza viruses and virus-like particles (virosomes). Effective deglycosylation of the glycoproteins was possible within 2 hours instead of the 16-hour protocol used for SDS-PAGE. Full factorial design of experiments was used to optimize the applied voltage, capillary cassette temperature and the hydrodynamic injection. The gel buffer was optimized to achieve baseline separation of all influenza proteins within 15 min. The electrophoretic protein profile obtained by the validated method was specific for different influenza strains and could be used for identification purposes. The hemagglutinin (HA) concentration could precisely and accurately be determined in all samples from the production process, which was not possible for the existing HPLC method. The sample throughput for the CGE method is almost 3 times faster as for single radial immunodiffusion assay (SRID). With the improved method, the HA concentration can be quantified in new influenza strains without the need for an additional validation or specific antibodies.

The development of an RP-UHPLC-UV method for quantitative adenovirus protein profiling was described in **Chapter 3**. The TFA concentration and the column temperature were identified as the critical method parameters with the highest risk to negatively impact the adenovirus protein separation and, therefore, TFA and the column temperature were optimized and controlled by design of experiments (DOE). All adenovirus proteins could be baseline separated within 17 min on a C4 column (300 Å, 1.7 µm, 2.1 x 150 mm) with a water-ACN gradient containing 0.175% w/v TFA as ion-pairing agent. The adenovirus test samples were directly injected into the UHPLC system without the need for sample pre-treatment and the viruses dissociated into the viral proteins upon contact with the acetonitrile/water mobile phase. The RP-UHPLC method was successfully validated for two purposes: confirmation of the identity of the test sample and detection of protein modifications or degradation products of the adenovirus vector. The method can detect changes in the adenovirus protein composition as a result of thermal or oxidative stress and impurities such as protein degradants, leachables, and host cell proteins can be detected.

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Chapter 4 described the development of capillary zone electrophoresis (CZE) for precise and accurate analysis of adenovirus samples containing variable amounts of cell debris, cell lysate, host cell proteins, host cell DNA, salts, detergents, and/or additives. The CZE method was proposed as an alternative to the current methods, qPCR and AEX-HPLC, as issues with the latter techniques were circumvented with the CZE method. Intact adenoviruses from upstream and downstream processing (USP and DSP) could directly be analysed by CZE and only samples with high amounts of host cell DNA required a simple benzonase sample pre-treatment. The separation voltage, the buffer composition and pH, and the capillary effective length were optimized by DOE as part of the AQbD approach. The CZE method was validated for the quantification of adenovirus throughout the production process. A great advantage of CZE is its compatibility with USP and DSP samples and their variable matrices. In contrast, anion exchange (AE) HPLC is only suitable for purified adenovirus samples. With a run time of only 3 min, CZE allows the analysis of 30 samples within 4 hours compared to 3 days by qPCR. The precision and accuracy also significantly improved compared to AE-HPLC and qPCR. The improved precision of the CZE method makes it possible to improve the formulation or production process, since smaller process improvements can be detected with adequate statistical confidence.

Chapter 5 summarized the analytical quality by design (AQbD) method development approach in detail for the work presented in Chapters 4 and 6. All steps of AQbD were fully applied and the approach and tools were designed and optimized to ensure a structured and trailer-made approach for method development. CZE was selected as the method of choice for adenovirus analysis after comparison of six analytical methodologies by using a technology selection tool. The criticality assessment and a risk assessment were successfully used to prioritize and focus the method development experiments according DOE (Chapter 4) and were also used as input to define the operator training and control strategy for the method during routine use (Chapter 6). Ultimately, all method parameters were defined as either non-critical or critical method parameters and for each critical method parameter mitigations were in place to control them. The CZE method was validated using a total error approach which gives a 90% probability that future results will also adhere to the requirements as defined in the analytical target profile.

Chapter 6 described the implementation of the validated CZE method from Chapter 4 for IPC samples from DSP. This chapter focuses on the last step of the AQbD process, method maintenance, in which the CZE method was implemented and evaluated during routine use. The adenovirus concentrations determined by CZE and qPCR were equivalent, as demonstrated by statistical analysis (CBLS regression) of results for 131 representative samples. The intermediate precision of the virus particle concentration was 6.9% RSD and the relative bias was 2.3%. The CZE method was intended to replace a qPCR method which requires three replicates in three analytical runs (during three days) to achieve an

intermediate precision of 8.1% RSD. The time from sampling until reporting results of the CZE method was less than 2 hours, indicating that the CZE method enables faster processing times in DSP. The CZE method has been implemented in 6 different labs, 8 instruments have been installed, and 20 operators have been trained. An extensive system suitability test and trending of critical data from the analytical method assured that after 525 analytical runs the precision and bias of the method still adhered to the original requirements from the analytical target profile and the number of invalid analyses was neglectable.