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Research Article

Implementation of at-line capillary zone electrophoresis for fast and reliable determination of adenovirus concentrations in vaccine manufacturing

A CZE method was validated and implemented for fast and accurate in-process determination of adenovirus concentrations of downstream process samples obtained during manufacturing of adenovirus vector-based vaccines. An analytical-quality-by-design approach was embraced for method development, method implementation, and method maintenance. CZE provided separation of adenovirus particles from sample matrix components, such as cell debris, residual DNA and proteins. The intermediate precision of the virus particle concentration was 6.9% RSD and the relative bias was 2.3%. In comparison, the CZE method is intended to replace a quantitative polymerase chain reaction method which requires three replicates in three analytical runs to achieve an intermediate precision of 8.1% RSD. Given that, in addition, the time from sampling till reporting results of the CZE method was less than 2 h, whereas quantitative polymerase chain reaction requires 3 days, it follows that the CZE method enables faster processing times in downstream processing.

Keywords:
Analytical quality by design / At-line IPC testing / Capillary zone electrophoresis lifecycle management / Virus quantification DOI 10.1002/elps.201900068

1 Introduction

Modified adenoviruses are being developed as gene-delivery vectors in vaccines against infectious diseases [1–3]. The viral DNA can be altered to render the virus non-replicating and to encode for the vaccine's antigen of interest, for example, against human immunodeficiency virus or Ebola [4, 5]. Upon vaccination, the antigen will be expressed in the human cells and presumably trigger an immune response which will subsequently confer protection against the infectious disease. The adenovirus vectors made by the AdVac® technology were modified such that they can not replicate in human cells but can be replicated to high titers using the modified PER.C6® human cell line [4, 5]. Downstream processing (DSP) for recovery and purification of adenovirus is described in ref. 6. In short, the DSP process comprises cell lysis, clarification and filtration, purification by anion exchange chromatography, and a buffer exchange step [7]. The purification steps aim to remove host cell DNA, non-encapsidated viral DNA, host cell proteins and viral proteins, and to achieve a high concentration of adenovirus [6, 7]. The virus particle concentration of an adenovirus vector-based vaccine product is an important quality attribute that should be monitored and controlled throughout the production process [7, 8]. The concentration of virus particles is often used to optimize and adapt DSP. In-process control (IPC) testing of the virus content is performed during manufacturing, in order to select DSP settings for subsequent most efficient purification [8, 9]. The virus particle concentration is an essential parameter to determine or adjust the virus load prior to anion exchange chromatography, to adjust the salt concentration prior to buffer exchange, and to dilute the final product to the target concentration [7].

The concentration of adenovirus particles in vaccine process intermediates can be determined using anion exchange (AEX) HPLC [10–12], quantitative polymerase chain reaction (qPCR) [13], or CZE [14, 15]. AEX-HPLC is relatively fast, but the method suffers from serious matrix effects when the test samples contain high salt concentrations or cell debris.

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Abbreviations: AEX, anion exchange; **AQbD**, analytical-quality-by-design; **ATP**, analytical target profile; **CBLS**, correlated bivariate least squares; **DSP**, downstream processing; **IPC**, In-process control; **qPCR**, quantitative polymerase chain reaction; **SSC**, system suitability control; **SST**, system suitability test; **vp/mL**, adenovirus particles per mL

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Color online: See the article online to view Figs. 1–6 in color.

Therefore, qPCR and CZE are the current methods of choice for the quantitative analysis of adenovirus in process intermediates [7, 13–15].

The analytical method for IPC testing of the virus content ideally should meet the following requirements: 1) accurate and precise, 2) compatible with the complex matrices of process intermediate samples, and 3) the time-to-result (i.e., the time between sampling and reporting of the analytical result) should be as short as possible. Imprecise or inaccurate measurement of the virus content could lead to, for example, overloading of the AEX column or an incorrect dilution of the final product [7]. As the production process is typically on-hold during an IPC test, long analysis times could lead to product degradation during this hold. Our research focused on an at-line testing approach which allows the adenovirus concentration of an IPC sample to be analyzed and reported within 4 h. For this, we implemented a CZE method providing fast determination of the virus particle concentration in DSP intermediates. The CZE method was aimed to replace a standard qPCR method, which takes at least one day to achieve a reliable result for one sample. In order to replace the qPCR method for IPC testing by CZE, equivalence between the CZE and qPCR methods was assessed by statistical methods.

An analytical-quality-by-design (AQbD) approach was embraced for the development, validation, and implementation of the CZE method. AQbD consists of six defined steps [16,17]: 1) definition of the analytical target profile (ATP) describing the objective of the test and the requirements, 2) technology selection, 3) definition of the critical method parameters by a criticality (risk) assessment, 4) method development by design of experiments (DOE), 5) method validation, and 6) method maintenance. Upon completion of the first five steps, a previously validated CZE method was proposed as an alternative to qPCR for the quantification of intact adenovirus particles in samples from upstream processing and DSP [14]. The ATP described an intermediate precision of $<$10% RSD of the adenovirus particle concentration for the IPC control, a bias of $<$10%, and a time-to-result of less than 4 h. This paper describes the last step of the AQbD process for the CZE method: the method maintenance. In this phase, the method is installed (i.e., transferred to the executing lab and implemented for routine use) and a control strategy is defined for the critical method parameters, to assure method performance according to requirements during routine use of the method.

2 Materials and methods

2.1 Chemicals and materials

Tricine (PN 93356) was obtained from Sigma Aldrich (Zwijndrecht, the Netherlands), tris(hydroxymethyl)aminomethane (PN 1083861000), polysorbate-20 (PN 655204), and benzonase (PN 1.01697) were from Merck Millipore (Amsterdam-Zuidoost, the Netherlands). Extended light path PVA capillaries (PN G1600-61239) of 50 μm id with a total length of 64.5 cm were from Agilent Technologies (Waldbronn, Germany). Formulation buffer, 1 M magnesium(II) chloride solution, and adenovirus crude harvest, lysed harvest, clarified harvest, anion-exchange product, diafiltration product, drug substance, drug product, and reference material (i.e., calibrant) were from Janssen Vaccines and Prevention BV (Leiden, the Netherlands). Forward primer (CMV, MGB) 5′-TGGGCGGTAGGCGTGTA-3′ (PN 4304972), reverse primer (CMV, MGB) 5′-CGATCTGACGGTTCACTAAACG-3′ (PN 4304972), and probe (CMV-2, MGB) 5′-VIC-TGGGAGGTCTATATAAGC-MGB-NFQ-3 (PN 4316032) were obtained from Applied Biosystems (Gent, Belgium).

2.2 Instrumentation and CZE method

All CZE experiments were performed on an Agilent 7100 Capillary Electrophoresis system comprising a UV-Visible DAD (Waldbronn, Germany). Data processing was performed with Chemstation software (B.04.03). Extended light path PVA-coated capillaries of 50 μm id (375 μm od) were cut to a total length of 33 cm. Capillary preconditioning was performed prior to each run with 10 mM phosphoric acid and BGE at 2.5 bar for 1 min each. The BGE consisted of 125 mM Tris, 338 mM tricine (pH 7.7), and 0.2% polysorbate-20. Sample injection was at 50 mbar for 5 s at the short end of the capillary (8.5 cm effective length). The separation voltage was −25 kV and the capillary was thermostated at 15°C. UV-absorbance detection was at 214 nm (4 nm bandwidth).

Prior to every CZE run a system suitability test (SST) is performed (see below, Section 3.3). The SST starts by filling the capillary with BGE and applying a voltage of −25 kV without performing an injection. This step is performed to check if the capillary is freely accessible for BGE and proper BGE should provide a current between 40 and 60 μA. Subsequently, two blanks (i.e., formulation buffer) are analyzed to check for system contamination. Next, an adenovirus system suitability control sample with a concentration of 1.0×10^{11} adenovirus particles per mL (vp/mL) is injected six times to check for peak area repeatability, migration time repeatability, and concentration. The adenovirus concentration of the control sample should be within 0.85 and 1.15×10^{11} vp/mL, which is based on the total error ($\leq$15% for 1.0×10^{11} vp/mL) of the CZE method. All these requirements need to be met to pass the SST. Adenovirus samples were diluted with formulation buffer, if needed, to fall within the validated range of 0.5×10^{11}–7.3×10^{11} vp/mL. The adenovirus concentration of the samples was determined by one-point calibration at 7.3×10^{11} vp/mL with an adenovirus reference material (i.e., calibrant).

Table 1. Overview of the between-run, within-run (method repeatability) and total variance (intermediate precision), the intermediate precision per testing format, and the bias of CZE and qPCR analysis of a clarified harvest sample (IPC)

A) Variances per test method in $\log_{10}$ vp/mL

	Technique	
Variances	CZE	qPCR
Between-run	1.5E-04	3.1E-03
Within-run (method repeatability)	7.4E-04	1.6E-03
Total (intermediate precision)	8.8E-04	4.7E-03

B) Intermediate precision per testing format in %RSD

	Technique	
Testing format	CZE	qPCR
One measurement in one analytical run ($n = 1$)	6.9	15.9
Three measurements in one analytical run ($n = 3$)	4.6	14.0
One measurement in three analytical runs ($n = 3$)	4.0	9.2
Three measurements in three analytical runs ($n = 9$)	2.6	8.1

C) Bias per test method

	Technique	
Bias	CZE	qPCR
Bias ($\log_{10}$ vp/mL)	1.0E-02	6.0E-02
Relative bias (%)	2.3	13.9

A) Between-run variance, within-run variance, and total variance in $\log_{10}$ vp/mL of the CZE and qPCR methods for the determination of the adenovirus particle concentration. **B**) Intermediate precision in %RSD for different testing formats using CZE and qPCR. **C**) Bias ($\log_{10}$ vp/mL) and relative bias (%) of the CZE and qPCR method for adenovirus particle concentration.

2.3 Method validation and determination of the testing format

The method repeatability (variation within a run), intermediate precision (sum of the variation within a run and the variation between runs), and the bias were determined for CZE and qPCR at five concentration levels in the range of 0.25 $\times$ 10^{11}–2.0 $\times$ 10^{11} vp/mL, with three measurements at each concentration level, repeated on three different days by two different operators (Table 1). Known amounts of adenovirus calibrant were spiked into the clarified harvest sample matrix at levels in the range of 0.25 $\times$ 10^{11}–2.0 $\times$ 10^{11} vp/mL. The intermediate precision acceptance criterion was ≤10% RSD for the adenovirus concentration. The bias acceptance criterion was <10% relative bias (difference between the measured concentration and the expected reference concentration).

The intermediate precision depends on the number of measurements and on the number of analytical runs, according to Eq. (1).

$$SD_{IP} = \sqrt{\frac{Var\_B}{p} + \frac{Var\_W}{n*p}} \quad (1)$$

where *Var_B* represents the variance between runs (variance of the day and operator combined), *Var_W* represents the variance within runs, p is the number of runs, and n is the number of measurements.

2.4 Equivalence study

Table 2 gives an overview of the process intermediates and final product tested in the equivalence study. Three measurements per sample in one analytical run (3 × 1 testing format) were performed by qPCR and one measurement per sample in one analytical run (1 × 1 testing format) was performed by CZE. The qPCR analyses were performed according to Ma et al. [13], with specific primers and probe as described above. The CZE analyses were performed according to van Tricht et al. [14]. The crude harvest and lysed harvest samples were treated with benzonase (0.2 units benzonase per mL in 31 mM $MgCl_2$ and incubated for 1 h at room temperature).

Correlated bivariate least squares (CBLS) regression [18] was used to demonstrate the equivalence of the CZE and qPCR method. CBLS is a regression model that takes as dependent variable the differences between two measurements of the same sample performed with both methods, and as predictor the mean of the measurements. Then the 95% prediction interval of the differences in virus particle concentration between qPCR and CZE are calculated for the entire concentration range. The prediction interval is the interval in which 95% of the future observations will fall. The CZE and qPCR methods were considered equivalent or interchangeable in practice if the prediction interval calculated for the differences in virus particle concentration fell within the equivalence limits of [−0.2 to 0.2] $\log_{10}$ vp/mL for the entire concentration range (i.e., the difference in concentration between CZE and qPCR for future measurements is maximally 0.2 $\log_{10}$ vp/mL). The BivRegBLS package in the R v.3.5.1 statistical software was used for the analyses.

3 Results and discussion

A typical result of CZE analysis of a clarified harvest IPC sample is shown in 1. The adenovirus at 2.2 min is baseline separated from sample matrix components, such as cell debris, residual DNA, and proteins. The paragraphs below describe the implementation of the CZE method, the determination of the testing format, the equivalence between CZE and qPCR, the reduction by of the time-to-result, and the method performance of the CZE method in routine use (over 500 runs).

3.1 Establishing the testing format based on method validation data

Currently, qPCR is the standard method for the determination of the concentration of virus particles in IPC samples

Table 2. Overview of process intermediates and the final product analyzed by qPCR and CZE, including number of sample lots tested, the mean virus particle concentration, and the SD per process intermediate and final product and per method

Process intermediates and final product	# lots	CZE		qPCR	
		Mean (log10 vp/mL)	SD (log10 vp/mL)	Mean (log10 vp/mL)	SD (log10 vp/mL)
1 - Crude harvest	13	11.14	0.12	11.16	0.10
2 - Lysed harvest	38	11.91	0.16	11.84	0.14
3 - Clarified harvest	8	11.66	0.16	11.68	0.16
4 - Anion exchange product	13	12.04	0.12	12.00	0.16
5 - Diafiltration product	9	11.77	0.13	11.79	0.15
6 - Drug substance	35	11.36	0.23	11.39	0.21
7 - Drug product	15	10.95	0.05	10.95	0.06

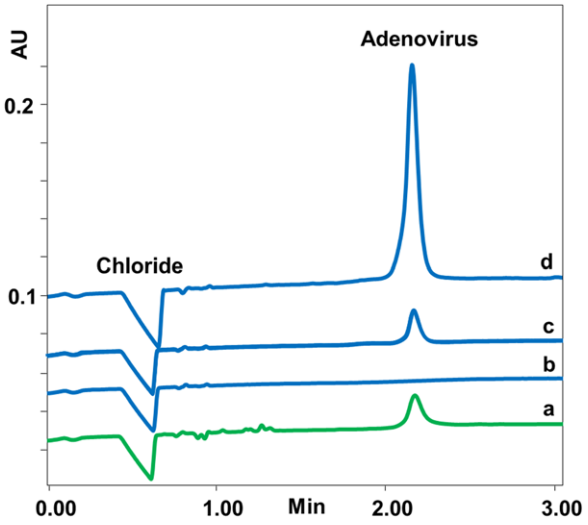

Figure 1. Electropherograms of a clarified harvest IPC sample (1.3×10^{11} vp/mL) (a), a blank formulation buffer (b), an adenovirus system suitability control sample (1×10^{11} vp/mL), six repeated analyses (overlaid) (c), and an adenovirus calibrant/assay control (7.3×10^{11} vp/mL) (d). Conditions: BGE: 125 mM Tris and 338 mM tricine (pH 7.7) with 0.2% polysorbate-20; PVA-coated capillary with effective length of 8.5 cm; applied voltage, −25 kV. Other conditions: see Materials and Methods.

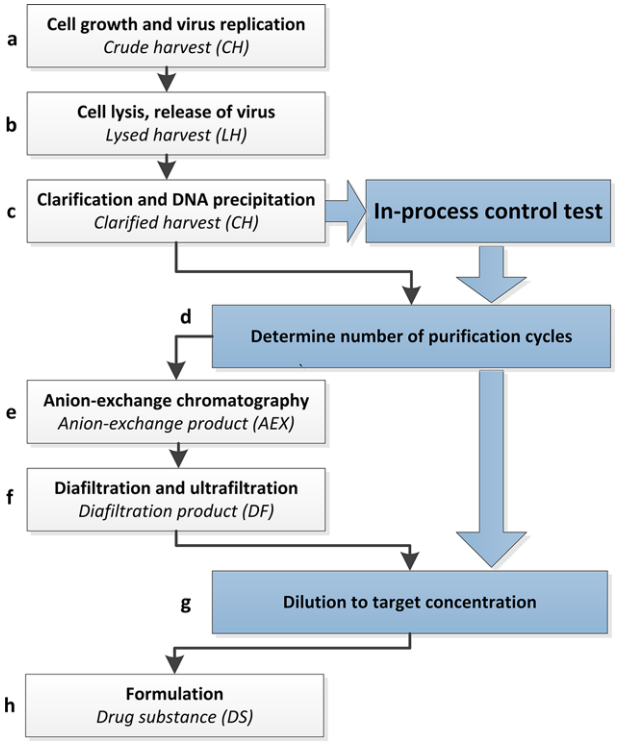

Figure 2. Overview of the DSP from crude harvest (a) to drug substance (h). The IPC result (c) is used to determine the number of purification cycles for the AEX chromatography (d and e) and the dilution of the diafiltration product (f and g) with formulation buffer to achieve the target concentration of the final drug substance (h).

from the clarified harvest. Fig. 2 gives an overview of the DSP. The adenovirus concentration determined in the IPC sample (2) serves two purposes. Firstly, the concentration is needed to determine the number of purification cycles for AEX chromatography (Fig. 2d and e). If the virus particle concentration is underestimated, for example, due to method bias or lack of precision of the reported value, then the AEX column might be overloaded and/or clogged. Secondly, the determined virus concentration is used to dilute the diafiltration product (Fig. 2f and g) with formulation buffer to the target concentration of the drug substance (Fig. 2h). If the total error (sum of the bias and the intermediate precision) of the IPC measurement is insufficient, then the drug substance might be too diluted or too concentrated and fails the concentration acceptance criterion.

Based on the requirements in the ATP, the method to determine the virus particle concentration in the IPC sample should have an intermediate precision of maximally 10% RSD and a bias of <10% in order to enable a reliable determination of the number of AEX purification cycles needed and to accurately dilute the final diafiltration product. Method repeatability (i.e., within-run variation), between-run variation, intermediate precision (sum of the within-run and between-run variation), and bias of the concentration of virus particles were assessed in method validation (Table 1A) for both CZE

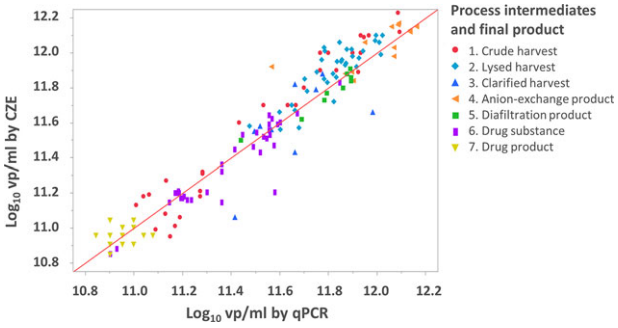

Figure 3. Comparison of the qPCR results (log_{10} vp/mL) vs. CZE results (log_{10} vp/mL) of six process intermediates and the final product. The red line represents the unit line.

and qPCR. In addition, CZE results were compared to those of qPCR obtained for the same IPC sample (clarified harvest). The results obtained by qPCR are log-normally distributed and therefore the precisions were calculated in log_{10} vp/mL for both methods, to allow direct comparison (Table 1A and C).

The intermediate precisions of the CZE and qPCR method were 6.9% RSD and 15.9% RSD, respectively (Table 1B). Based on the between run and within run variances determined in the method validations, it can be calculated (Eq. (1)) that three measurements need to be performed by qPCR in three analytical runs to obtain a result with a precision of 8.1% RSD. With CZE, a single measurement is sufficient to have a precision of 6.9% RSD (Table 1B). It takes up to 3 days to perform the three qPCR analyses needed, which means that the production process is on-hold for 3 days awaiting the qPCR results. With CZE, the holding time is much shorter since only a single measurement needs to be performed. The relative bias was 2.3 and 13.9% for CZE and qPCR, respectively. Hence, the CZE method meets the ATP requirement for intermediate precision of <10% RSD, when employing a testing format of one measurement in one analytical run. CZE also meets the ATP requirement for bias (<10%). The total error (sum of the bias and the intermediate precision) of the CZE method for a single measurement was estimated to be <15% based on the intermediate precision and the relative bias according to [19].

3.2 Equivalence of CZE and VP-qPCR

Figure 3 and Table 2 show the results for 131 samples of six different process intermediates and the final product analyzed by CZE and qPCR. CBLS regression [18] was used to demonstrate equivalence between the concentration of virus particles determined by CZE vs. qPCR. The acceptance limits for the difference between the CZE and qPCR results were set at [−0.2 to 0.2] log_{10} vp/mL, as these are considered to demonstrate equivalence between different qPCR results, for example, when a new calibrant is introduced. The 95% prediction interval of the difference between CZE and qPCR results was [−0.18 to 0.16] in the entire measured range and thus within the acceptance limits ([−0.2 to 0.2] log_{10} vp/mL) for all types of process intermediates and across the range of virus particle concentrations. In other words, 95% of the future results of CZE or qPCR will have a difference of maximally ±0.2 log_{10} vp/mL independent of the sample type or concentration. Therefore, it can be concluded that the CZE and qPCR methods are interchangeable in practice for the quantification of the adenovirus concentration.

3.3 System suitability testing

The CZE method adhered to all requirements from the ATP (time to result <4 h, intermediate precision <10%RSD, and bias <10%) and was implemented to routinely determine the virus particle concentration in DSP intermediates. An important purpose of AQbD is to be well in control of the critical method parameters of an analytical method during routine use. The criticality assessment was revised after method validation and the equivalence study to determine if any critical method parameter of the CZE method was not yet adequately controlled. The capillary coating was identified as the critical method parameter with the highest risk to impact the accuracy of the virus particle concentration. The capillary coating degrades due its exposure to the sample matrix components, such as DNA, protein and cell debris, resulting in adenovirus adsorption to the capillary wall. The rate of degradation of the capillary coating is variable and can therefore not be predicted. To control the capillary coating, the system suitability test was developed with the aim to verify the method performance prior to each analytical run (Fig. 4) guarantying a precision of <5% RSD on the adenovirus peak area and a total error (sum of the bias and intermediate precision) of <15% on the virus particle concentration, see Fig. 4. In addition to the SST, the calibrant is measured as a control sample after every five test samples and at the end of each sample sequence (1 and Fig. 4). Decrease of the adenovirus concentration of the control sample during the sequence indicates degradation of the capillary coating and/or adenovirus adsorption. A decrease or increase of the adenovirus concentration of 10% is accepted. Only the test sample results before the last valid analysis of the control sample are processed and reported.

Routine use of the CZE method ($n = 525$ analytical runs) showed that the SST failure rate is about 20% ($n = 105$). In one out of five runs troubleshooting and a retest of the SST are needed before samples can be analyzed. Although 20% of the SST runs fail, still in 99.4% of the cases the sample data was generated on the same day. With the strict SST requirements, we prevented that samples were analyzed unnecessarily on a CE system that was not working properly (i.e., not adhering to the ATP requirements). The causes of SST failure were monitored and in 50% ($n = 53$) of the cases failure was due to instrument malfunction. Other causes were; 27% ($n = 28$) material defects (e.g., capillary coating), 15% ($n = 16$) software issues, 4% ($n = 4$) unclarities in the test procedure, and 4% ($n = 4$) operator errors. Continuous improvement projects

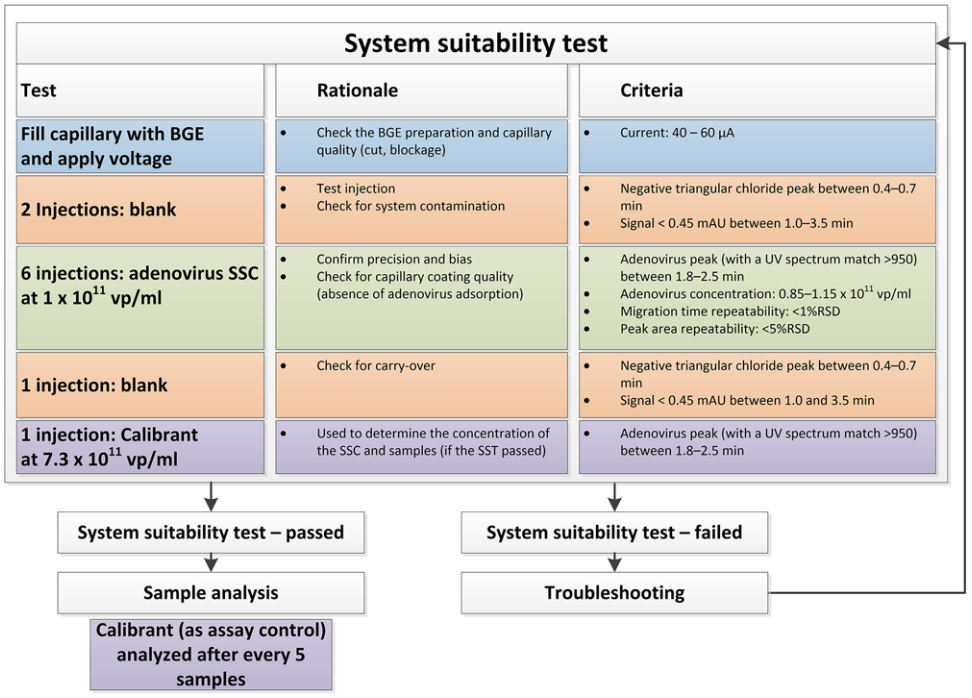

Figure 4. System suitability test to assure proper performance of the CZE method during an analytical run.

are ongoing to prevent or solve the causes and to lower the invalid rate of the SST, e.g., by retraining of operators.

3.4 Reducing the time-to-result

The analytical target profile (ATP) describes a time-to-result of less than 4 h. This is the total time it should take from sampling until reporting the processed and reviewed analytical result. Prior to test sample analysis, an SST needs to be performed, comprising ten control-sample injections with a total analysis time of 70 min, including capillary cleaning and conditioning, as described above (Fig. 4). Ideally in practice, the result of the SST is already available before the test sample is received from production, to shorten the time-to-result by about 70 min and to allow for troubleshooting in case the SST fails, without causing delays in production. An experiment was set up to demonstrate that the SST can performed at any time during the day by testing the SST, as shown in Fig. 4, at t = 0, t = 8, t = 24 and t = 48 h. In addition, a control sample, typically analyzed together with the samples upon receipt, was also analyzed at these time points and the virus concentration obtained at each time point was compared to t = 0 h. All SSTs passed the criteria independent of the time point. The control samples, analyzed together with the test samples, showed a maximum difference of 10% in virus concentration between 24 h and the initial SST results at t = 0 h. Therefore, it was concluded that the SST can be performed at the start of the day and the test samples can be analyzed within 24 h together with the control sample (criterion: maximum difference of 10% in the adenovirus concentration compared to the SST), see Fig. 5.

With this approach, the time-to-result for the analysis of an IPC sample is 90 min in total: 15 min to receive the sample, 10 min to analyze the IPC sample (Fig. 1a) and a control sample (Fig. 1d), 15 min for data processing, 15 min for result reporting, and 30 min for review (Fig. 5). The SST is typically run in the morning and the IPC sample from production can be received at any time during the day. In this way, the process hold time is maximally 2 h (Fig. 5).

3.5 Method performance during routing use—control charts

In addition to system suitability testing, the method performance during routine use was controlled by monitoring for each result–the migration time and peak area RSD% ($n = 6$) of the adenovirus peak, and the concentration of the system suitability control sample with defined control limits. For diagnostic purposes, the following parameters were also recorded in time for each individual CE system: capillary lot number, number of injections per capillary, the CE current during each analysis, the adenovirus peak width, and the regression slope of the calibrant. Control charts were updated after each method run to verify that the method performance was consistently as expected, meeting the criteria of the system suitability test and the requirements of the original ATP, see Fig. 6.

So far, 18,108 samples have been analyzed in 525 analytical runs. Three out of the 525 runs were invalid (invalid rate of 0.6%) meaning that SST passed the criteria, but the control samples during the run did not. The test sample results in those specific runs could not be generated or reported on the

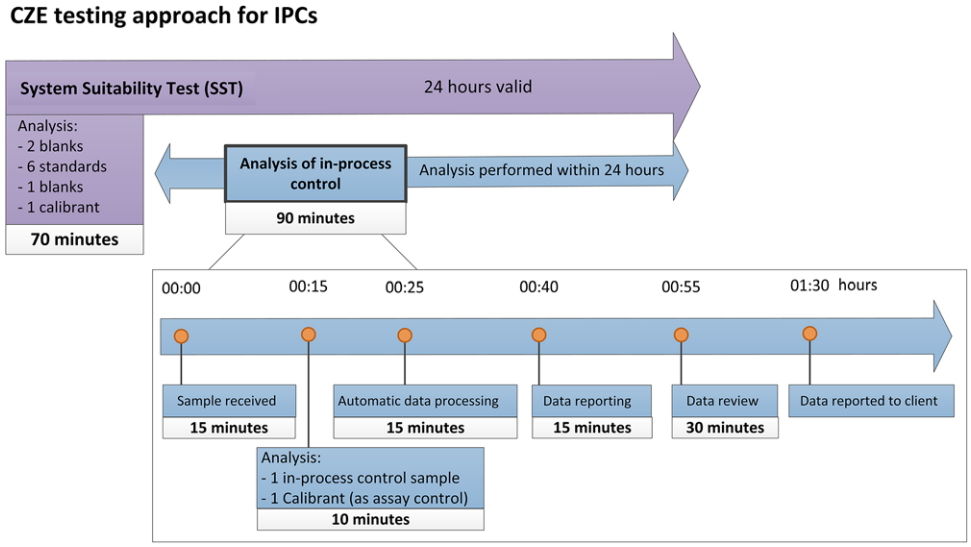

Figure 5. CZE approach for in-process control testing. The SST (70 min, ten injection) is run prior to receipt of the process samples. After that, the IPC sample can be analyzed within 24 h. Upon IPC sample receipt, the test sample and a control sample are analyzed. The data are processed and reported only if both the test sample and control sample criteria are met.

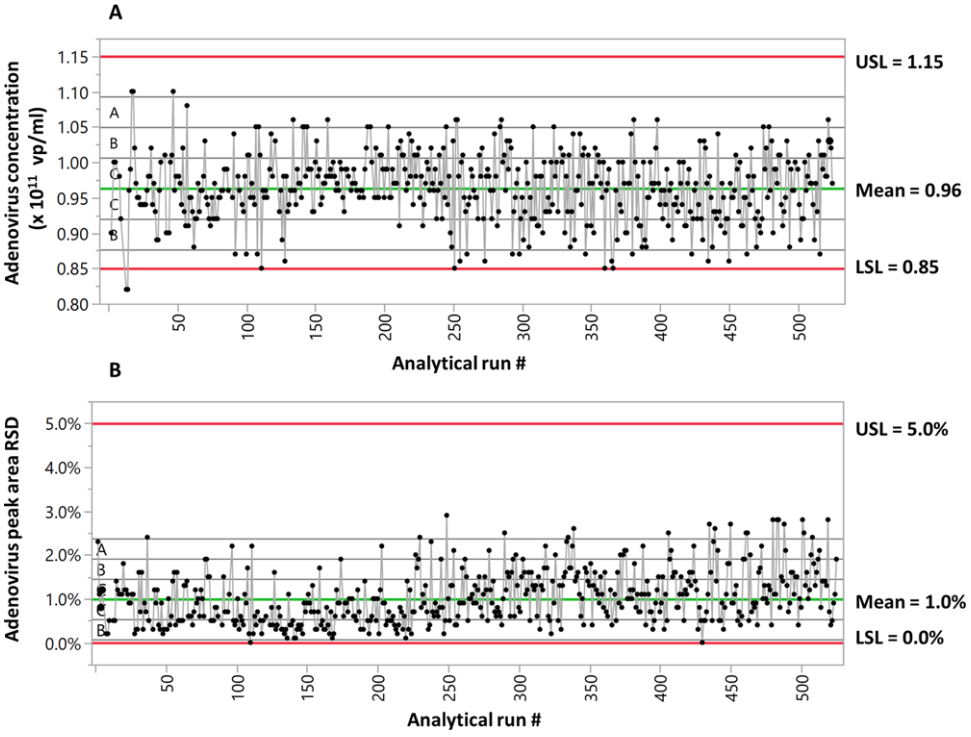

Figure 6. Control charts of (A) the adenovirus concentration (vp/mL) and (B) the adenovirus peak area RSD. USL = upper specification limit, LSL = lower specification limit. The letters A, B, and C correspond with the SD zones. A = 3 * SD, B = 2 * SD and C = 1 * SD.

same day. The control plot, in Fig. 6A, shows that the mean adenovirus concentration is 0.96 × 10^{11} vp/mL (a relative bias of 4%, n = 525) and 99.6% of the data points fall within the acceptance limits of 0.85–1.15 × 10^{11} vp/mL. No trends were observed and the total error of the CZE method was still <15% after 525 runs. Figure 6B shows the control plot of the repeatability (SD) of the adenovirus peak area with control limits of 0–5% RSD. The average RSD is 1.0% over 525 runs and 100% of the analysis had a RSD% result lower than the upper acceptance limit (5%) assuring the repeatability requirements as defined in the ATP.

4 Concluding remarks

A CZE method was successfully implemented for quantification of the adenovirus concentration in an IPC sample from the DSP. Analytical-quality-by-design was used to validate and implement the test method and was used to define a control strategy for the critical method parameters. The CZE method has been implemented in six different labs, eight 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, over 2 years, the precision and bias of the method still adhered to the original requirements from the analytical target profile.

The intermediate precision of the CZE method was 7% RSD (testing format: one measurement in one analytical run) and the relative bias on the virus particle concentration of the IPC was 2.3%. The current qPCR method requires a testing format of three measurements in three analytical runs to achieve an intermediate precision of 8% RSD. This means that the production process can be on hold for up to three days awaiting the qPCR result. As described in this paper, the adenovirus concentrations determined by CZE and qPCR are equivalent, as demonstrated by statistical analysis (CBLS regression) of results for 131 representative samples. Since statistical analysis showed that CZE and qPCR are interchangeable in practice for quantification of the adenovirus concentration, and given that the IPC concentration could be determined much faster by CZE – with a time-to-result of less than 2 h, from sampling the clarified harvest (the IPC) to reporting the adenovirus concentration – it is concluded that the CZE method allows for accurate, precise, and fast determination of virus particle concentration, thereby enabling a faster determination of the number of purification cycles of the AEX chromatography in the DSP and the accurate dilution of diafiltration product to the final drug substance.

The authors have declared no conflict of interest.

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