Transgene's proposal to participate in Assignment of Particle concentration.
RFP 8.0

Standard operating procedure for the determination of adenovirus particle concentration by AE-HPLC.

1. OBJECTIVE

Determination of adenovirus particle concentration by anion exchange HPLC.

2. PRINCIPLE

Adenovirus samples are injected on a anion exchange column and eluted with a linear saline gradient. The resulting adenovirus peak is quantified at 260 nm by comparison to a standard curve.

3. MATERIALS AND METHODS

3.1 Materials

1-ml Resource Q column (Pharmacia Biotech)
Adjustable pipettes: 10-100µL, 20-200µL, 200-1000µL
Sterile pipette tips, with aerosol filters for 10-100µL,
20-200µL and 200-1000µL
3-ml flasks with caps for 717 autosampler (Waters or equivalent)

3.2 Equipment

A 600S chromatography system equipped with a 717 plus autosampler and a 996 photodiode array detector (Waters or equivalent) piloted by Millenium 32 software.
Biosafety cabinet (laminar flow hood) suitable for BL-2 containment

3.3 Reagents

Buffer A : 50 mM Hepes, 300 mM NaCl, pH 7.5, filtered with 0.2 µm filter
Buffer B : 50 mM Hepes, 1.5 M NaCl, pH 7.5, filtered with 0.2 µm filter
Buffer C : 50 mM Hepes, Tween 80 54mg/l, pH 7.5, filtered with 0.2 µm filter
2 doses of SDTQC01 standard .
2 doses of ADTG 13383W1RIQC01 reference

3.4 Method

3.4.1 Sample preparation

3.4.1.1 Standard preparation

Open the ampoules and homogenize the suspension by gentle pipetting. Transfer the two doses content in single 3-ml plastic vial for 727 autosampler. Cap the vial.

3.4.1.2 Reference preparation

Open the ampoule and homogenize the suspension by gentle pipetting. Transfer the viral suspension in a 3-ml plastic vial for 727 autosampler. Cap the vial.

3.4.1.3 Test sample preparation

If necessary, based upon the estimated concentration, properly dilute the samples in buffer C in order to obtain peak areas in the calibration

curve range. Perform the dilutions in triplicate. Transfer the sample in a 3-ml plastic vial for 727 autosampler. Cap the vial.
Record the dilutions on the appropriate form.

3.4.2 AE-HPLC run

3.4.2.1 Set-up

Equilibrate the column in buffer A at the flow rate of 1 ml/min for 10 min.
Inject the samples according to the following sequence :
1: Blank (1ml of buffer C)
2. Standard (70 µL, 0.44 x 10^{10} particles)
3. Standard (160 µL, 1.00 x 10^{10} particles)
4. Standard (470 µL, 2.94 x 10^{10} particles)
5. Standard (790 µL, 4.95 x 10^{10} particles)
6. Standard (1300 µL, 8.14 x 10^{10} particles)
7: Reference of known particles concentration
8 and following positions: samples to be analyzed in triplicates
Position after the last test sample: Second injection of Reference.
Record and print the sample set table

3.4.2.2 Run

For each injection apply the following conditions (flow rate 1 ml/ min.).:
1. Equilibration: 100 % buffer A for two minutes
2. Linear gradient ranging from 0 to 25 % buffer B in 10 minutes
3. 2 minutes hold in 25 % buffer B
4. 2.6 minutes wash with 100% buffer B
5. 9 minutes- equilibration in buffer A

3.4.2.3 Monitoring and data acquisition

Monitor the elution with the PDA scanning from 210 to 300 nm.

4. RESULTS

4.1.1 Data processing

1. Extract the chromatograms at 260 nm from PDA data and integrate the peaks.
2. Plot the peak areas of the standard in function of the number of injected particles to generate the standard curve.
3. Calculate the concentration of the test sample from this curve.

4.1.2 Report

Record and print all the results in the pre-established format

5. VALIDITY

The assay is valid if the following criteria are met :

1. The slope of standard curve is within the pre-established limits
2. The correlation coefficient r^2 is > 0.99
3. The reference concentration is within the pre-established limits for both measurements
4. The peak of the test sample is symmetric with the expected retention time +/- 0.18 min.
5. The peak area for test samples are within the calibration curve range

6. REFERENCES

7. Shabram *et al.*, (1997), Hum. Gene Ther., **8**, 453-465.