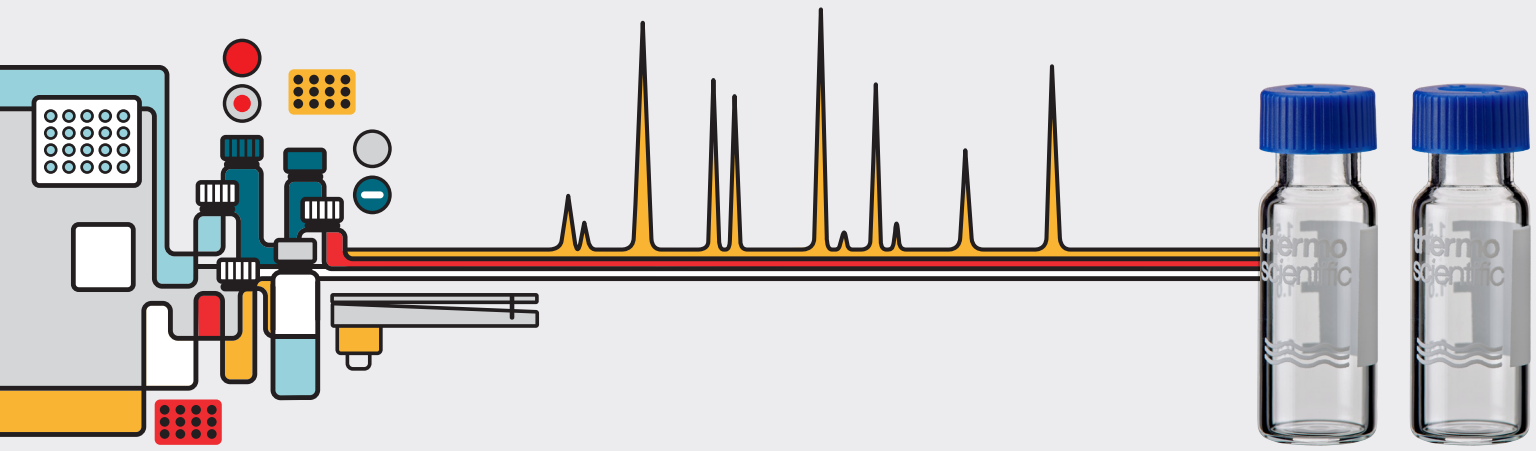

Vials and caps

Choosing the right sample vial for analysis of AAV to prevent sample loss

Authors

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Keywords

Viral capsid, adeno-associated virus, gene therapy, size exclusion chromatography, sample vials, low binding vials

Key benefits

Analysis of viral capsids remains very challenging for numerous reasons. Especially limited sample availability makes it difficult to maintain high sensitivity and repeatability. In this regard, using the correct sample vials for capsid analysis via liquid chromatography can constitute an important step allowing for highly reproducible results.

Goal

To demonstrate the benefit of using low-binding glass vials compared to standard polypropylene vials for viral capsid analysis.

Introduction

Gene therapy is the most recent and promising area of biopharmaceutical research; it refers to either the supply or suppression of a specific gene that is lacking in the patient or causing the disease, respectively. In both cases, the treatment works through the delivery of therapeutic DNA to target cells. The delivery strategy is based on both viral and non-viral vectors, with the most common being adeno-associated virus (AAV). Hence, analytical technologies that are able to monitor the production and correct assembly of viral capsids, as well as encapsulation of the gene used to treat the disease, have become of pivotal importance for the further development of this promising therapeutic area.

Characterization of AAVs includes a number of critical parameters that need to be assessed during production, such as viral protein ratio to assure transduction efficiency and correct genome delivery and release,[1-3] or vector yield to ensure enough of the therapeutic gene is administered. Among others, one important step post upstream production of AAVs is to accurately determine the titer of the viral capsids that will be used in the following production processes.

In this study, we performed SEC-FLR analysis to determine viral capsid titer following upstream purification. Purified and concentrated reference AAV5 material was sourced commercially. To mimic conditions after affinity purification, samples were diluted to gain a titer of 1e11 capsids/mL. Diluted samples were then transferred into plastic vials for further analysis. Following SEC-FLR analysis, we observed a rapid decrease in sample recovery, affecting evaluation of the sample. A comparison of the performance for SEC-FLR analysis using the same sample stored in plastic versus glass low-binding vials was performed and allowed an assessment of the level of sample loss caused by the use of improper vials.

Experimental

Sample preparation

Samples were diluted in 50 mM Na_3PO_4, 300 mM NaCl to reach a concentration of 1e11 capsids/mL. Following dilution, samples were transferred into either glass or plastic vials.

Glass vials

- Thermo Scientific™ SureStop™ convenience kit, GOLD grade glass vial (screw thread) (P/N **6PKG592W**)
- Thermo Scientific™ SureSTART™ polyspring glass insert (P/N **6PME03C1SP**)

Cap

- Thermo Scientific™ SureStop™ (P/N **6PSC9ST1**)

Plastic vials

- Thermo Scientific™ SureStop™ plastic screw thread vial (P/N **6ESV9-04PP**)

Equipment

Thermo Scientific™ Vanquish™ Flex UHPLC system, constituted by the following modules:

- Quaternary Pump F (P/N **VF-P20-A**)
- Split Sampler HT (P/N **VH-A10-A**)
- Column Compartment H (P/N **VH-C10-A**)
- Fluorescence Detector F (P/N **VF-D50-A**)

Chromatography conditions

Parameter	Setting
Column	Thermo Scientific™ MabPac™ SEC-1 2.1 × 150 mm (P/N **088790**)
Solvents	50 mM sodium phosphate monobasic monohydrate 300 mM NaCl
Gradient	Isocratic
Flow rate	0.075 mL/min
Column temperature	25 °C
Detector	$\lambda_{ex/em}$= 340/280 nm

Software

- Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) 7.2.10

Results and discussion

Characterization of AAVs produced for gene therapy involves a number of analytical assays to determine their features and titer. The upstream process for production of AAVs is challenging, and for this reason, good recovery and extremely sensitive methods are usually required. In this study, we demonstrate the importance of appropriate selection of HPLC vials to obtain consistent and accurate results.

AAVs were transferred in either SureSTART HPLC plastic vials or low-binding glass vials (Figure 1) and titer was assessed through SEC-FLR analysis as indicated in the experimental section.

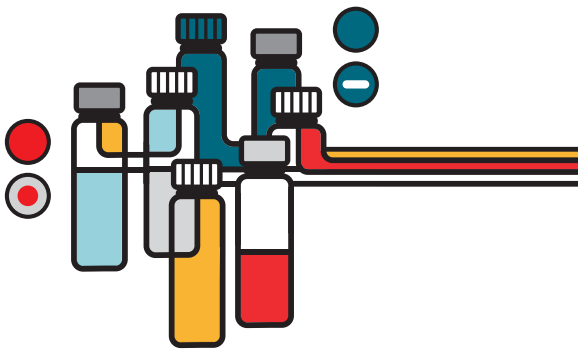

Figure 1. GOLD grade glass vial (screw thread)

For the first measurement in triplicate, a %RSD value of 13.5 was observed for the sample stored in plastic vials, while in glass vials the value was as low as 0.7 and peak area was ~10% higher. For this reason, we started to investigate reproducibility of the measurement at different time points for 12 h to investigate potential loss of sample due to adsorption of AAV capsids onto the material of the vials. The analysis was performed in triplicate at time 0 and after 2, 6, and 12 hours, while the sample was left in the vial in the autosampler at 5 °C.

From Figure 2, it is possible to observe a clear trend for the sample stored in plastic vials, suggesting adsorption of the sample onto the vial material. After 12 hours the sample showed a concentration 90% lower than at time zero. At the same time, the overlay prepared in the same test of experiments for the sample stored in glass vials was almost unchanged, with a slight decrease only in the period between 6 and 12 hours showing a maximum loss of 15% of the starting material.

The material of the vials was influencing the accuracy of the analysis as well (Figure 3). In plastic vials, %RSD values range between 5.0 and 26.6, while the same test performed in glass vials showed %RSD values generally lower than 2, with only the last time point showing %RSD = 4.2.

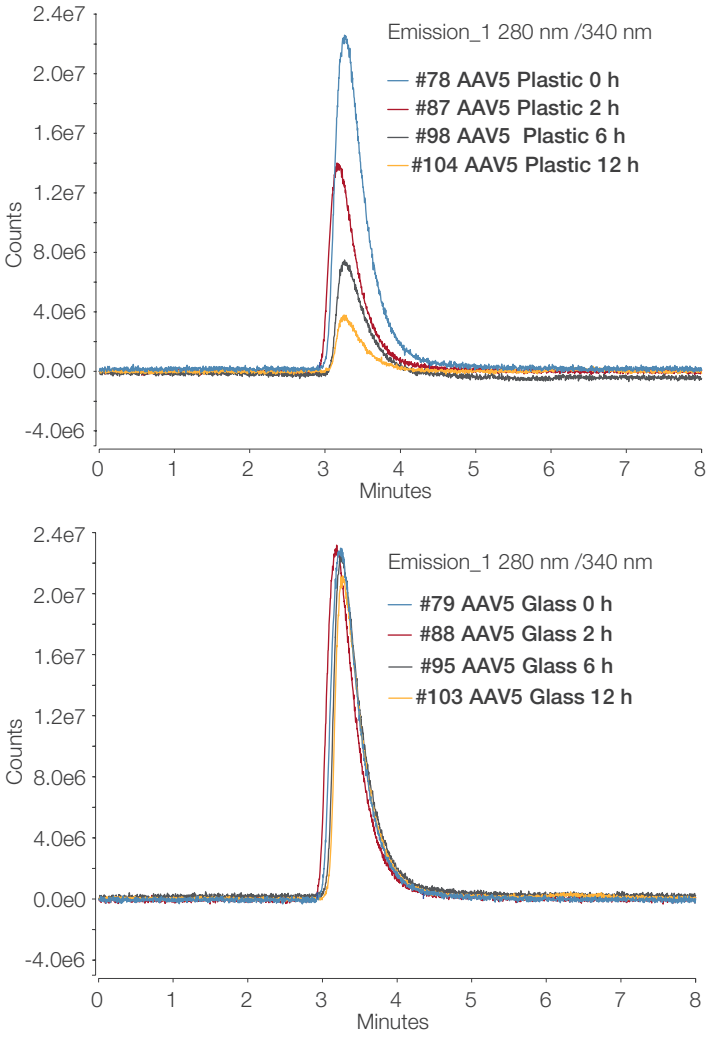

Figure 2. SEC-FLR analysis of AAV5. Top panel shows the overlay of analysis performed along 12 h for an AAV5 sample stored in plastic vials, while the bottom panel shows the same analysis performed on sample stored in glass vials.

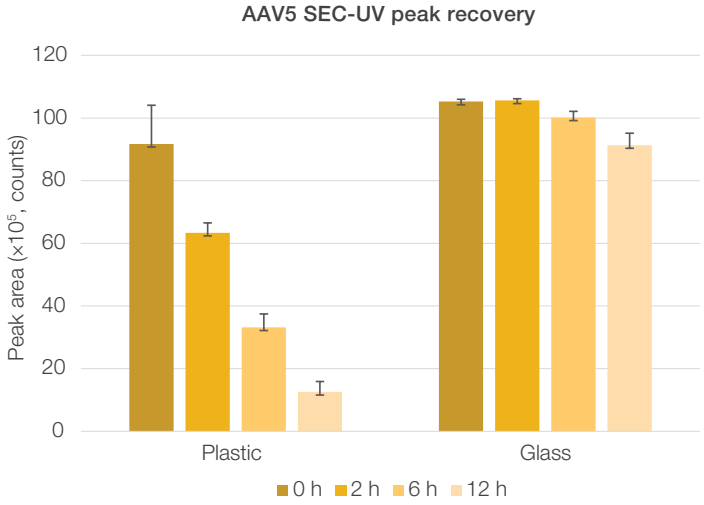

Figure 3. Bar graph plot of the peak area obtained after SEC-FLR analysis of AAV sample stored in plastic or glass vials at different time points

Conclusion

In this study, we demonstrated the advantage of using low-binding glass vials for AAV analysis. Analogous results were generated using different AAV serotypes, all reporting similar results when the two vial-typologies were compared during 12 hours of sample storage. The material of the HPLC vial affected not only the correct evaluation of the concentration of viral capsids, but also the accuracy of the result and mainly the reproducibility of the results over a short period of time, with SEC-FLR peak intensity being reduced by ~90% in plastic vials. This study highlights the need for proper evaluation of consumables when working and developing methods for this new class of biopharmaceuticals.

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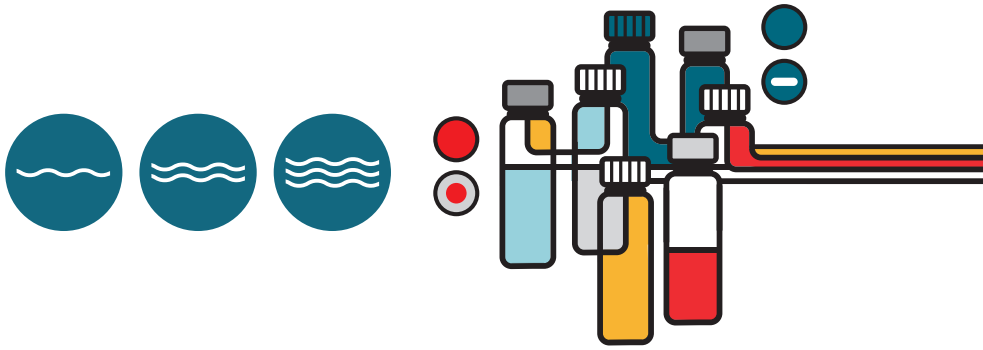

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