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Optimization of Adeno-Associated Virus Peptide Mapping Using Trapped Ion Mobility Spectrometry

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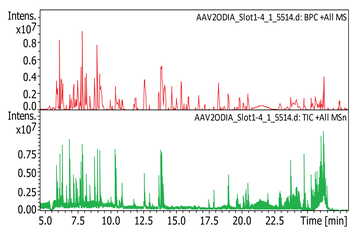
Introduction

Recombinant adeno-associated viruses (AAV) have emerged as one of the most popular platforms for gene therapy strategies and vaccine development. Structural characterization of biotherapeutic proteins via peptide mapping is a common technique in the biopharmaceutical industry to confirm the product identity, purity and stability. Post translation modification (PTMs) in capsid proteins are known to regulate several aspects of virus' potency and tropism in the host. Several recent studies[1] attempted to optimize peptide mapping of biotherapeutics to increase reproducibility and preserve the integrity of PTMs through decreasing the number of sample preparation steps. Nano-flow liquid chromatography and mass spectrometry are key analytical tools that can overcome these challenges and serve as a fast and robust analytical technique.

A.

AAV serotype	AAV 2 DDA	AAV 6 DDA	AAV 2 DIA	AAV 6 DIA
VP1	97%	96%	97%	95%
VP2	89%	94%	96%	98%
VP3	94%	92%	96%	97%

B.



C.

Fig. 1. A. Peptide coverage of the serotypes AAV 6 and AAV 8. An average of 94% peptide sequence coverage is seen for the 3 viral proteins. B. BPC MS and TIC MSn traces show AAV 2 peptides well resolved on the C18 column. C. Sequence coverage of AAV capsid proteins. Excellent sequence coverage was obtained for the unique N-terminal peptide sequence of the VP1 (697-734 aa), VP2 (565-598 aa), and the VP3 common region (499-533 aa).

Methods

AAV 2 was used as the model serotype for peptide mapping method optimization, the same optimized method was used for AAV 6. For peptide mapping, the sample was denatured by RapiGest™ (Waters), reduced with 2.5 mM dithiothreitol, and alkylated using 5 mM Iodoacetamide, followed by digestion (2 hr, 37ºC) using trypsin and chymotrypsin (Promega). 100 ng of digest was injected on a 25 cm C18 column (50 µm id, 1.5 µm particle, Gen 3 IonOptics column) using a nanoElute2 nanoHPLC (Bruker) coupled to a timsTOF HT (Bruker) via a CaptiveSpray 2 (Bruker) ionization source. Peptides were eluted with a 30-minute reverse phase gradient. Several DDA and dia-PASEF settings were evaluated. For the dia-PASEF acquisition, a window placement scheme as shown in Fig. 4A was used. Data are processed with Spectronaut and SpectroMine (Biognosys). For directDIA™ analysis of dia-PASEF runs, we used AAV 2 and AAV 6 Uniprot FASTA files.

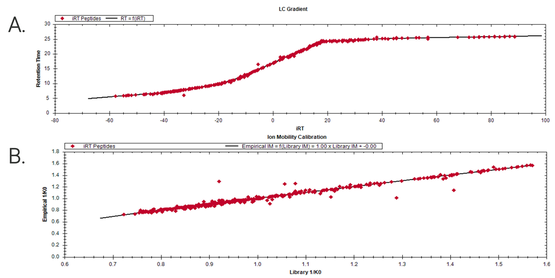


Fig. 2. A. iRT Calibration Chart B. The Ion Mobility Calibration plot.

Results

- An analytical method for peptide mapping of different AAV serotypes (AAV 2, 6) was developed using a nanoElute 2 (Bruker) coupled to a timsTOF HT (Bruker) via a CaptiveSpray 2 ionization source.
- The AAV peptide digestion protocol was robust and fast, completed in less than 2 hours. The combination of chymotrypsin and trypsin gave better results than other proteases.
- The timsTOF HT used for this study enabled enhanced ion loading capacity resulting in an increased dynamic range for the eluting peptides, providing additional analytical depth while retaining the specificity and sensitivity delivered by the TIMS separation.

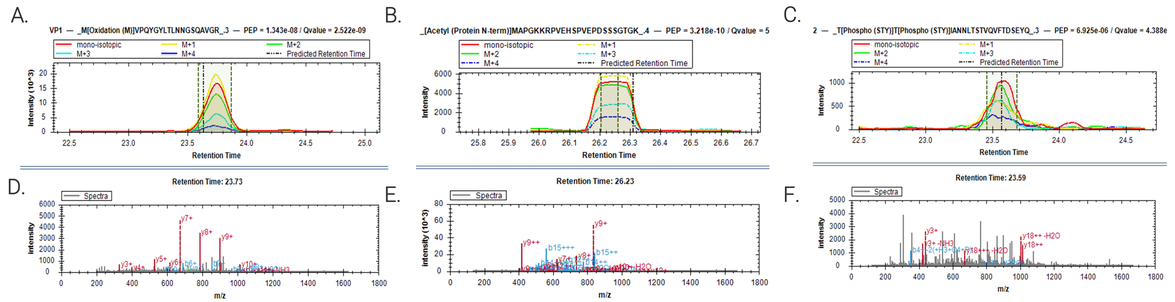


Fig. 3. MS1 XIC of modified peptides A. Oxidation B. Acetylation C. Phosphorylation and MS2 spectra of modified peptides D. oxidation, E. acetylation and F. phosphorylation.

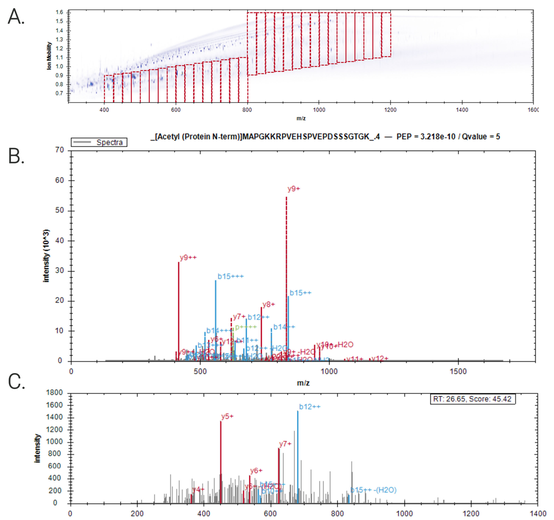


Fig. 4 A. DIA windows of the acquisition B. MSMS fragment spectra of the same peptide in DIA and C. DDA showing high quality in the DIA spectra.

- High quality MS and MSMS spectra were obtained resulting in 98% sequence coverage of AAV 2, 6 (Fig. 1).
- The iRT calibration chart shows the non-linear transformation from library iRT to actual predicted retention times (Fig. 2A).
- The Ion Mobility Calibration plot shows the empirical ion mobility as a function of the ion mobility values in the library (Fig. 2B).

- Several PTMs were identified including deamidation, oxidation, phosphorylation, and acetylation (Fig. 3).
  - PTMs can alter the gas phase structure of peptides. TIMS provides the collisional cross section (CCS) allowing for an unbiased alignment of precursor and fragment ions information, thus adding confidence when identifying modified peptides.
  - When combined with trapped ion mobility, the additional separation dimension reduces complexity. The sequential elution of separated ion packages from the TIMS device allows for more efficient ion usage.

Conclusion

- PASEF-based scan modes were evaluated to develop a fast, robust, high throughput characterization and PTM analysis of AAV serotypes.
- dia-PASEF acquisition was preferred over DDA. dia-PASEF typically gave better quantification than DDA due to the consistent availability of precursor fragments across runs (Figure 4).
- Indeed, our results showed ~10% better coverage when dia-PASEF mode is used and allowed identification of several PTMs
- The mass and mobility selectivity of dia-PASEF enabled the creation of highly optimized DIA window schemes.

Pharma/Biopharma

[1]Hiroaki Oyama, et al. (2021). Human Gene Therapy. 32:1403-1416

The authors declare no competing financial interest