Viral Vector Analytical Workflows

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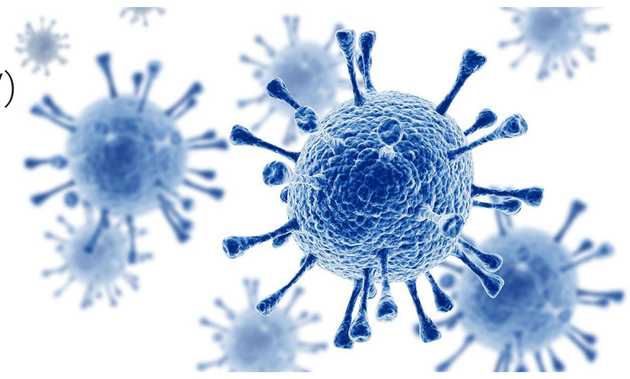


Viral vector is the most effective means of gene transfer to modify specific cell type or tissue and can be manipulated to express therapeutic genes. Several virus types are currently being investigated for use to deliver genes to cells to provide either transient or permanent transgene expression. Adeno-Associated Virus Vectors (AAV) and Lentivirus (LV) are the mostly utilised viral vectors for gene therapy.

Herby an overview of RSSL capabilities is shown alongside some examples of analytical methods to enhance viral vector characterisation and facilitate process development optimisation and regulatory approval.

Cell and gene therapy is an exciting area of bio-pharmaceuticals that offers the potential to treat, prevent, or cure diseases for which there is currently no other therapeutic option.

Cell therapies involve altering cells outside of the body so that they can restore normal function when introduced back into the patient. Gene therapy, on the other hand, involves changing or restoring the genetic function of a cell by introducing new genetic material, typically delivered by a vector that targets specific patient cells in-vivo.

Adeno-associated virus (AAV) and Lentiviral vectors are the most commonly used viral vectors for cell and gene therapy. AAV are a family of non-enveloped parvoviruses that are non-pathogenic, replication-defective and package a single stranded viral DNA. They have emerged as the most popular gene transfer vehicle for in-vivo gene therapy, largely owing to their high infectivity and low-pathogenicity. Lentiviral vectors (LVs) are potent tools for the delivery of genes of interest into mammalian cells and are commonly utilised for the treatment of monogenic diseases and adoptive therapies such as chimeric antigen T-cell (CAR-T) therapy. Lentiviral vectors are engineered to modify the viral genome to remove its pathogenic properties, while retaining the essential elements necessary for efficient gene transfer. The viral genes, responsible for replication and pathogenicity, are replaced with the therapeutic gene of interest, making lentiviral vectors safe and useful vehicles for gene delivery

Viral Vector Analytical toolbox at RSSL

Attribute class	Quality Attribute	Method
Content	Viral genome titer Protein titer	qPCR ELISA
Identity	Capsid identity Sequence identity	LC-MS and LC-MSMS; SDS-PAGE NGS, dPCR
Product related impurities	Capsid purity & VP1:VP2:VP3 ratio Empty:Full Integrity	CE-SDS, LC-UV LC-anion exchange chromatography CE-SDS
Process related impurities	Residual host cell DNA Residual plasmid DNA Residual host cell proteins Residual gene expression Others	qPCR qPCR ELISA and LC-MS mRNA by RT-PCR LC, GC, ELISA, LC-MS.....
Potency	Transgene expression Infectivity	mRNA by RT-qPCR tcID50
Safety	Sterility, Endotoxins, Mycoplasma, adventitious agents	Sterility testing and identity by MALDI-ToF; dPCR, cultured methods

AAV VP1:VP2:VP3 separation and quantification by CE-SDS (UV or LIF)

Recombinant adeno-associated virus is a leading platform in human gene therapy. The adeno-associated virus (AAV) capsid is composed of three viral proteins (VPs): VP1, VP2, and VP3. CE-SDS (Capillary Electrophoresis Sodium Dodecyl Sulfate) technology is successfully applied for AAV capsid protein analysis in the cell and gene therapy industry because of its automated separation of viral proteins with higher resolution, quantitation capability, better reproducibility and is less labor-intensive than traditional SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis).

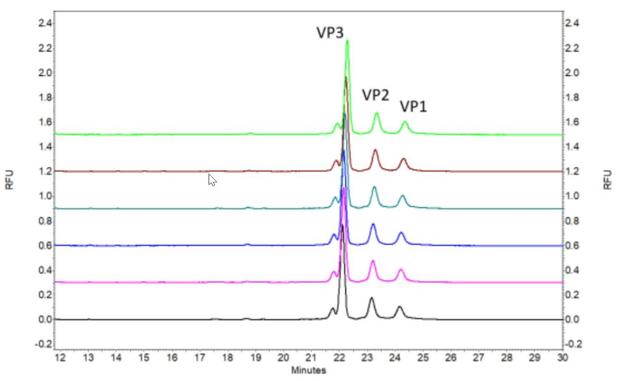


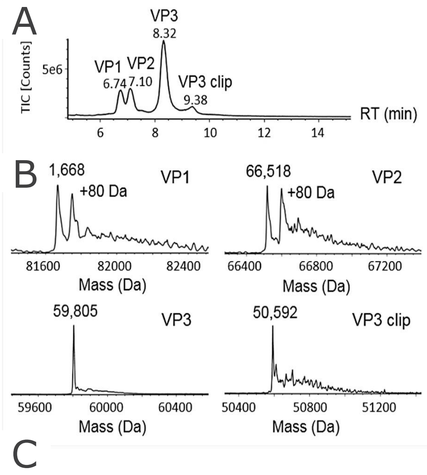
Figure 1. Electropherogram of VP1, VP2 and VP3 from AAV 8 obtained by CE-SDS[1]

References:

1 Purity Analysis of Adeno-Associated Virus (AAV) Capsid Proteins using CE-LIF Technology (sciex.com)
2_Optimizing Adeno-Associated Virus (AAV) Capsid Protein Analysis Using UPLC and UPLC-MS | Waters
3 J. Pharm and Biom Analysis, 207, 2021, 114427

Identification of capsid proteins

Full characterization, including sequence and post-translational modification (PTM) identification of viral proteins is required to ensure the safety, quality, and efficacy of AAV products. Alongside SDS-PAGE, Western Blot and ELISA, Mass Spectrometry coupled with liquid chromatography offer the advantage of confirming the protein sequence, identify and locate PTM



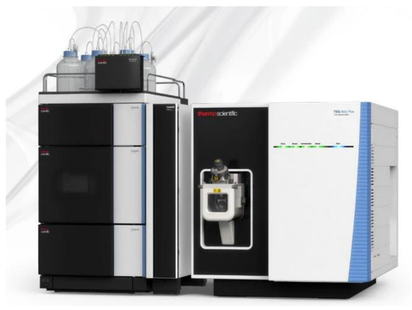


Figure 2. A. Total ion chromatogram showing separation of VP proteins from AAV8. C4 column, mobile phase water and acetonitrile 0.1%DFA. B. Deconvoluted spectra of VP1, VP2 and VP3[2]. C. Sequence confirmation of VP1, VP2 and VP3 by LC-MSMS[3]

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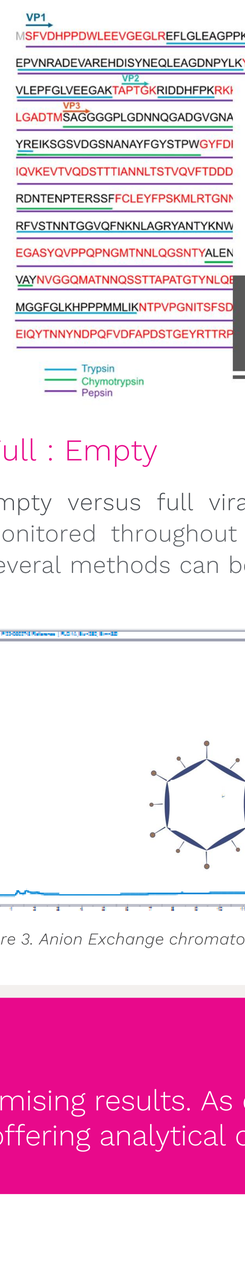

Modification	Peptide sequence	Confidence	Recovery	Average % abundance	SD
S1+Acetylation	SFVDHPPDWLEE	100.00	3.19	100.00	0.00
S193+Acetylation	SAGGGGPLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRT	100.00	40.09	95.39	0.71
N55+Deamidation	EAGPPKPKPNQQHQDQARGLVLPGYNYLGPGNGLDRGEPVNRADE	100.00	13.38	39.13	3.81
Q118+Succinimide	QAKKRVLEPFGL	100.00	6.87	34.53	3.46
Q99+Succinimide	QEKLADDTSFGGNLGKAVF	100.00	6.79	28.92	5.62
Q587+Succinimide	QEIVPGSVW	100.00	37.79	17.91	10.34
D38+Isomerization	EAGPPKPKPNQQHQDQARGLVLPGYNYLGPGNGLDRGEPVNRADE	100.00	5.41	13.45	0.64
M596+Oxidation	QEIVPGSVWM	100.00	95.22	13.20	3.29
-N571+Deamidation	LITSESETQPVNRVAYNVGGQMATNNQSSTTAPATGTYNLQEIVPGSVW	100.00	83.92	12.05	1.51
N92+Deamidation	VAREHDISYNEQLEAGDNPYLKYNHADAEF	98.83	7.23	9.41	0.75
E543+Amidation	MIFNSQPANPGTTATYLEGNM	90.94	3.08	3.66	4.27
M568+Oxidation	LITSESETQPVNRVAYNVGGQMATNNQSSTTAPATGTYNL	100.00	72.10	2.39	0.85
M394+Oxidation	EYFPSKM	100.00	47.39	2.33	0.61
M634+Oxidation	AKIPETGAHFHPSPAMGGFGLKHPPPMML	100.00	396.18	2.14	0.44
M468+Oxidation	NKNLAGRYANTYKNWFPGPMGRTQGWNLGSGVNRASVSA	100.00	58.92	1.97	0.38
N49+Deamidation	EAGPPKPKPNQQHQDQARGLVLPGYN	100.00	7.06	1.62	1.33
D317+Isomerization	VKEVTVQDSTTTIANNLTST	100.00	12.25	1.51	0.51
-N33+Deamidation	EAGPPKPKPNQQHQDQARGLVLPGYN	100.00	13.37	1.47	0.22
M494+Oxidation	FATTNRMELEGAS	100.00	63.12	1.33	0.37
N55+Succinimide	EAGPPKPKPNQQHQDQARGLVLPGYNYLGPGNGLDRGEPVNRADE	100.00	3.63	1.23	0.12
R436+Methylation	YRFVSTNNTGGVQFNKNLAGRYANT	100.00	56.92	1.20	0.10

Full : Empty

Empty versus full viral vector is an important critical quality attribute that needs to be monitored throughout the development and production of the viral vector based therapy. Several methods can be used including anion exchange chromatography.

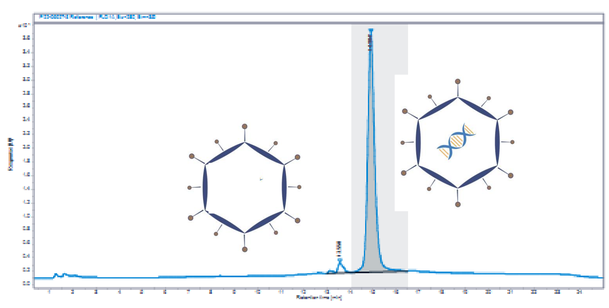

Figure 3. Anion Exchange chromatography of a viral vector vaccine

Summary

Cell and Gene Therapies are becoming more successful, with increased investment and promising results. As development progress, define analytical workflows to support process optimization and facilitate regulatory approval. RSSL is addressing those needs by offering analytical orthogonal solutions to unravel those complex molecules. Here an example of our solutions for viral vectors is provide

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