

Supplementary Information

Boosting the Separation of Adeno-Associated Virus Capsid Proteins by Liquid Chromatography and Capillary Electrophoresis Approaches

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Table S1. List of investigated columns and their properties.

LC Mode	Name	Provider	Column chemistry	Column dimensions (mm)	Particle (μm) and pore (\AA) size
RPLC	ACQUITY BEH C4	Waters	C4	2.1 x 150	1.7 μm , 300 \AA
	BioResolve RP mAb	Waters	Polyphenyl	2.1 x 50	2.7 μm , 450 \AA
	YMC-Triart Bio C4	YMC	Bioinert C4	2.1 x 50	1.7 μm , 300 \AA
	ProSwift RP-4H	Thermo	Polymeric	1.0 x 50	Monolith
HILIC	ACQUITY Glycoprotein BEH Amide	Waters	Amide	2.1 x 150	1.7 μm , 300 \AA
HIC	BioPro HIC HT	YMC	Butyl	4.6 x 33	2.3 μm
	MABPac HIC-10	Thermo	Alkylamide	4.6 x 100	5.0 μm , 1000 \AA
	TSK-Gel Ether-5PW	Tosoh	Ethyl	2.0 x 75	10 μm , 1000 \AA
	TSK-Gel Butyl-NPR	Tosoh	Butyl	4.6 x 100	2.5 μm

Table S2. Optimized RPLC multi-step gradients applied for the analyses of VPs obtained from different rAAV serotypes, namely rAAV2, rAAV5, rAAV8, and rAAV9. MPA: H₂O/ACN (66/34 for AAV8 and 65/35 for AAV2, AAV5, and AAV9) + 0.1% TFA; MPB: H₂O/ACN (64/36 for AAV8 and 63/37 for AAV2, AAV5, and AAV9) + 0.1% TFA.

VPs from rAAV2		VPs from rAAV5		VPs from rAAV8		VPs from rAAV9	
Time (min)	%B	Time (min)	%B	Time (min)	%B	Time (min)	%B
0	0	0	0	0	0	0	0
1.0	23.9	1.0	39.3	1.0	37.7	1.0	40.4
3.0	23.9	3.0	39.3	3.0	37.7	3.0	40.4
3.1	29.7	3.1	44.5	3.1	41.6	3.1	44.6
5.1	29.7	5.1	44.5	5.1	41.6	5.1	44.6
5.2	33.1	5.2	46.1	5.2	51.4	5.2	45.4
7.2	33.1	7.2	46.1	7.2	51.4	7.2	45.4
10.0	100	7.3	49.2	7.3	54.0	7.3	53.5
		9.3	49.2	9.3	54.0	9.3	53.5
		10.0	100	9.4	58.9	9.4	57.3
				11.4	58.9	11.4	57.3
				11.5	61.3	11.5	62.9
				13.5	61.3	13.5	62.9
				15.0	100	15.0	100

Table S3. Optimized HILIC multi-step gradients applied for the analyses of VPs obtained from different rAAV serotypes, namely rAAV2, rAAV5, rAAV8, and rAAV9. MPA: [ACN/IPA (80/20, v/v)] / H₂O (79/21, v/v) + 0.1% TFA; MPB: [ACN/IPA (80/20, v/v)] / H₂O (67/33, v/v) + 0.1% TFA.

VPs from rAAV2		VPs from rAAV5		VPs from rAAV8		VPs from rAAV9	
Time (min)	%B	Time (min)	%B	Time (min)	%B	Time (min)	%B
0	41.3	0	38.5	0	36.5	0	41.0
2.00	41.3	2.00	38.5	2.50	36.5	2.00	41.0
2.01	41.6	2.01	39.2	2.51	37.0	2.01	41.3
4.01	41.6	4.01	39.2	4.51	37.0	3.01	41.3
4.02	41.9	4.02	39.8	4.52	37.4	3.02	41.5
6.02	41.9	6.02	39.8	6.52	37.4	4.02	41.5
6.03	42.2	6.03	40.5	6.53	37.8	4.03	41.8
8.03	42.2	8.03	40.5	8.53	37.8	5.03	41.8
8.04	42.5	8.04	41.1	8.54	38.3	5.53	46.4
10.04	42.5	10.04	41.1	10.54	38.3	6.53	46.4
10.54	47.7	10.54	44.9	10.55	38.7	6.54	47.0
12.54	47.7	12.54	44.9	12.55	38.7	7.54	47.0
12.55	48.5	12.55	46.6	13.55	45.0	7.55	47.7
14.55	48.5	14.55	46.6	15.55	45.0	8.55	47.7
14.56	49.3	14.56	48.2	15.56	47.1	8.56	48.3
16.56	49.3	16.56	48.2	17.56	47.1	9.56	48.3
16.57	50.1	16.57	49.9	17.57	49.2	9.57	49.0
18.57	50.1	18.57	49.9	19.57	49.2	10.57	49.0
18.58	51.0	18.58	51.6	19.58	51.2	10.58	49.6
20.58	51.0	20.58	51.6	21.58	51.2	11.58	49.6
20.59	51.8	20.59	53.2	21.59	53.3	11.6	41.0
22.59	51.8	22.59	53.2	23.59	53.3	20.0	41.0
22.6	41.3	22.6	38.5	23.6	55.4		
30.0	41.3	30.0	38.5	26.1	55.4		
				26.2	36.5		
				40.0	36.5		

Table S4. Optimized HIC multi-step gradients applied for the analyses of VPs obtained from different rAAV serotypes, namely rAAV2, rAAV5, rAAV8, and rAAV9. MPA: 100 mM phosphate buffer solution + 1.5 M (NH₄)SO₄, pH 6.8; MPB: 100 mM phosphate buffer solution, pH 6.8.

VPs from rAAV2		VPs from rAAV5		VPs from rAAV8		VPs from rAAV9	
Time (min)	%B	Time (min)	%B	Time (min)	%B	Time (min)	%B
0	0	0	0	0	0	0	0
1.0	26.5	1.0	55.1	1.0	39.0	1.0	28.5
3.0	26.5	3.0	55.1	3.0	39.0	3.0	28.5
3.1	28.6	3.1	60.8	3.1	39.7	3.1	31.0
5.1	28.6	5.1	60.8	5.1	39.7	5.1	31.0
10.0	100	5.2	63.6	5.2	40.3	5.2	33.5
		7.2	63.6	7.2	40.3	7.2	33.5
		10.0	100	7.3	41.0	7.3	36.0
				9.3	41.0	9.3	36.0
				9.4	41.4	9.4	38.5
				11.4	41.4	11.4	38.5
				15.0	100	15.0	100

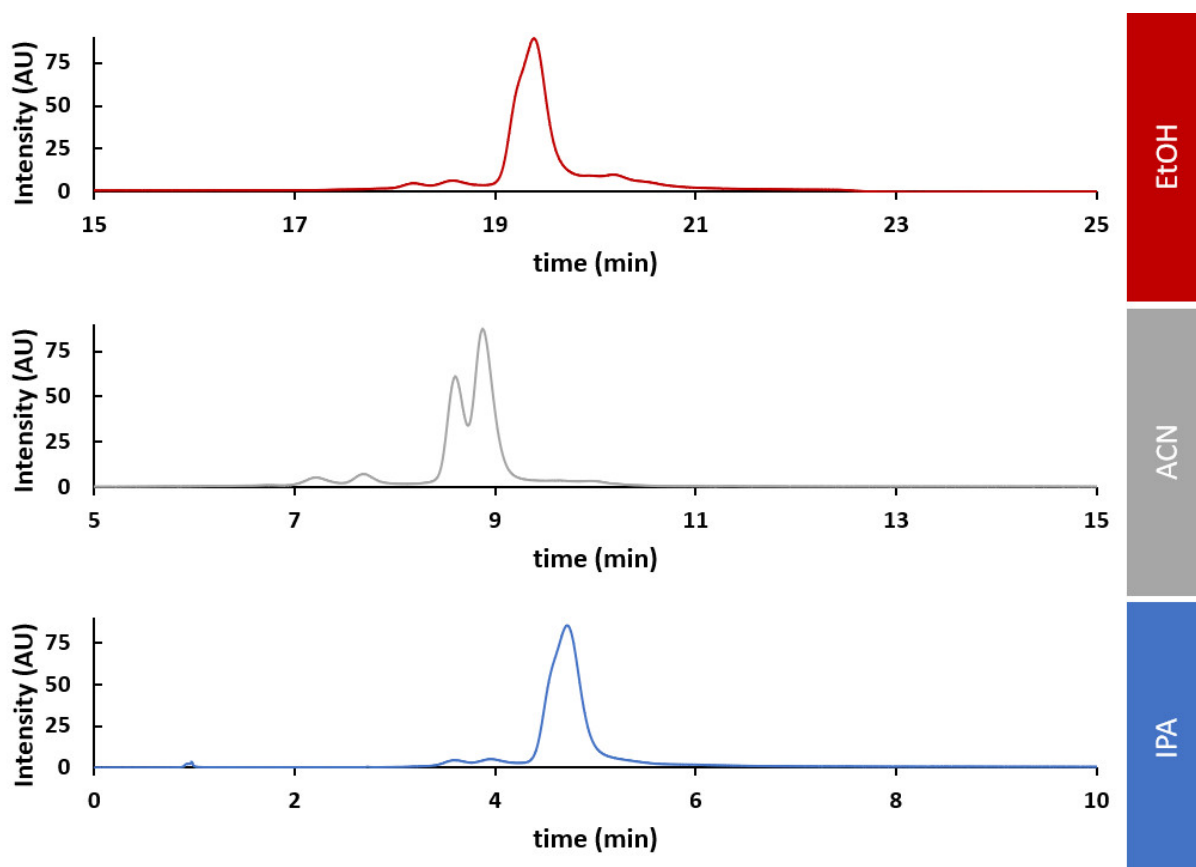


Figure S1. Effect of solvent selection on RPLC analysis of rAAV8 VPs. Comparison of IPA, ACN, and MeOH mobile phases on an Acquity UPLC Protein BEH C4 column (flow rate 0.4 mL/min, 2 μ L injected, 70°C) with a 20 min gradient from 27 to 30 %B for IPA, 36 to 39 %B for ACN, and 38 to 41 %B for EtOH). MPA: H₂O + 0.1% TFA; MPB: EtOH (red), ACN (grey), or IPA (blue) + 0.1% TFA.

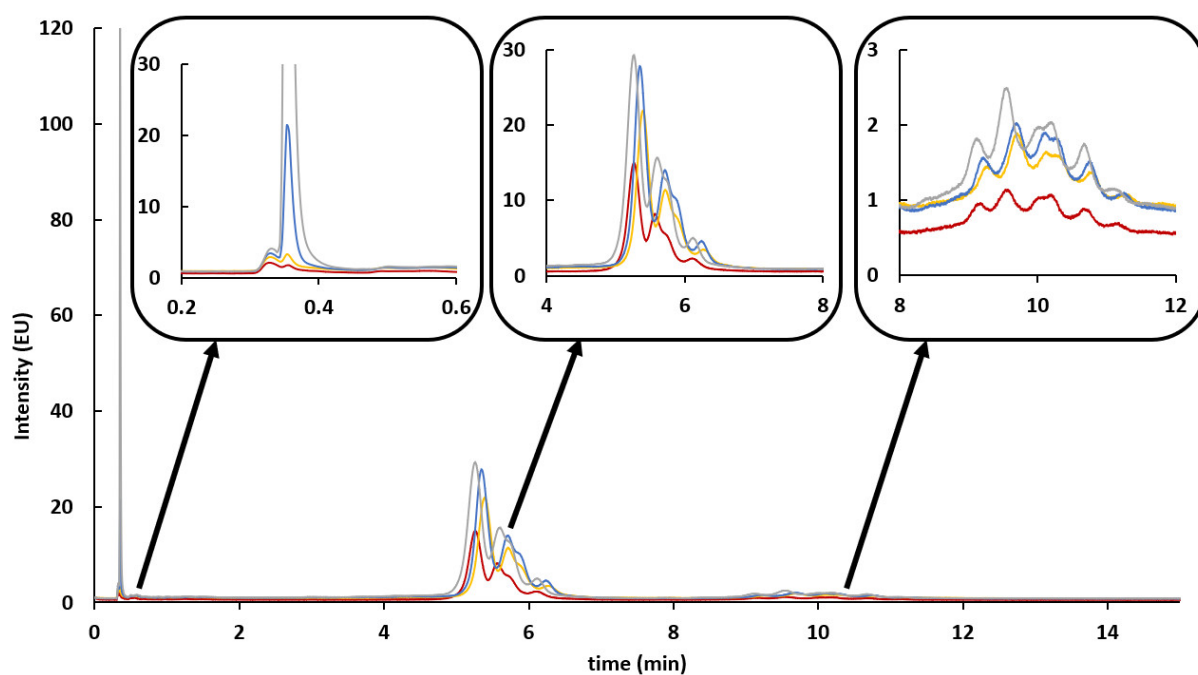


Figure S2. Effect of injection volume on HILIC analysis of rAAV8 VPs: comparison of 0.2 μL (red), 0.3 μL (yellow), 0.4 μL (blue), and 0.5 μL (grey) on Waters ACQUITY Glycoprotein BEH Amide column with a 15 min gradient from 0 to 100 %B. MPA: [ACN/IPA (80/20, v/v)] / H₂O (75/25, v:v) + 0.1%TFA; MPB: [ACN/IPA (80/20, v/v)] / H₂O (70/30, v/v) + 0.1% TFA.