CLAIMS

1. A method to characterize capsid viral proteins VP1, VP2 and VP3 in an adeno-associated virus (AAV) particle, the method comprising:

subjecting the AAV particle to a column for a liquid chromatography, wherein the column is maintained at 70°C to 90 °C to denature the capsid viral proteins, wherein the liquid chromatography comprises a first mobile phase that comprises 0.05% to 0.15% trifluoroacetic acid by volume;

wherein the ratio of VP1, VP2 and VP3 capsid proteins are determined by ultraviolet (UV)-visible spectroscopy, and the protein mass is determined by mass spectrometer.

2. The method of claim 1, wherein individual masses of VP1, VP2 and VP3 capsid proteins are determined by the mass spectrometry.

3. The method of claim 1 or 2, wherein the ratio of the VP1, VP2 and VP3 capsid proteins is determined by comparing ultraviolet chromatogram (UV) of the VP1, VP2 and VP3 capsid proteins.

4. The method of any one of the preceding claims, wherein the liquid chromatography is a reverse phase liquid chromatography.

5. The method of claim 4, wherein the reverse phase liquid chromatography is performed using a C18 column, a C8 column, or a C4 column.

6. The method of claim 5, wherein the column comprises particles of 1.2-3.5 µm, optionally, wherein the column comprises particles of 1.7 µm or 1.8 µm.

7. The method of any one of claims 5 and 6, wherein the column is from 50 mm to 300 mm long and has an internal diameter of from 1 mm to 4.6 mm.

8. The method of any one of claims 1-7, wherein the AAV particle is of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV 13, AAVrh10, AAVrh74 serotype, or any naturally occurring, recombinant, or synthetic AAV particle.

9. The method of any one of claims 1-8, wherein the liquid chromatography is performed at 75°C to 85 °C.

10. The method of claim 1, wherein the liquid chromatography comprises a second mobile phase that comprises trifluoroacetic acid in the mixture of acetonitrile and water, optionally wherein the second mobile phase comprises from 0.05 to 0.15% trifluoroacetic acid by volume.

11. The method of claim 10, wherein the second mobile phase comprises 80-95% of acetonitrile by volume, optionally wherein the second mobile phase comprises 90% of acetonitrile and 10% water by volume.

12. The method of claim 10 or 11, wherein the percentage of the second mobile phase, as compared to a combination of the first mobile phase and the second mobile phase, in the liquid chromatography is increased over time.

13. The method of claim 12, wherein the percentage of the second mobile phase is increased from 10% to 40%, and then from 40% to 45%, by volume, optionally wherein the percentage of the second mobile phase is increased: from 10% to 40%, by volume, over 3 min; and then from 40% to 45%, by volume, over 30 min.

14. The method of claim 13, wherein the percentage of the second mobile phase, in the combination of the first mobile phase and the second mobile phase, is subsequently increased to 100%, by volume, over 1 min, optionally wherein the percentage of the second mobile phase, in the combination of the first mobile phase and the second mobile phase, is subsequently decreased to 10%, by volume, over 1 min.

15. The method of any one of the preceding claims, wherein the mass spectrometry is performed using a fragmentor voltage of 125-350 V.

16. The method of any one of the preceding claims, wherein the mass spectrometry is performed using a capillary voltage of 3-6 kV.

17. The method of claim 1, wherein the characterization further comprises determining a post translational modification of at least one of VP1, VP2 and VP3 capsid proteins.

18. The method of claim 17, wherein the post translational modification comprises one or more of loss of amino acid, glycosylation, sialylation, acetylation, phosphorylation, deamidation, oxidation, formylation, hydroxylation, methylation, and sulfation, optionally wherein the post translational modification comprises one or more of loss of N-terminal methionine, deamidation, loss of threonine, phosphorylation, and acetylation.

19. The method of any one of claims 1-18, wherein the method is performed with a gradient program listed in Table 2.

20. The method of claim 17, wherein the characterization uses a buffer comprising Tris-HCl, optionally acetonitrile, and/or optionally methionine.

21. The method of claim 20, wherein the buffer comprises Tris-HCl at 5mM to 50 mM, 5%-20% acetonitrile, and methionine at 1 mM to 50 mM, optionally wherein the buffer comprises Tris-HCl at 20 mM, 5%-10% acetonitrile, and methionine at 10 mM.

22. The method of claim 20, wherein the Tris-HCl is at pH 7.5.

23. The method of claim 17, wherein the post-translation modification comprises deamidation at one or more of N263, N514, N57, N502, N254, and N94 of AAV8 or its equivalent residue at AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV9, AAV10, AAV11, AAV12, AAV 13, AAVrh10, AAVrh74, or a recombinant or synthetic AAV particle.

24. An AAV composition comprising an AAV capsid that comprises deamidation at one or more of N57, N255, N256, and N263 of AAV. Rh74 or the equivalent residues of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV 13, AAVrh10, or a recombinant or synthetic AAV particle.

25. An AAV composition comprising an AAV capsid that comprises oxidation at one or more of M437, M473, M526, M544, M560, and M637 of AAV. Rh74 or the equivalent

residues of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, CAAV11, AAV12, AAV 13, AAVrh10, or a recombinant or synthetic AAV particle.