Claims:

1. A method for purifying recombinant a disintegrin-like and metallopeptidase with thrombospondin type 1 motif 13 (ADAMTS13) protein from a sample comprising ADAMTS13 protein and non-ADAMTS13 impurities, the method comprising: chromatographically contacting the sample with hydroxyapatite under conditions that allow said ADAMTS13 protein to appear in a flow through fraction from said hydroxyapatite by said ADAMTS13 protein not binding to said hydroxyapatite while said non-ADAMTS13 impurities are retained on said hydroxyapatite.

2. The method according to claim 1, further comprising chromatographically contacting said flow through fraction with a cation exchange/hydrophobic interaction resin that binds said ADAMTS13 protein; and eluting said ADAMTS13 protein from said cation exchange/hydrophobic interaction resin with an elution buffer.

3. The method according to claim 1 or 2, further comprising chromatographically contacting said sample with an anion exchange resin and eluting said ADAMTS13 protein from said anion exchange resin before chromatographic contact with said hydroxyapatite.

4. The method according to any one of claims 1-3, further comprising concentrating said ADAMTS13 protein in said sample by ultrafiltration; and stabilizing said ADAMTS13 protein by diafiltration exchange into a buffer comprising calcium ions and zinc ions before chromatographic contact with said hydroxyapatite.

5. The method according to any one of claims 2-4, further comprising, following contact with said hydroxyapatite or said cation exchange/hydrophobic interaction resin, the step of preparing said ADAMTS13 protein for cation exchange by reducing buffer conductivity.

6. The method according to claim 5, wherein said preparing step is performed by ultrafiltration/diafiltration.

7. The method according to claim 5, wherein said preparing step is performed by dialysis, said dialysis being performed once or twice.

8. The method according to claim 5, wherein said preparing step is performed by gel filtration.

9. The method according to any one of claims 1-8, further comprising subjecting said ADAMTS13 protein to at least one virus inactivation or virus removal step.

10. The method according to claim 9, wherein said virus inactivation step comprises adding a solvent-detergent mixture comprising a non-ionic detergent and an organic solvent to said ADAMTS13 protein.

11. The method according to claim 10, wherein said ADAMTS13 protein is immobilized during said virus inactivation step.

12. The method according to claim 11, wherein said ADAMTS13 protein is immobilized on a cation exchange resin.

13. The method according to claim 10, wherein said solvent-detergent mixture comprises 1% TRITONX-100, 0.3% Tri-N-butyl phosphate, and 0.3% TWEEN 80.

14. The method according to claim 9, wherein said virus removal step comprises filtering said ADAMTS13 protein with a nanofilter to remove viruses and/or viral particles.

15. The method according to any one of claims 5-8, further comprising subjecting said ADAMTS13 protein to at least one virus inactivation or virus removal step, wherein said virus inactivation or virus removal step is performed after said preparing step.

16. The method according to claim 12, further comprising eluting said ADAMTS13 protein from said cationic exchange resin using gradient elution, said gradient elution using a first buffer having low salt content and a second buffer having higher salt content.

17. The method according to claim 12, further comprising eluting said ADAMTS13 protein from said cationic exchange resin using step elution.

18. The method according to claim 17, wherein said step elution comprises eluting said ADAMTS13 protein from said cationic exchange resin with a storage buffer.

19. The method according to claim 18, wherein said storage buffer has a pH of greater than 7.0 and comprises less than 10 mM calcium ions, a buffering compound, 0.05% non-ionic detergent, and a salt.

20. The method according to claim 18 or 19, wherein there is no ultrafiltration, diafiltration, or buffer exchange step subsequent to the cation exchange chromatography step.

Claims

1. A method for purifying recombinant a disintegrin-like and metallopeptidase with thrombospondin type 1 motif 13 (ADAMTS13) protein from a sample comprising ADAMTS13 protein and non-ADAMTS13 impurities, the method comprising:

chromatographically contacting the sample with hydroxyapatite under conditions that allow said ADAMTS13 protein to appear in a flow through fraction from said hydroxyapatite by said ADAMTS13 protein not binding to said hydroxyapatite while said non-ADAMTS13 impurities are retained on said hydroxyapatite;

subsequently chromatographically contacting said flow through fraction with a mixed mode cation exchange/hydrophobic interaction resin that binds said ADAMTS13 protein; and

eluting said ADAMTS13 protein from said mixed mode cation exchange/hydrophobic interaction resin with an elution buffer.

2. The method according to claim 1, further comprising chromatographically contacting said sample with an anion exchange resin and eluting said ADAMTS13 protein from said anion exchange resin before chromatographic contact with said hydroxyapatite.

3. The method according to any one of claims 1-2, further comprising concentrating said ADAMTS13 protein in said sample by ultrafiltration; and stabilizing said ADAMTS13 protein by diafiltration exchange into a buffer comprising calcium ions and zinc ions before chromatographic contact with said hydroxyapatite.

4. The method according to any one of claims 2-3, further comprising, following contact with said hydroxyapatite or said mixed mode cation exchange/hydrophobic interaction resin, a preparing step for preparing said ADAMTS13 protein for cation exchange by reducing buffer conductivity.

5. The method according to claim 4, wherein said preparing step is performed by ultrafiltration/diafiltration.

6. The method according to claim 5, wherein said preparing step is performed by dialysis, said dialysis being performed once or twice.

7. The method according to claim 5, wherein said preparing step is performed by gel filtration.

8. The method according to any one of claims 1-7, further comprising subjecting said ADAMTS13 protein to at least one virus inactivation or virus removal step.

9. The method according to claim 8, wherein said virus inactivation step comprises adding a solvent-detergent mixture comprising a non-ionic detergent and an organic solvent to said ADAMTS13 protein.

10. The method according to claim 9, wherein said ADAMTS13 protein is immobilized during said virus inactivation step.

11. The method according to claim 10, wherein said ADAMTS13 protein is immobilized on a cation exchange resin.

12. The method according to claim 9, wherein said solvent-detergent mixture comprises 1% TRITONX-100, 0.3% Tri-N-butyl phosphate, and 0.3% TWEEN 80.

13. The method according to claim 8, wherein said virus removal step comprises filtering said ADAMTS13 protein with a nanofilter to remove viruses and/or viral particles.

14. The method according to any one of claims 4-7, further comprising subjecting said ADAMTS13 protein to at least one virus inactivation or virus removal step, wherein said virus inactivation or virus removal step is performed after said preparing step.

15. The method according to claim 11, further comprising eluting said ADAMTS13 protein from said cationic exchange resin using gradient elution, said gradient elution comprising using a first buffer having low salt content and a second buffer having higher salt content.

16. The method according to claim 11, further comprising eluting said ADAMTS13 protein from said cationic exchange resin using step elution.

17. The method according to claim 16, wherein said step elution comprises eluting said ADAMTS13 protein from said cationic exchange resin with a storage buffer.

18. The method according to claim 17, wherein said storage buffer has a pH of greater than 7.0 and comprises less than 10 mM calcium ions, a buffering compound, 0.05% non-ionic detergent, and a salt.

19. The method according to claim 17 or 18, wherein there is no ultrafiltration, diafiltration, or buffer exchange step subsequent to the cation exchange chromatography step.