columns can be used. The use of CHT columns, however, has a limited lifetime in the production process and can be expensive and possibly lead to downtime when CHT resins need to be replaced. As such, Applicants sought to develop additional chromatography purification methods to, e.g., increase resin lifetime and/or maintain the functional aspects of purification, i.e., increase the purity of dulaglutide and removal of impurities from the production process of dulaglutide.

SUMMARY

[0001] The present disclosure generally relates to methods for purifying dulaglutide compositions using a hydrophobic interaction chromatography (HIC) column. Such methods comprise a loading step, a washing step, and an eluting step. The loading step comprises loading the dulaglutide composition in-line with a buffer B solution onto the HIC column in a downflow direction. The washing step comprises washing the HIC column with a buffer mixture of a buffer A and the buffer B solutions. The eluting step comprises eluting dulaglutide from the HIC column using a linear gradient of a percentage of the buffer A and buffer B solutions, which is followed by an isocratic hold based on a UV absorbance reading of an eluate from the HIC column indicating a protein-containing material is eluting from the HIC column. The methods further comprise maintaining the isocratic hold and then dropping the buffer B solution to 0% for dulaglutide to elute from the HIC column.

[0001a] In an aspect, there is provided a method of purifying a dulaglutide composition, the method comprising:

loading the dulaglutide composition in-line with a buffer B solution onto a hydrophobic interaction chromatography (HIC) column in a downflow direction, wherein dulaglutide from the dulaglutide composition binds to the HIC column;

washing the HIC column with a buffer mixture of a buffer A solution and the buffer B solution; and

eluting dulaglutide from the HIC column using a linear gradient of a percentage of the buffer A and buffer B solutions, wherein the linear gradient is followed by an isocratic hold based on a UV absorbance reading of an eluate from the HIC column indicating a protein-containing material is eluting from the HIC column, and maintaining the isocratic hold until a front side cut is made, and then dropping the buffer B solution to 0% for dulaglutide to elute from the HIC column.

[0002] In some examples, a method for purifying dulaglutide compositions using a hydrophobic interaction chromatography (HIC) column comprises loading the dulaglutide composition in-line with a buffer B solution at a volume ratio ranging from 62.7% to 72.5% and at a linear rate ranging from 80 cm/hr -160 cm/hr onto the HIC column in a downflow direction, wherein dulaglutide from the dulaglutide composition binds to the HIC column and the composition comprises a plurality of compounds from a fermentation process of mammalian cells. The method further comprises washing the HIC column with a

buffer mixture comprising from 15% to 25% of a buffer A solution and from 75% to 85% of the buffer B solution in the downflow direction at a linear rate ranging from 80 cm/ hour - 160 cm/ hour for a duration of 2.7 column volumes (CV) - 3.3 CV. The method then comprises eluting dulaglutide from the HIC column using a linear gradient comprising from 67.5% of the buffer B solution to 10% of the buffer B solution for a duration of 10 CV - 12 CV, which is followed by an isocratic hold based on the UV absorbance reading of an eluate at A 280 nm with a range from 0.1 AU/cm to 0.5 AU/cm indicating a protein-containing material is eluting from the HIC column. The isocratic hold is maintained for 1.25 CV – 1.75 CV, at which point a front side cut is made and mainstream collection begins with an elution gradient dropping the buffer B solution from its isocratic hold to 0%.

[0002a] In another aspect, there is provided a method of purifying a dulaglutide composition, the method comprising:

loading the dulaglutide composition in-line with a buffer B solution at a volume ratio ranging from 62.7% to 72.5% and at a linear rate ranging from 80 cm/hr -160 cm/hr onto a hydrophobic interaction chromatography (HIC) column in a downflow direction, wherein dulaglutide from the dulaglutide composition binds to the HIC column and the composition comprises a plurality of compounds from a fermentation process of mammalian cells;

washing the HIC column with a buffer mixture comprising from 15% to 25% of a buffer A solution and from 75% to 85% of the buffer B solution in the downflow direction at a linear rate ranging from 80 cm/ hour - 160 cm/ hour for a duration of 2.7 column volumes (CV) - 3.3 CV; and

eluting dulaglutide from the HIC column using a linear gradient comprising from 67.5% of the buffer B solution to 10% of the buffer B solution for a duration of 10 CV - 12 CV, wherein the linear gradient is followed by an isocratic hold based on the UV absorbance reading of an eluate at A 280 nm with a range from 0.1 AU/cm to 0.5 AU/cm indicating a protein-

containing material is eluting from the HIC column, maintaining the isocratic hold with a fixed % buffer B solution for 1.25 – 1.75 CV, making a front side cut is with the buffer B solution at 0%, collecting a dulaglutide-containing eluate until a UV absorbance reading of the eluate at A 280 nm is within a range from 0.125 AU/cm to 0.25 AU, and making a back side cut when the UV absorbance reading of the eluate at A 280 nm is within 0.125 AU/cm to 0.25 AU.

[0002b] In another aspect, there is provided dulaglutide produced by the method as described herein.

[0002c] In another aspect, there is provided a composition comprising dulaglutide produced by the method as described herein.

[0003] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the disclosure, as claimed.

[0004] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate one (several) example(s) of the disclosure and together with the description, serve to explain the principles of the present disclosure.

BRIEF DESCRIPTION OF DRAWINGS

[0005] Exemplary embodiments are illustrated in referenced figures of the drawings. It is intended that the embodiments and figures disclosed herein are to be considered illustrative rather than restrictive.

[0006] FIG. 1 illustrates a unit operation for purifying a dulaglutide composition using a HIC column according to some embodiments herein.

[0007] FIG. 2 is a chromatogram overlay of an embodiment of the present disclosure for purifying a dulaglutide composition.

WHAT IS CLAIMED IS:

1. A method of purifying a dulaglutide composition, the method comprising:

loading the dulaglutide composition in-line with a buffer B solution onto a hydrophobic interaction chromatography (HIC) column in a downflow direction, wherein dulaglutide from the dulaglutide composition binds to the HIC column;

washing the HIC column with a buffer mixture of a buffer A solution and the buffer B solution; and

eluting dulaglutide from the HIC column using a linear gradient of a percentage of the buffer A and buffer B solutions, wherein the linear gradient is followed by an isocratic hold based on a UV absorbance reading of an eluate from the HIC column indicating a protein-containing material is eluting from the HIC column, and maintaining the isocratic hold until a front side cut is made, and then dropping the buffer B solution to 0% for dulaglutide to elute from the HIC column.

2. The method of claim 1, wherein loading the dulaglutide composition with the buffer B solution is at a volume ratio ranging from 62.7% to 72.5% of the buffer B solution.

3. The method of claim 1 or 2, wherein loading the dulaglutide composition in-line is at a target load ranging from 10 grams – 20 grams of dulaglutide per liter of HIC resin and wherein loading the dulaglutide composition is at a linear rate ranging from 80 cm/hr - 160 cm/hr.

4. The method of any one of claims 1-3, wherein the buffer A solution comprises 20 mM Tris at a pH ranging from 7.7-8.3 and wherein the buffer B solution comprises 20 mM Tris, 400 mM sodium sulfate at a pH ranging from 7.7-8.3.

5. The method of any one of claims 1-4, wherein the wash buffer mixture comprises from 15% to 25% of the buffer A solution and from 75% to 85% of the buffer B solution.

6. The method of any one of claims 1-5, wherein the wash buffer mixture comprises 20% of the buffer A and 80% of the buffer B solution.

7. The method of any one of claims 1-6, wherein the wash buffer mixture comprises 20 mM Tris, 320 mM sodium sulfate solution at a pH ranging from 7.7-8.3.

8. The method of any one of claims 1-7, wherein washing is at a linear rate ranging from 80 cm/ hour - 160 cm/ hour of the buffer mixture with 2.7 column volumes (CV) - 3.3 CV of the buffer mixture.

9. The method of any one of claims 1-8, wherein washing of the HIC column is with 3.0 CV of the buffer mixture.

10. The method of any one of claims 1-9, wherein the linear gradient ranges from 67.5% to 72.5% of the buffer B solution at a start setpoint and ranges from 5% to 50% of the buffer B solution at an end setpoint.

11. The method of any one of claims 1-10, wherein eluting dulaglutide with the linear gradient is with 10 column volumes (CV) - 12 CV of the buffer A and buffer B solutions.

12. The method of any one of one claims 1-11, wherein eluting dulaglutide with the linear gradient is with 11.5 CV of the buffer A and B solutions.

13. The method of any one of claims 1-12, wherein eluting dulaglutide with the linear gradient is at a linear rate of 80 cm/hr - 160 cm/hr.

14. The method of any one of claims 1-13, wherein initiation of the isocratic hold is based on the UV absorbance at A 280 nm and an UV signal ranging from 0.1 AU/cm to 0.5 AU/cm.

15. The method of any one of claims 1-14, wherein initiation of the isocratic hold is based on the UV absorbance at A 280 nm and the UV signal at about 0.25 AU/cm.

16. The method of any one of claims 1-14, wherein maintaining the isocratic hold is with 1.25 – 1.75 CV of the buffer B solution.

17. The method of any of claims 1-16, wherein maintaining the isocratic hold is with about 1.5 CV of the buffer B solution.

18. The method of claim 17, wherein the front side cut is with the buffer B solution at 0% and wherein collection of dulaglutide-containing eluate is initiated when the front side cut is made.

19. The method of claim 18, wherein said collection continues until a UV absorbance reading of the eluate at A 280 nm is within a range from 0.125 AU/cm to 0.25 AU.

20. The method of claim 19, wherein said collection continues until a UV signal of about 0.25 AU/cm is reached.

21. The method of any one of claims 1-20, wherein the dulaglutide composition comprises a plurality of compounds from a fermentation process of mammalian cells.

22. The method of any one of claims 1-21, wherein the dulaglutide composition is a downstream product of an anionic exchange chromatography column.

23. The method of claim 21, wherein the plurality of compounds comprises host cell proteins.

24. The method of any one of claims 1-23, wherein the HIC column comprises a highly cross-linked agarose matrix with phenyl ligands, has a diameter ranging from 50 cm to 200 cm, has a packed bed height ranging from 15 cm to 30 cm and comprises two 0.2 micron filters upstream of the HIC column.

25. The method of any one of claims 1-24, further comprising before loading, equilibrating the HIC column in a downflow direction with 2 CV of a linear gradient at 32.5% of the buffer A and 67.5% of the buffer B solutions.

26. The method of any one of claims 1-25, wherein eluting further comprises initiating a mainstream product collection step by making the front side cut and ending the mainstream product collection step by making a back side cut at a UV absorbance of 0.125 AU/cm or more at A 280 nm.

27. The method of any one of claims 1-26, further comprising one or more steps of: regenerating the HIC column, sanitizing the HIC column, and storing the HIC column.

28. The method of claim 27, wherein regenerating the HIC column comprises washing the HIC column with 0.01 N NaOH for 3.0 CV in an upflow direction after each elution step.

29. The method of claim 27, wherein sanitizing the HIC column comprises washing the HIC column in an upflow direction with 1 N NaOH for 2.0 CV with a target contact static hold time of not less than 30 minutes.

30. The method of claim 27, wherein storing the HIC column comprises washing the HIC column with a storage buffer solution of 0.01 N NaOH with at least 2 CV in an upflow direction, when the HIC column is held for more than 24 hours before loading the dulaglutide composition.

31. A method of purifying a dulaglutide composition, the method comprising:

loading the dulaglutide composition in-line with a buffer B solution at a volume ratio ranging from 62.7% to 72.5% and at a linear rate ranging from 80 cm/hr -160 cm/hr onto a hydrophobic interaction chromatography (HIC) column in a downflow direction, wherein dulaglutide from the dulaglutide composition binds to the HIC column and the composition comprises a plurality of compounds from a fermentation process of mammalian cells;

washing the HIC column with a buffer mixture comprising from 15% to 25% of a buffer A solution and from 75% to 85% of the buffer B solution in the downflow direction at a linear rate ranging from 80 cm/ hour - 160 cm/ hour for a duration of 2.7 column volumes (CV) - 3.3 CV; and

eluting dulaglutide from the HIC column using a linear gradient comprising from 67.5% of the buffer B solution to 10% of the buffer B solution for a duration of 10 CV - 12 CV, wherein the linear gradient is followed by an isocratic hold based on the UV absorbance reading of an eluate at A 280 nm with a range from 0.1 AU/cm to 0.5 AU/cm indicating a protein-containing material is eluting from the HIC column, maintaining the isocratic hold with a fixed % buffer B solution for 1.25 – 1.75 CV, making a front side cut is with the buffer B solution at 0%, collecting a dulaglutide-containing eluate until a UV absorbance reading of the eluate at A 280 nm is within a range from 0.125 AU/cm to 0.25 AU, and making a back side cut when the UV absorbance reading of the eluate at A 280 nm is within 0.125 AU/cm to 0.25 AU.

32. The method of claim 31, wherein the HIC column comprises a highly cross-linked agarose matrix with phenyl ligands, has a diameter ranging from 50 cm to 200 cm, and has a packed bed height ranging from 15 cm to 30 cm.

33. The method of claim 31 or 32, wherein the HIC column comprises two 0.2 micron filters upstream of the HIC column.

34. The method of any one of claims 31-33, wherein the plurality of compounds in the composition comprises host cell proteins.

35. The method of claim 34, wherein the purification process reduces the concentration of host cell proteins in the composition ranging from 30-70 ppm to about 10 ppm.

36. The method of any of claims 31-35, wherein the concentration of dulaglutide present in the mainstream product after elution from the HIC column ranges from 2.15 g/L to 6.51 g/L.

37. The method of claim 36, wherein the concentration of host cell proteins in the mainstream product after elution from the HIC column ranges from 2 ppm to 11 ppm.

38. Dulaglutide produced by the method of any one of the preceding claims.

39. A composition comprising dulaglutide produced by the method of any one of claims 1-37.