

We claim:

1. A protein drug product comprising:
an Fc fusion protein and an excipient, wherein the protein drug product comprises between 0.05% and 30 0% (w/w) of intermediate high molecular weight protein drug impurities selected from the group consisting of at least one of (i) monomer with extra H2L3 light chains, (ii) monomer with extra H2L4 chains and (iii) monomer with Fab fragment complexes.
2. The protein drug product of claim 1, wherein the Fc fusion protein is a Trap protein.
3. The protein drug product of any one of claims 1-2, wherein the drug product comprises between 0.05% to 25% w/w of intermediate high molecular weight protein drug impurities.
4. The protein drug product of any one of claims 1-2, wherein the drug product comprises between 0.05% to 15% w/w of intermediate high molecular weight protein drug impurities.
5. The protein drug product of any one of claims 1-2, wherein the drug product comprises between 0.05% to 10% w/w of intermediate high molecular weight protein drug impurities.
6. The protein drug product of any one of claims 1-2, wherein the drug product comprises between 0.05% to 5% w/w of intermediate high molecular weight protein drug impurities.
7. A method for characterizing intermediate high molecular weight protein drug product impurities comprising:

deglycosylating a protein drug product sample, wherein the protein is an Fc fusion protein;

separating protein components of the protein drug product sample by native size exclusion chromatography using an aqueous mobile phase,

analyzing the separated protein components by mass spectrometry to characterize intermediate high molecular weight protein drug product impurities in the protein drug product sample, wherein 0.05% and 30 0% (w/w) of intermediate high molecular weight protein drug impurities are selected from the group consisting of at least one of (i) monomer with extra H2L3 light chains, (ii) monomer with extra H2L4 chains and (iii) monomer with Fab fragment complexes.

8. The method of claim 7, wherein the protein drug product sample is from a fed-batch culture.

9. The method of claim 7 or 8, wherein the Fc fusion protein is a Trap protein.

10. A method of producing an antibody, comprising:

culturing cells producing the antibody in a cell culture;

obtaining a sample from the cell culture;

characterizing and quantifying intermediate high molecular weight impurities in the sample according to the method of any one of claims 7-9; and

modifying one or more culture conditions of the cell culture to reduce the amount of characterized low molecular protein drug impurities produced during cell culture of the antibody.

11. The method of claim 10, wherein the one or more conditions of the cell culture that are changed to reduce the amount of intermediate high molecular weight protein drug impurities are selected from the group consisting of pH, cell

density, amino acid concentration, osmolality, growth factor concentration, agitation, gas partial pressure, surfactants, or combinations thereof.

12. The method of claim 10 or 11, wherein the cells are selected from the group consisting of bacterial cells, yeast cells, Chinese Hamster Ovary (CHO) cells (e.g. CHO Kl , DXB-11 CHO, Veggie-CHO), COS cells (e.g COS-7), retinal cells, Vero cells, CV1 cells, kidney cells (e.g. HEK293, 293 EBNA, MSR 293, MDCK, HaK, BHK21), HeLa cells, HepG2 cells, WI38 cells, MRC 5 cells, Colo25 cells, HB 8065 cells, HL-60 cells, lymphocyte cells, e.g. autologous T cells, Jurkat (T lymphocytes) or Daudi (B lymphocytes), A431 (epidermal) cells, U937 cells, 3T3 cells, L cells, C127 cells, SP2/0 cells, NS-0 cells, MMT cells, stem cells, tumor cells, and a cell line derived from any of the aforementioned cells.

13. The method of claim 10 or 11, wherein the cells are hybridoma cells or quadroma cells.

14. The antibody produced by the method of any one of claims 10 to 13.

15. The antibody of claim 14, comprising 0.05 and 30.0% (w/w) of intermediate high molecular weight protein drug impurities.

16. A system for characterizing intermediate high molecular weight drug impurities, comprising:

a native size exclusion chromatography system comprising a size exclusion column linked to a mobile phase column comprising an aqueous mobile phase,

wherein the size exclusion chromatography column is used to separate protein components of a protein drug product sample comprising an Fc fusion protein, wherein the size exclusion column is in fluid communication with a Nano-ESI mass spectrometry system and the mass spectroscopy system is used

to analyze between 0.05% and 30 0% (w/w) of intermediate high molecular weight protein drug impurities selected from the group consisting of at least one of (i) monomer with extra H2L3 light chains, (ii) monomer with extra H2L4 chains and (iii) monomer with Fab fragment complexes.

17. A method for characterizing charge variant drug impurities, comprising:

deglycosylating a protein drug product sample, wherein the protein is an Fc fusion protein;

separating protein components of the protein drug product sample by native strong cation exclusion chromatography using an aqueous mobile phase;

analyzing the separated protein components by Nano-ESI mass spectrometry to characterize charge variant protein drug product impurities in the protein drug product sample, wherein the protein drug product impurities are 0.05% and 30 0% (w/w) of intermediate high molecular weight protein drug impurities selected from the group consisting of at least one of (i) monomer with extra H2L3 light chains, (ii) monomer with extra H2L4 chains and (iii) monomer with Fab fragment complexes.

18. The method of claim 17, wherein the protein drug product sample is from a fed-batch culture.

19. The method of claim 17 or 18, wherein the Fc fusion protein is a Trap protein.

20. The method of any one of claims 7-9 and 17-19, wherein the aqueous mobile phase comprises ammonium acetate and ammonium bicarbonate.