CLAIMS

1. A method for characterizing low molecular weight protein drug product impurities comprising:

i) deglycosylating a protein drug product sample, wherein the protein drug product is selected from an antibody, a fusion protein, recombinant protein, or a combination thereof,

ii) separating protein components of the protein drug product sample by hydrophilic interaction chromatography; and

iii) analyzing the separated protein components by mass spectroscopy to characterize low molecular weight protein drug product impurities in the protein drug product sample, wherein the low molecular weight protein drug product impurities is selected from free light chain, half antibody, H2L, H2, HL, HC, or a combination thereof, and comprises between 0.05% and 30.0% (w/w) of low molecular weight protein drug impurities

2. The method of claim 1, wherein the protein drug product sample is from a fed-batch culture.

3. The method of any one of claims 1 or 2, wherein the method removes N-linked glycans from the protein drug product.

4. The method of any one of claims 1 or 2, wherein the hydrophilic interaction chromatography is performed under denaturing conditions.

5. The method of any one of claims 1 or 2, wherein the method further comprises a step of reducing the protein drug product sample.

6. The method of any one of claims 1 or 2, wherein the protein drug product is an IgG1 antibody.

7. The method of claim 6, wherein the protein drug product is a recombinant IgG1 mAb (mAb-1) antibody.

8. A protein drug product comprising between 0.05% and 30.0% (w/w) of low molecular weight protein drug impurities obtainable by the method of any one of claims 1 or 2.

9. The protein drug product of claim 8, wherein the drug product comprises between 0.05% to 25% w/w of low molecular weight protein drug impurities.

10. The protein drug product of claim 8, wherein the drug product comprises between 0.05% to 15% w/w of low molecular weight protein drug impurities.

11. The protein drug product of any one of claims 8-10, wherein the drug product comprises between 0.05% to 10% w/w of low molecular weight protein drug impurities.

12. The protein drug product of any one of claims 8-10, wherein the drug product comprises between 0.05% to 5% w/w of low molecular weight protein drug impurities.

13. A method of producing an antibody, comprising:

i) culturing cells producing the antibody in a cell culture;

ii) obtaining a sample from the cell culture;

iii) characterizing and quantifying low molecular weight impurities in the sample according to the method of any one of claims 1 or 2, and

iv) 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.

14. The method of claim 13, wherein the one or more conditions of the cell culture that are changed to reduce the amount of low 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.

15. The method of claim 13 or 14, wherein the cells are selected from the group consisting of bacterial cells, yeast cells, Chinese Hamster Ovary (CHO) cells, COS cells, retinal cells, Vero cells, CV1 cells, kidney cells, HeLa cells, HepG2 cells, WI38 cells, MRC 5 cells, Colo25 cells, HB 8065 cells, HL-60 cells, lymphocyte cells, , 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.

16. The method of any one of claims 13-15, wherein the cells are hybridoma cells or quadroma cells.

17. The method of claim 15, wherein the CHO cells are selected from the group of cells consisting of CHO K1, DXB-11 CHO, and Veggie-CHO cells.

18. The method of claim 15, wherein the COS cells are COS-7 cells.

19. The method of claim 15, wherein the kidney cells are selected from the group of cells consisting of HEK293, 293 EBNA, MSR 293, MDCK, HaK, BHK21.

20. The method of claim 15, wherein the lymphocyte cells are selected from the group of cells consisting of autologous T cells, Jurkat (T lymphocytes), and Daudi (B lymphocytes).

21. A system for characterizing low molecular weight drug impurities, comprising:

a hydrophilic interaction liquid chromatography system comprising a hydrophilic interaction liquid chromatography (HILIC) column linked to at least two mobile phase columns, wherein

the HILIC column is in fluid communication with a mass spectroscopy system, wherein: the protein drug product is selected from an antibody, a fusion protein, recombinant protein, or a combination thereof;

the mass spectroscopy system is used to analyse low molecular weight protein drug product impurities in the protein drug product sample; and

the low molecular weight protein drug product impurities are selected from free light chain, half antibody, H2L, H2, HL, HC or a combination thereof and comprises between 0.05% and 30.0% (w/w) of low molecular weight protein drug impurities.

22. Use of the method according to any one of claims 1 or 2 for determination of stability and forced degradation studies of a protein drug product.

23. Use of a system according to claim 17 for determination of stability and forced degradation studies of a protein drug product.