

CLAIMS

1.A method for producing an output mixture of two or more different antibodies having a difference in their amino acid sequences, which results in a difference in the charge properties, hydrophobic properties or affinity of the two or more antibodies, so that they interact differently with a chromatography resin, which difference enables separation of the antibodies by chromatography, wherein

- the two or more different antibodies are present in said output mixture at, a desired or predetermined concentration ratio, and the total amount of antibody in the output mixture is 1 g/L or more; and

- the method comprises the steps of:

a. providing an input mixture wherein the two or more different antibodies are not present at the desired or predetermined concentration ratio;

b. separating the two or more antibodies by chromatography , wherein

I. the conditions of the chromatography step are adjusted so that the total binding capacity for a given antibody under these conditions is adequate to retain the amount of that antibody, which is required in order to provide the output mixture, thereby producing an eluate and a flow-through, or

II. the conditions of the chromatography step are adjusted so that the total binding capacity for a given antibody under these conditions is adequate to retain the amount of each antibody that is in excess to the amount needed to provide the output mixture, thereby producing an eluate and a flow-through;

and

c. recovering the two or more antibodies in the amounts required to provide the output mixture by collecting the eluate and discarding the flow-through produced in i), or discarding the eluate and collecting the flow-through produced in ii) thereby obtaining the output mixture;

wherein when

- the two or more antibodies have a difference in the charge properties, they are separated using an ion exchange resin,

- the two or more antibodies have a difference in hydrophobic properties, they are separated using a hydrophobic interaction resin or a mixed mode resin with a hydrophobic component to the interaction, and

- the two or more antibodies have a difference in affinity, they are separated by use of an affinity resin.

2. The method according to claim 1, wherein the ion exchange resin is selected from a cation exchange resin, an anion exchange resin or a mixed mode resin with an ionic component to the interaction.

5 3. The method according to claim 1, further comprising processing said output mixture to produce a drug substance, wherein the two or more different antibodies are present at, the concentration ratio specified in claim 1.

10 4. The method according to claim 1, further comprising processing said output mixture to produce a drug product, wherein the two or more different antibodies are present at, the concentration ratio specified in claim 1.

15 5. The method according to any of the preceding claims, wherein said output mixture is processed without any additional means or measures for changing or substantially changing the ratio between the concentrations of the antibodies, to produce a drug substance or drug product in which the relative amounts of the two or more antibodies and the ratio between the concentrations of the two or more antibodies are in accordance with an applicable drug product specification.

6. The method according to any of the preceding claims, wherein each binding specificity and/or each antibody charge variant in the input mixture is also found in the output mixture.

7. The method according to any of the preceding claims, wherein step (c) comprises recovering the two or more antibodies in the same pool or fraction, thereby obtaining the output mixture.

20 8. The method according to any of the preceding claims, wherein the least abundant of said two or more different antibodies is present in an amount which is at least 1% (w/w), 2% (w/w), 3% (w/w), 4% (w/w), 5%(w/w), 6% (w/w), 7% (w/w), 8%(w/w), 9% (w/w) or 10% (w/w) of the amount of the most abundant of the said two or more different antibodies.

25 9. The method according to any of the preceding claims, wherein the two or more antibodies are present in such amounts that the ratio (w/w) between the amounts of any two antibodies is between 1:5 and 5:1, such as between 1:4 and 5:1, 1:3 and 5:1, 1:2 and 5:1, 1:1 and 5:1, 2:1 and 5:1 3:1 and 5: 1, 3:4 and 5:1, 1:5 and 4:1, 1:5 and 3:1, 1:5 and 2:1, 1:5 and 1:1, 1:5 and 1:2, 1:5 and 1:3, 1:5 and 1:4, 1:4 and 4:1, 1:4 and 3:1, 1:4 and 2:1, 1:4 and 1:1, 1:4 and 1:2, 1:4 and 1:3, 1:3 and 4:1, 1:3 and 3:1, 1:3 and 2:1,

1:3 and 1:1, 1:3 and 1:2, 1:2 and 4:1, 1:2 and 3:1, 1:2 and 2:1, 1:2 and 1:1, 1:1 and 4:1, 1:1 and 3:1, or such as between 1:1 and 2:1.

10. The method according to any of the preceding claims comprising 2-10 different antibodies.

5 11. The method according to any of the preceding claims, which comprises recovering the two or more antibodies in the amounts required to provide an output mixture, wherein the total amount of antibody is 1.5 g/L or more, such as 2 g/L or more, 3 g/L or more, 4 g/L or more, 5 g/L or more, 7 g/L or more, 8 g/L or more, 9 g/L or more or such as 10 g/L or more.

10 12. The method according to any of the preceding claims, which comprises recovering the two or more antibodies in the amounts required to provide an output mixture wherein the total amount of antibody is 1-20 g/L, such as 1.5-20 g/L, 2-20 g/L, 3-20 g/L, 4-20 g/L, 5-20 g/L, 7-20 g/L, 8-20 g/L, 9-20 g/L, or such as 10-20 g/L.

13. The method according to any of the preceding claims, wherein at least one of said two or more antibodies is an antibody binding an antigen expressed on the surface of a tumor, such as a metastatic, solid tumor or such as a metastatic, locally advanced tumor, or such as a hematologic tumor.

15 14. The method according to any of the preceding claims, wherein at least one of said two or more antibodies is an antibody binding an antigen associated with or expressed during an immune or autoimmune disease, an inflammatory disease, a cardiovascular disease, a disease in the central nervous system (CNS) or a musculo-skeletal disease.

20 15. The method according to any of the above claims, wherein the separation of the two or more antibodies is done by a single chromatography step using a single chromatography resin.

16. The method according to claim 15, wherein the single chromatography resin is a preparative chromatography resin.

17. The method according to any of the preceding claims, wherein the separation of the two or more antibodies is done by use of a mixture of chromatography resins at a predetermined ratio.

25 18. The method according to any of the preceding claims, wherein the method comprises an initial step of determining the separability of the two or more antibodies by chromatography and where the different antibodies are inseparable then modifying the amino acid sequence of one or more of the antibodies to obtain separability by chromatography.

19. The method according to claim 18, wherein the modification of the amino acid sequence of one or more of the antibodies is selected from: amino acid substitutions, additions or deletions in one or more of the antibodies or a combination hereof.

20. The method according to claim 19, wherein the modification comprises one or more amino acid substitutions in the heavy chain variable region and/or in the light chain variable region of one or more of the antibodies wherein the substitution is at a position selected from the group comprising: 1, 6, 17, 24, 48, 75, 90, 93, 96, 97 in the heavy chain variable region and/or from the group comprising: 1, 4, 47, 48, 51, 68, 74, 80, 90, 93, and 95 in the light chain variable region, wherein the numbering is according to the IMGT numbering of IgG1 variable regions.

21. The method according to claim 20, wherein the one or more substitutions introduce an amino acid which has a different charge than the wild type amino acid at the corresponding position.

22. The method according to any of claims 20-21, wherein the one or more amino acid substitutions comprises an E345K substitution in the heavy chain constant region using the EU numbering system.

23. The method according to any of claims 18-21, wherein modifying the one or more antibodies comprises introducing at least one amino acid substitution in a kappa light chain constant region of one or more of the antibodies wherein the substitution eliminates binding to an affinity resin and wherein the substitution is selected from the group comprising V110D, V110R, V110E, V110H, V110K, V110N, V110P, V110Q, V110W and E143D using the EU numbering system and wherein the chromatography uses the affinity resin for which the substitution eliminates binding.

24. The method according to any of claims 18-19, wherein modifying the one or more antibodies comprises introducing at least one amino acid substitution which is an S12P substitution in the light chain variable region when using IMGT for numbering, wherein the substitution eliminates binding to an affinity resin and wherein the chromatography uses the affinity resin for which the substitution eliminates binding.

25. The method according to any of claims 18-22, wherein modifying the one or more antibodies comprises introducing at least one amino acid substitution in said one or more of the antibodies wherein the substitution is in the CH1 domain and comprises an S157T and/or a T164S mutation using the EU numbering system and wherein the substitution eliminates binding to an affinity resin and wherein the chromatography uses the affinity resin for which the substitution eliminates binding.

26. The method according to any of claims 18-24, wherein modifying the one or more antibodies comprises introducing at least one amino acid substitution in the heavy chain constant region of said one or more antibodies wherein the substitution is selected from the group comprising M252A, S254M, E380A, E380M, E382A, E382L, S426M, M428G, M428T, M428V, H433D, N434A, N434G, N434S, M428A using the EU numbering system and wherein the substitution eliminates binding to an affinity resin and wherein the chromatography uses the affinity resin for which the substitution eliminates binding.
27. The method according to any of the preceding claims, wherein the process leads to reproducible results between different batches of the output mixture, such that the two or more different antibodies are present at, the desired or predetermined concentration ratio.
28. The method according to any of the preceding claims, wherein the two or more different antibodies are selected from the group comprising IgG1, IgG2, IgG3 or IgG4 antibodies or a combination thereof.
29. The method according to any of the preceding claims, wherein at least one of said two or more different antibodies is a monoclonal antibody.
30. The method according to any of the preceding claims, wherein all of said two or more different antibodies are monoclonal antibodies.
31. The method according to any of the preceding claims, wherein at least one of the two or more different antibodies is specific for a target on a tumor cells, such as a target selected from the group consisting of erbB1 (EGFR), erbB2 (HER2), erbB3, erbB4, MUC-I, CD19, CD20, CD4, CD38, CD138, CXCR5, c-Met, HERV-envelop protein, periostin, Bigh3, SPARC, BCR, CD79, CD37, EGFrvIII, U-CAM, AXL, Tissue Factor (TF), CD74, EpCAM and MRP3.
32. The method according to any of claims, wherein at least one of the two or more different antibodies is specific for a target on an effector cell, such as, CD1, CD3, CD4, CD8, FcgammaRIII (CD16), CD25, CD89, CD32, CD32a, FCeRI, CD40, or FcgammaRI (CD64).