

CLAIMS

1. A method for producing an output mixture of two or more different antibodies having a difference in their amino acid sequences, which difference enables separation of the antibodies by chromatography, wherein
- the two or more different antibodies are present in said output mixture at, ~~or essentially at,~~ a desired or predetermined concentration ratio; and
 - the method comprises the steps of:
 - a. providing an input mixture wherein the two or more different antibodies are not present at, ~~or essentially at,~~ the desired or predetermined concentration ratio;
 - b. separating the two or more antibodies by chromatography using an action principle selected from the group consisting of adsorption chromatography, ion exchange chromatography, size exclusion chromatography, affinity chromatography, hydrophobic interaction chromatography, metal chelate chromatography, normal phase chromatography, reversed phased chromatography, or mixed mode chromatography;
 - c. recovering the two or more antibodies in the amounts required to provide the output mixture, wherein
 - i) the chromatography has produced an eluate and a flow-through and the two or more antibodies are recovered in the same pool or fraction by
 - I. collecting the eluate and discarding the flow-through when the conditions of the chromatography have been adjusted so that the total binding capacity for a given antibody under these conditions is adequate to retain the amount of that antibody required to provide the output mixture, or
 - II. discarding the eluate and collecting the flow-through when the conditions of the chromatography have been adjusted so that the total binding capacity for a give 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,
 - ii) the two or more antibodies are recovered in multiple pools or fractions, and said multiple pools or fractions or parts of said multiple pools or fractions are combined,
- thereby obtaining the output mixture.

2. 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, ~~or essentially at,~~ the concentration ratio specified in claim 1.

3. 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, ~~or essentially at,~~ the concentration ratio specified in claim 1.

4. 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.

5. 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.

6. The method according to any of claims 1 to 4, wherein step (c) comprises recovering the two or more antibodies in multiple pools or fractions, and combining said multiple pools or fractions or parts of said multiple pools or fractions, thereby obtaining the output mixture.

~~7. The method according to any one of claims 1-3, wherein the chromatography in step (b) produces an eluate and a flow-through and the output mixture is produced by:~~

- ~~i) Collecting the eluate and discarding the flow-through; or~~
- ~~ii) Discarding the eluate and collecting the flow-through.~~

~~8. The method according to claim 7, wherein step (b) comprises adjusting the conditions of the chromatography step 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.~~

~~9. The method according to claim 7, wherein step (b) comprises adjusting the conditions of the chromatography step 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.~~

~~10.~~ 7. 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.

~~11-8.~~_____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.

~~12-9.~~_____The method according to any of the preceding claims comprising 2-10 different antibodies.

~~13-10.~~_____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.

~~14-11.~~_____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.

~~15-12.~~_____The method according to any of the preceding claims, wherein the difference in the amino acid sequence of said two or more different antibodies results in a difference in the charge properties of the two or more antibodies so that the two or more antibodies interact differently with a chromatography resin.

~~16-13.~~_____The method according to any of claims 1-~~12~~15, wherein the difference in the amino acid sequence of said two or more different antibodies results in a difference in the hydrophobic properties of the two or more antibodies so that the two or more antibodies interact differently with a chromatography resin.

~~17-14.~~_____The method according to any of the preceding claims, wherein the difference in the amino acid sequence of said two or more different antibodies results in a difference in affinity for a chromatography resin.

~~18.~~15._____The method according to any of the preceding claims, wherein the chromatography resin is selected from the group comprising: affinity resin, ion exchange resin, hydrophobic interaction resin or mixed mode resin.

~~19.~~_____The method according to any of the preceding claims, wherein the method comprises the separation of the two or more antibodies and depletion of excess of one or more of the antibodies to recover the predetermined ratio of the two or more different antibodies.

~~20.~~16._____The method according to any of the preceding claims, wherein the two or more different antibodies of the output mixture are recovered in a single pool in step c.

~~21.~~17._____The method according to any of the preceding claims, wherein the two or more antibodies in step b are separated into different fractions and wherein the fractions which contain one of the antibodies at a purity of at least 80% are subsequently pooled at the predetermined concentration ratio of the different antibodies to recover the output mixture.

~~22.~~18._____The method according to any of the preceding claims, comprising
i) separating in step (b) the two or more antibodies into different fractions, and selecting for each antibody one or more fractions containing that antibody at a purity of at least 80%; and
ii) providing said output mixture by pooling volumes of the selected fractions, the size of the volumes being adjusted to provide the predetermined concentration ratio of said two or more antibodies.

~~23.~~19._____The method according to any of the preceding claims comprising a further step of determining the concentration of the antibodies in each fraction prior to the pooling of the antibodies.

~~24.~~20._____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.

~~25.~~21._____The method according to claim 20~~24~~, wherein the single chromatography resin is a preparative chromatography resin.

~~26.~~22._____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.

~~27.~~23._____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.

5 ~~28-24.~~_____The method according to claim ~~2327~~, 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.

10 ~~29-25.~~_____The method according to claim ~~2428~~, 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.

15 ~~30-26.~~_____The method according to claim ~~2529~~, 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.

15 ~~31-27.~~_____The method according to any of claims ~~2529-2630~~, wherein the one or more amino acid substitutions comprises an E345K substitution in the heavy chain constant region using the EU numbering system.

20 ~~32-28.~~_____The method according to any of claims ~~2327-2731~~, 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.

25 ~~33-29.~~_____The method according to any of claims ~~2327-2833~~, 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.

30 ~~34-30.~~_____The method according to any of claims ~~2327-2833~~, 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.

35-31. The method according to any of claims ~~2327-3034~~, 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.

36-32. 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, ~~or essentially at,~~ the desired or predetermined concentration ratio.

37-33. 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.

38-34. The method according to any of the preceding claims, wherein at least one of said two or more different antibodies is a monoclonal antibody.

39-35. The method according to any of the preceding claims, wherein all of said two or more different antibodies are monoclonal antibodies.

40-36. 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.

41-37. 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, FcεRI, CD40, or FcgammaRI (CD64).

42. ~~A mixture of two or more different antibodies, said mixture being obtainable by the method of any of the above claims.~~

43. ~~The mixture according to claim 42, wherein the two or more different antibodies are present at, or essentially at, a desired or predetermined concentration ratio.~~

44. ~~A mixture of two or more different antibodies having a predetermined ratio of the two or more different antibodies, which antibodies have a difference in size, charge, hydrophobicity or affinity for a chromatography resin.~~

45. ~~The mixture according to any of claims 42-44, wherein the least abundant of said two or more different antibodies is present in an amount which is at least 1% (w/w) of the amount of the most abundant of the said two or more different antibodies.~~

46. ~~The mixture according to any one of claims 42-45, 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.~~

47. ~~The mixture according to any one of claims 42-46, wherein each of said two or more different antibodies is an active pharmaceutical ingredient.~~

48. ~~The mixture according to any one of claims 42-47 comprising 2-10 different antibodies.~~

49. ~~The mixture according to any of claims 42-48, wherein at least one of said two or more different antibodies is a monoclonal antibody.~~

50. ~~The mixture according to any of claims 42-49, wherein all of said two or more different antibodies are monoclonal antibodies.~~

51. ~~The mixture according to any of claims 42-50, wherein at least one of said one or more antibodies is a bispecific or multispecific antibody.~~

52. ~~The mixture of two or more different antibodies according to claim 42, wherein at least one of the antibodies comprise at least one amino acid substitution in the heavy chain variable region and/or in the light chain variable region wherein the substitution is at one or more positions 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 IgG variable regions.~~

~~53. A pharmaceutical composition comprising the mixture of any of claims 42-52 as an active ingredient, such as an active pharmaceutical agent.~~

~~54. The pharmaceutical composition according to claim 53, which is sterile and having one or more of the following characteristics:~~

~~A physiologically acceptable pH, such as a pH, which is between 5 and 8;~~

~~An osmolality, which is 600 mOsm/kg or lower; and~~

~~A level of aggregates, which is such that 10% by weight or less of the antibodies in the composition are present in the form of aggregates.~~

~~55. The pharmaceutical composition according to claim 53 or 54, which is isotonic or substantially isotonic, such as a composition having an osmolality, which is from 290-300 mOsm/kg, such as 295 mOsm/kg.~~

~~56. Use of a mixture of two or more different antibodies according to any of claims 42-52 for the manufacture of a medicament for the treatment of a disease.~~

~~57. The use according to claim 56, wherein the disease is cancer, a tumor, an immune or autoimmune disease, an inflammatory disease, a cardiovascular disease, a disease in the central nervous system (CNS), a musculo-skeletal diseases or an infectious disease~~