

WHAT IS CLAIMED IS

1. A method for quantifying an amount and purity of a heterodimeric antibody in a sample comprising a mixture of the heterodimeric antibody, a first homodimeric antibody impurity, and a second homodimeric antibody impurity,

wherein the heterodimeric antibody and the first homodimeric antibody impurity bind to a first affinity matrix, the second homodimeric antibody impurity does not substantially bind to the first affinity matrix and binds to a second affinity matrix, and the heterodimeric antibody has a lower affinity to the first affinity matrix than does the first homodimeric antibody impurity,

wherein the heterodimeric antibody is a bispecific antibody comprising one unsubstituted CH3 domain and one H435R/Y436F-substituted CH3 domain (FcFc* antibody), the first homodimeric antibody impurity is a monospecific antibody comprising two unsubstituted CH3 domains (FcFc antibody), and the second homodimeric antibody impurity is a monospecific antibody comprising two H435R/Y436F-substituted CH3 domains (Fc*Fc* antibody), the first affinity matrix comprises protein A (protein A affinity matrix) and the second affinity matrix comprises protein G (protein G affinity matrix), the unsubstituted CH3 domain binds to both protein A and protein G, the H435R/Y436F-substituted CH3 domain binds to protein G but does not substantially bind to protein A, Fc denotes a heavy chain having the unsubstituted CH3 domain, and Fc* denotes a heavy chain having the H435R/Y436F-substituted CH3 domain,

said method comprising the steps of:

a. applying the sample to the protein A affinity matrix in a chromatography system under conditions wherein the FcFc* antibody and the FcFc antibody bind to the protein A affinity matrix, wherein the chromatography system comprises the protein A affinity matrix, the protein G affinity matrix, a switch valve, and a detector, and the protein A affinity matrix is serially connected to the protein G affinity matrix via the switch valve;

b. washing the protein A affinity matrix under a first set of conditions with the switch valve in a position so that flow-through is directly applied onto the protein G affinity matrix and the Fc*Fc* antibody in the flow-through binds to the protein G affinity matrix;

c. switching the switch valve to disconnect the protein A affinity matrix from the protein G affinity matrix and to connect the protein A affinity matrix directly to the detector, then eluting the FcFc* antibody from the protein A affinity matrix under a second set of conditions while the FcFc

antibody is retained on the protein A affinity matrix, and determining the amount of the eluted FcFc* antibody;

d. eluting the retained FcFc antibody from the protein A affinity matrix through the detector under a third set of conditions and determining the amount of the eluted FcFc antibody;

e. switching the switch valve back to serially connect the protein A affinity matrix to the protein G affinity matrix and the protein G affinity matrix to the detector, then eluting the Fc*Fc* antibody from the protein G affinity matrix through the detector under the third set of conditions, and determining the amount of the eluted Fc*Fc* antibody, and

f. quantifying the amount of the heterodimeric antibody in the sample as in step (c) and the purity of the heterodimeric antibody in the sample, wherein the purity of the heterodimeric antibody is determined by the ratio of the amount of the FcFc* antibody to the sum of the amounts of the FcFc antibody, the FcFc* antibody, and the Fc*Fc* antibody.

2. The method of claim 1, wherein the first set of conditions comprises a first pH, the second set of conditions comprises a second pH, and the third set of conditions comprises a third pH.

3. The method of claim 2, wherein the second pH is lower than the first pH, and the third pH is lower than the second pH.

4. The method of claim 2 or 3, wherein the first pH is from pH 5.0 to pH 7.4, the second pH is from pH 4.3 to pH 5.6, and the third pH is from pH 2.0 to pH 2.8.

5. The method of any one of claims 1 to 4, wherein the first set of conditions, the second set of conditions, and the third set of conditions comprise a mobile phase modifier, and the mobile phase modifier is a salt buffer selected from LiCl, NaCl, KCl, MgCl₂, and CaCl₂ buffers.

6. A chromatography system comprising a first affinity matrix, a second affinity matrix, and a detector for quantifying an amount and purity of a heterodimeric antibody in a sample comprising a mixture of the heterodimeric antibody, a first homodimeric antibody impurity, and a second homodimeric antibody impurity,

wherein the first affinity matrix is serially connected to the second affinity matrix via a switch valve,

wherein the heterodimeric antibody and the first homodimeric antibody impurity bind to the first affinity matrix and the second homodimeric antibody impurity does not substantially bind to the first affinity matrix and binds to the second affinity matrix,

wherein the heterodimeric antibody has a lower affinity to the first affinity matrix than does the first homodimeric antibody impurity,

wherein the heterodimeric antibody is a bispecific antibody comprising one unsubstituted CH3 domain and one H435R/Y436F-substituted CH3 domain (FcFc* antibody), the first homodimeric antibody impurity is a monospecific antibody comprising two unsubstituted CH3 domains (FcFc antibody) and the second homodimeric antibody impurity is a monospecific antibody comprising two H435R/Y436F-substituted CH3 domains (Fc*Fc* antibody),

wherein the first affinity matrix comprises protein A (protein A affinity matrix) and the second affinity matrix comprises protein G (protein G affinity matrix),

wherein the unsubstituted CH3 domain binds to both protein A and protein G, the H435R/Y436F-substituted CH3 domain binds to protein G but does not substantially bind to protein A,

wherein Fc denotes a heavy chain having the unsubstituted CH3 domain and Fc* denotes a heavy chain having the H435R/Y436F substituted CH3 domain, and each of the protein A affinity matrix, the protein G affinity matrix, and the detector are connected via the switch valve.

7. The chromatography system of claim 6, wherein the detector comprises an HPLC column equipped with a UV detector, a charge aerosol detector, and/or a mass-spectrometer.