

What is claimed is:

1. A method for identification of a site of fragmentation of an antibody, said method comprising:

contacting a sample including at least one fragment of an antibody to a chromatographic system having a mixed-mode size-exclusion chromatography resin with an additional functionality;

selecting a concentration of salt for a mobile phase on the basis of separating the at least one fragment of the antibody by charge and/or hydrophobicity;

washing said mixed-mode size-exclusion chromatography resin using said mobile phase to provide an eluent with the at least one fragment of the antibody;

determining molecular weight data of the at least one fragment of the antibody in said eluent using a mass spectrometer coupled to said chromatographic system; and

correlating the molecular weight data of the at least one fragment of the antibody to data obtained from at least one known protein standard to identify the site of fragmentation of the antibody.

2. The method of claim 1, wherein the mobile phase has ammonium acetate, ammonium bicarbonate, or ammonium formate, or combinations thereof.
3. The method of claim 1, wherein the mobile phase has a total concentration of less than about 600 mM of ammonium acetate and ammonium bicarbonate.
4. The method of claim 1, wherein the mobile phase has a flow rate of from 0.2 mL/min to 0.4 mL/min.
5. The method of claim 1, wherein the amount of the sample loaded onto the mixed-mode size-exclusion chromatography resin is from 10 µg to 100 µg.
6. The method of claim 1, wherein the antibody is a monoclonal antibody.

7. The method of claim 1, wherein the antibody is a bispecific antibody.
8. The method of claim 1, wherein the antibody is a therapeutic antibody.
9. The method of claim 1, wherein the site of fragmentation of the antibody is in the hinge region of the antibody.
10. The method of claim 1, wherein the site of fragmentation of the antibody is in a constant immunoglobulin domain.
11. The method of claim 1, wherein the site of fragmentation of the antibody is in a variable domain of the antibody.
12. The method of claim 1, wherein the additional functionality is a charge-charge interaction functionality.
13. The method of claim 1, wherein the additional functionality is a hydrophobic interaction functionality.
14. The method of claim 1, wherein the at least one fragment is a F(ab)₂ fragment.
15. The method of claim 1, wherein the at least one fragment is a Fc fragment.
16. A method for identification of a fragment of an antibody, said method comprising:

contacting a sample including the fragment of the antibody to a chromatographic system having a mixed-mode size-exclusion chromatography resin with hydrophobic functionality;

selecting a concentration of salt for a mobile phase on the basis of separating the fragment of the antibody by hydrophobicity;

washing said mixed-mode size-exclusion chromatography resin using said mobile phase to provide an eluent with the fragment of the antibody;

determining molecular weight data of the fragment of the antibody in said eluent using a mass spectrometer coupled to said chromatographic system; and

correlating the molecular weight data of the fragment of the antibody to data obtained from at least one known protein standard to identify the fragment.

17. A method for quantification of a fragment of an antibody, said method comprising:

contacting a sample including the fragment of the antibody to a chromatographic system having a mixed-mode size-exclusion chromatography resin with hydrophobic functionality;

selecting a concentration of salt for a mobile phase on the basis of separating the fragment of the antibody by hydrophobicity;

washing said mixed-mode size-exclusion chromatography resin using a mobile phase to provide an eluent with the fragment of the antibody; and

quantifying the amount of the fragment of the antibody using a mass spectrometer coupled to said chromatographic system.