

We claim:

1. A method for identifying scrambled disulfide bonds in a protein drug product, comprising:

preparing peptide standards comprising regions of the protein drug product containing one or more disulfide bonds, wherein a first peptide standard comprises a first scrambled disulfide bond, and a second standard comprises a second and different scrambled disulfide bond, and wherein the first and second peptide standards have different, known liquid chromatography retention times;

digesting a sample of protein drug product into peptides,

analyzing a sample containing the protein drug product peptides and the peptide standards using an LC-MS² system, wherein peptides detected at the retention time of the first standard indicate the presence of scrambled disulfide bond of the first peptide standard is present in the protein drug product, and peptides detected at the retention time of the second peptide standard retention time indicates the presence of the scrambled disulfide bonds of the second peptide standard are present in the protein drug product.

2. The method of claim 1, wherein the scrambled disulfide bonds are selected from the group consisting of crossed disulfide bonds, crisscrossed disulfide bonds, and intra-chain disulfide bonds.

3. The method of claims 1 or 2, wherein the method includes peptide standard containing a parallel disulfide bond.

4. The method of any one of claims 1-3, wherein the disulfide bonds in the peptide standards are formed through an inducing the oxidation reaction that comprises oxidation with Cu²⁺.

5. The method of claim 4, wherein the molar ratio of peptide:Cu²⁺ for formation of disulfide bonds is 5:1.

6. The method of claim 1, wherein digesting the sample comprises tryptic digestion or dual-enzyme digestion.

7. The method of any one of claims 1-6, wherein the protein drug product comprises an antibody or an antigen binding fragment thereof, a recombinant protein, a fusion protein, or a combination thereof.

8. The method of any one of claims 1-7, wherein the disulfide bond is in the hinge region of an antibody.

9. A method of producing a protein drug product, comprising:

producing the protein drug product in a cell culture, wherein regions of the protein drug product are comprised of peptide standards, wherein at least one of the peptide standards comprises scrambled disulfide bonds;

identifying the scrambled disulfide bonds of the protein drug product according to the method of any one of claims 1-8, wherein at least one of the scrambled disulfide bonds are crossed disulfide bonds, and

modifying one or more cell culture, purification or excipient conditions to reduce the amount of crossed hinge disulfide bonds of the protein drug product to less than 1.0%.

10. The method of claim 9, wherein the one or more cell culture, purification or excipient conditions that are modified are selected from the group consisting of temperature, pH, oxygen levels, reactive oxygen species, surfactants, or combinations thereof.

11. The method of any one of claims 9-10, wherein the protein drug product is selected from the group consisting of an antibody, a fusion protein, recombinant protein, or a combination thereof.

12. ~~A pharmaceutical composition comprising monoclonal antibodies having~~ The method of any one of claims 9-11, wherein the protein drug product has less than 30% scrambled disulfide bonds.

13. The ~~method~~composition of claim 12, wherein the protein drug product is monoclonal antibody and wherein the disulfide bonds are in the hinge region of the antibody.