We claim:

1. A method for identifying free thiols in a protein drug product, comprising:

labeling a sample comprising a protein drug product with a first label comprising a sulfhydryl-reactive iodoacetyl group, an MS-neutral spacer arm, and a first MS/MS reporter having a unique reporter ion mass;

removing excess first label;

denaturing and reducing the sample;

labeling the sample with a second label comprising a sulfhydryl-reactive iodoacetyl group, an MS-neutral spacer arm, and a second MS/MS reporter having a unique reporter ion mass that is different from the first label;

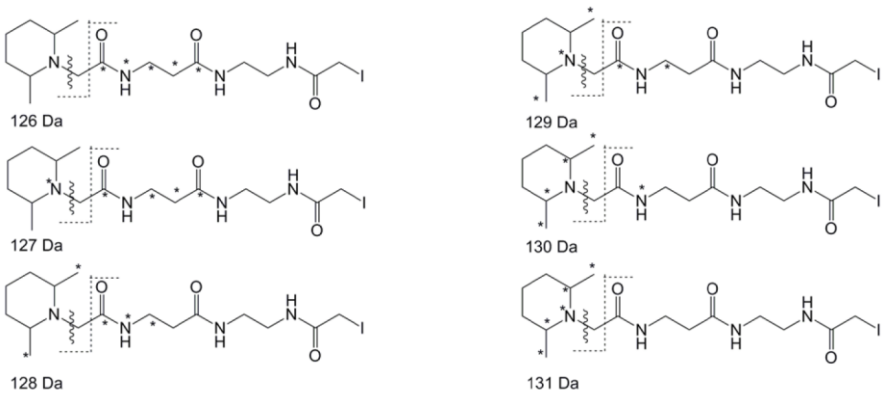
enzymatically digesting the sample;

analyzing the sample using an UPLC-MS^2 system comprising an ethylene bridged hybrid column or a charged surface hybrid column, and a formic acid buffer mobile phase; and

quantifying the first MS/MS reporter and the second MS/MS reporter, wherein the amount of the first MS/MS reporter correlates to the amount of free thiols in the protein drug product and the amount of the second MS/MS reporter correlates to the amount of bound thiols in the protein drug product, wherein the protein drug product has less than 5 free thiols and does not show a potential for disulfide bond breakage.

2. The method of claim 1, wherein the first MS/MS reporter has a mass of 128 Da and the second MS/MS reporter has a mass of 131 Da.

3. The method of claim 1, wherein the first and second labels are selected from the group consisting of



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4. The method of any one of claims 1-3, wherein the method provides complete coverage of all 32 cysteine residues in an IgG molecule.

5. The method of any one of claims 1-3, wherein the method covers the 16 cysteine residues on the heavy and light chains in an IgG molecule.

6. The method of any one of claims 1-3, wherein the method covers the 5 cysteine residues on each light chain of an IgG molecule.

7. The method of any one of claims 1-3, wherein the method covers the 11 cysteine residues on each heavy chain of an IgG molecule.

8. A method of identifying disulfide heterogeneity in a protein drug product comprising the steps of:

labeling a sample comprising a protein drug product with a first label comprising a sulfhydryl-reactive iodoacetyl group, an MS-neutral spacer arm, and a first MS/MS reporter having a unique reporter ion mass;

removing excess first label;

denaturing and reducing the sample;

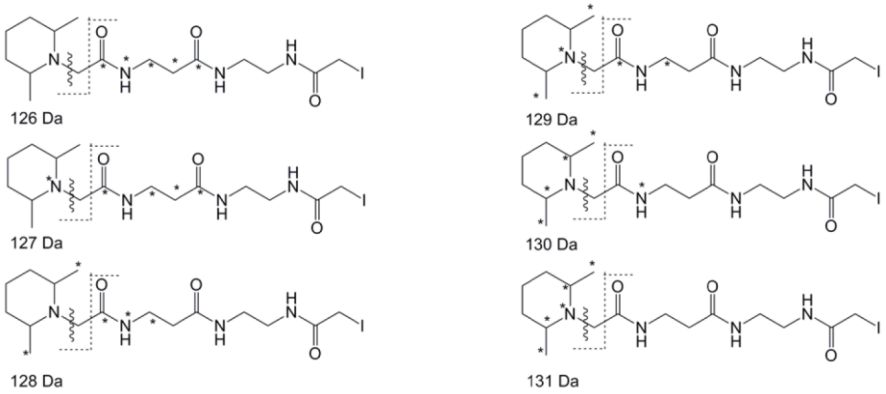
labeling the sample with a second label comprising a sulfhydryl-reactive iodoacetyl group, an MS-neutral spacer arm, and a second MS/MS reporter having a unique reporter ion mass that is different from the first label;

enzymatically digesting the sample;

analyzing the sample using an UPLC-MS^2 system comprising an ethylene bridged hybrid column or a charged surface hybrid column, and a formic acid buffer mobile phase; and

quantifying the first MS/MS reporter and the second MS/MS reporter, wherein the amount of the first MS/MS reporter correlates to the amount of free thiols in the protein drug product and the amount of the second MS/MS reporter correlates to the amount of bound thiols in the protein drug product, wherein the detection of a free thiol indicates the protein drug product has disulfide heterogeneity, wherein the protein drug product has less than 5 free thiols and does not show a potential for disulfide bond breakage.

9. The method of claim 8, wherein the first and second labels are selected from the group consisting of



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10. The method of any one of claims 8 or 9, wherein the method provides complete coverage of all 32 cysteine residues in an IgG molecule.

11. The method of any one of claims 8-10, wherein the method covers the 16 cysteine residues on the heavy and light chains in an IgG molecule.

12. The method of any one of claims 8-10, wherein the method covers the 5 cysteine residues on each light chain of an IgG molecule.

13. The method of any one of claims 8-10, wherein the method covers the 11 cysteine residues on each heavy chain of an IgG molecule.

14. A method for selecting a protein drug product comprising the steps of:

labeling a sample comprising a protein drug product with a first label comprising a sulfhydryl-reactive iodoacetyl group, an MS-neutral spacer arm, and a first MS/MS reporter having a unique reporter ion mass;

removing excess first label;

denaturing and reducing the sample;

labeling the sample with a second label comprising a sulfhydryl-reactive iodoacetyl group, an MS-neutral spacer arm, and a second MS/MS reporter having a unique reporter ion mass that is different from the first label;

enzymatically digesting the sample;

analyzing the sample using an UPLC-MS^2 system comprising an ethylene bridged hybrid column or a charged surface hybrid column, and a formic acid buffer mobile phase;

quantifying the first MS/MS reporter and the second MS/MS reporter, wherein the amount of the first MS/MS reporter correlates to the amount of free thiols in the protein drug product and the amount of the second MS/MS reporter correlates to the amount of bound thiols in the protein drug product, wherein the protein drug product has less than 5 free thiols and does not show a potential for disulfide bond breakage; and

selecting the protein product if it has less than 5 free thiols or no free thiols.

15. The method of claim 14, wherein the selected protein drug product contains less than 5 free thiols, less than 4 free thiols, or less than 3 free thiols in an IgG molecule.

16. The method of claim 15, wherein the free thiol is in a light chain of an IgG molecule.

17. The method of claim 15, wherein the free thiol is in a heavy chain of an IgG molecule.

18. The method of claim 15, wherein at least one free thiol is in a light chain and at least one free thiol is in a heavy chain of an IgG molecule.

19. A pharmaceutical composition comprising the protein product selected in any one of claims 14-18.

20. The method of claim 1, wherein the sample is analyzed for 90 minutes to 150 minutes.

21. The method of claim 1 further comprising equilibrating the sample.

22. The method of claim 21, wherein the sample is equilibrated for 40 minutes.

23. The method of claim 1, wherein the first MS/MS reporter has a unique mass of 126 Da, 127 Da, 128 Da, 129 Da, 130 Da or 131 Da, and the second MS/MS reporter has a unique mass of 126 Da, 127 Da, 128 Da, 129 Da, 130 Da, or 131 Da, wherein the first and second MS/MS reporters can be differentiated on MS/MS spectra.