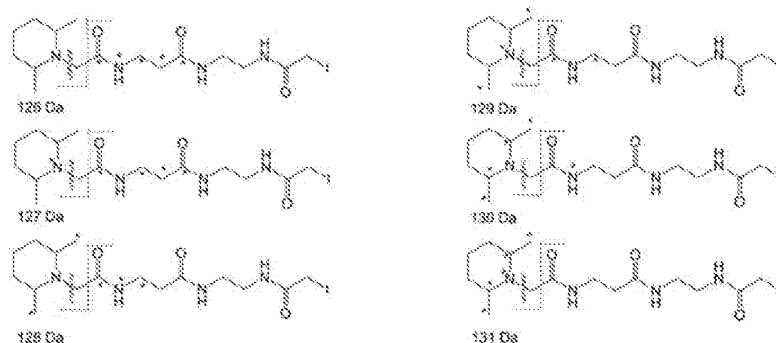


We claim:

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;
 - enzymatically digesting the sample;
 - analyzing the sample using an UPLC-MS² system comprising 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.
2. The method of claim 1, wherein the first MS/MS reporter has a mass of 128 and the second MS/MS reporter has a mass of 131.

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

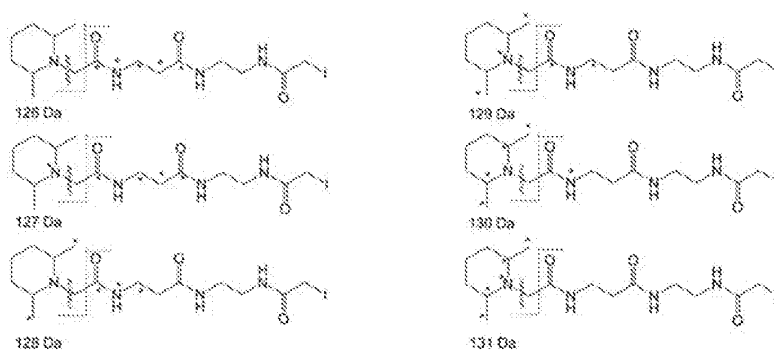


4. The method of any one of claims 1-3, wherein the method provides complete coverage of all 32 cysteine residues in an IgG molecule.
5. The method of any one of claims 1-3, wherein the method covers the 16 cysteine residues on the heavy and light chains in an IgG molecule.
6. The method of any one of 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. The method of any one of claims 1-3, wherein a threshold of 5-6% free thiols was set, and wherein a result below the threshold does not show a potential for disulfide bond breakage.
9. 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;
 enzymatically digesting the sample;
 analyzing the sample using an UPLC-MS² system comprising 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. .

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



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

12. The method of any one of claims 9-11, wherein the method covers the 16 cysteine residues on the heavy and light chains in an IgG molecule.

13. The method of any one of claims 9-11, wherein the method covers the 5 cysteine residues on each light chain of an IgG molecule.
14. The method of any one of claims 9-11, wherein the method covers the 11 cysteine residues on each heavy chain of an IgG molecule.
15. 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;
enzymatically digesting the sample;
analyzing the sample using an UPLC-MS² system comprising 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; and
selecting the protein product if it has little or no free thiols.
16. The method of claim 15, wherein the selected protein drug product contains less than 5 free thiols, less than 4 free thiols, less than 3 free thiols, or less than 2 free thiols in an IgG molecule.
17. The method of claim 16, wherein the free thiol is in a light chain of an IgG molecule.
18. The method of claim 16, wherein the free thiol is in a heavy chain of an IgG molecule.

19. The method of claim 16, 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.
20. A pharmaceutical composition comprising the protein product selected in any one of claims 15-20.