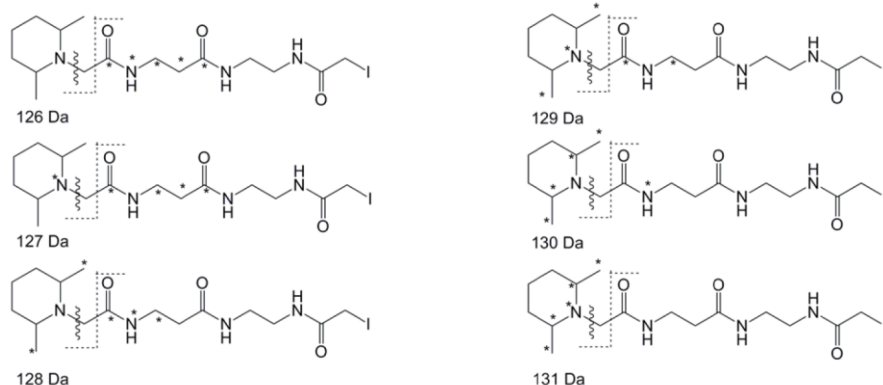


We claim:

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, 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.
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. 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;

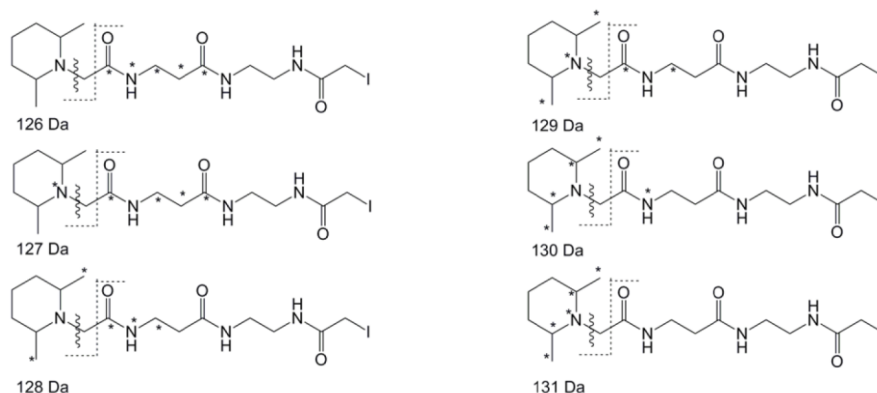
labeling the sample with a second label comprising a sulfhydryl-reactive iodoacetyl group, an MS-neutral spacer arm, and a second MS/MS reporter having a unique reporter ion mass;

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



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;
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, 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; and
selecting the protein product if it has little 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 14-19.
20. The method of claim 1, wherein the UPLC-MS² system comprises an ethylene bridged hybrid (BEH) column and a formic acid buffer mobile phase.
21. The method of claim 1, wherein the sample is analyzed for about 90 minutes to about 150 minutes.
22. The method of claim 1 further comprising equilibrating the sample.
23. The method of claim 22, wherein the sample is equilibrated for 40 minutes.