

FIG. 1

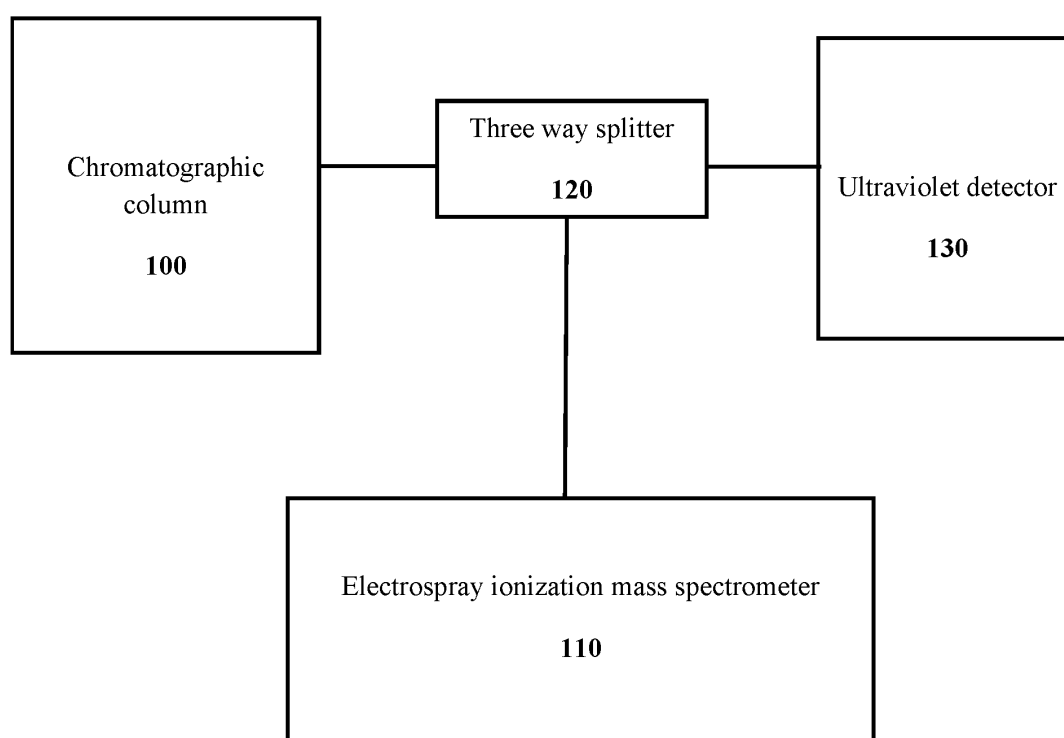


FIG. 2

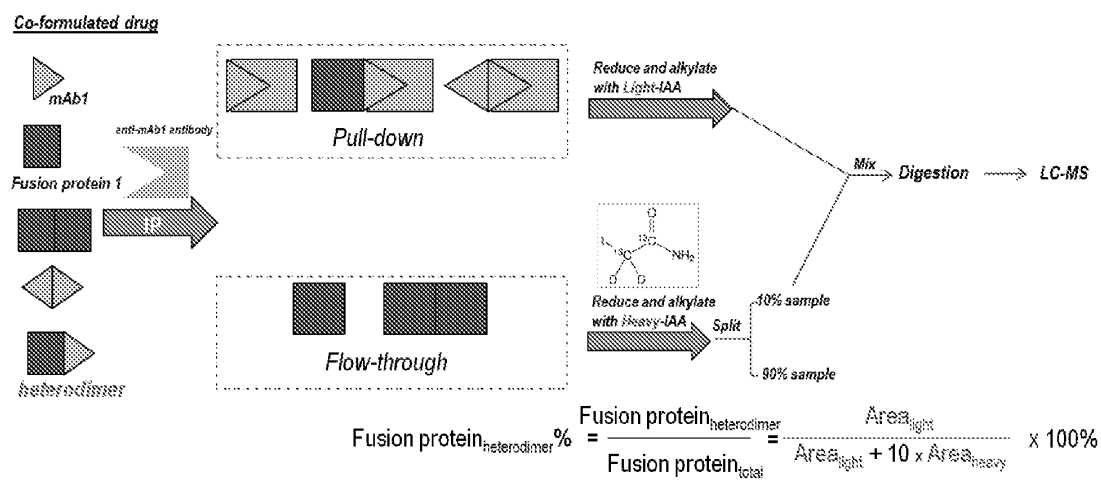


FIG. 3

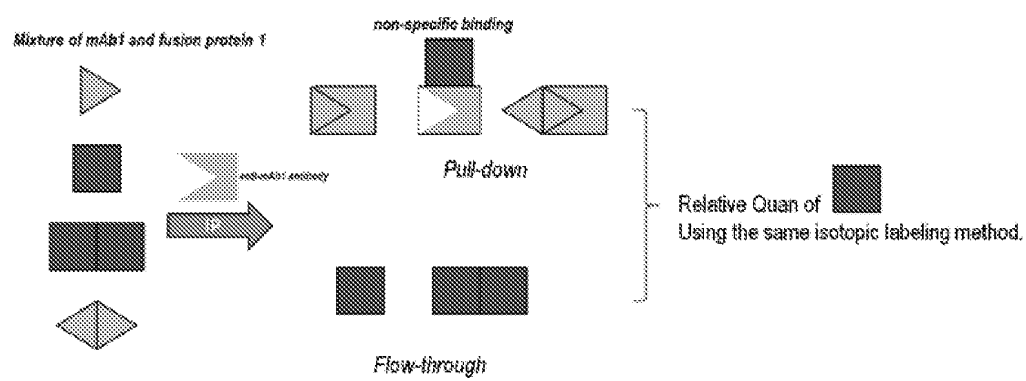


FIG. 4

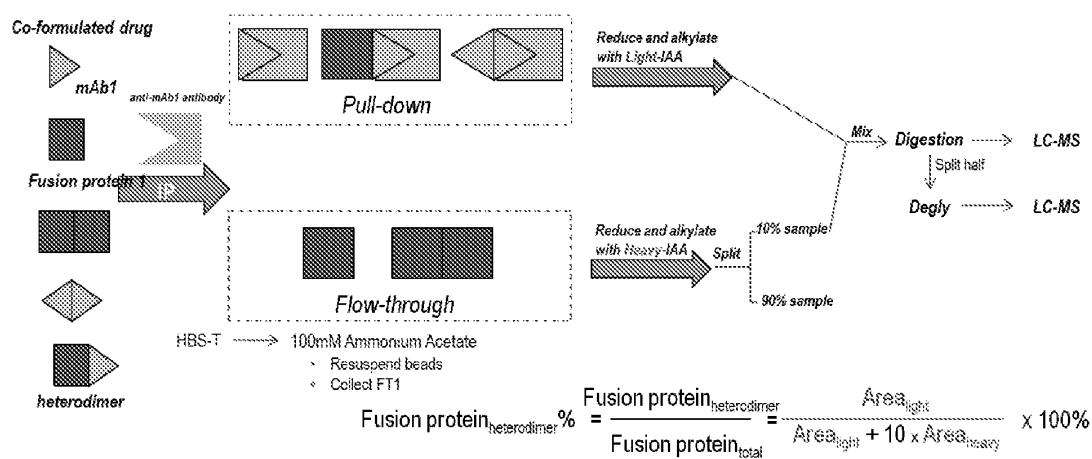


FIG. 5

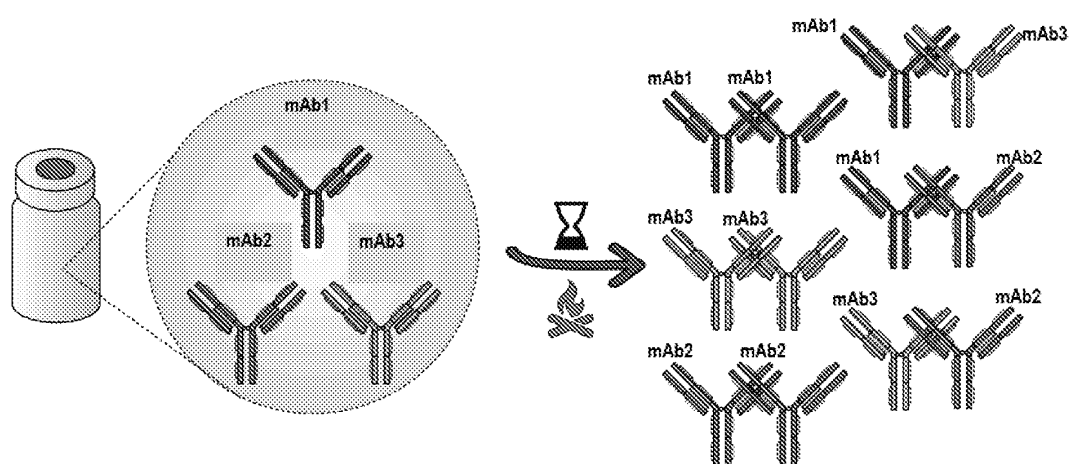
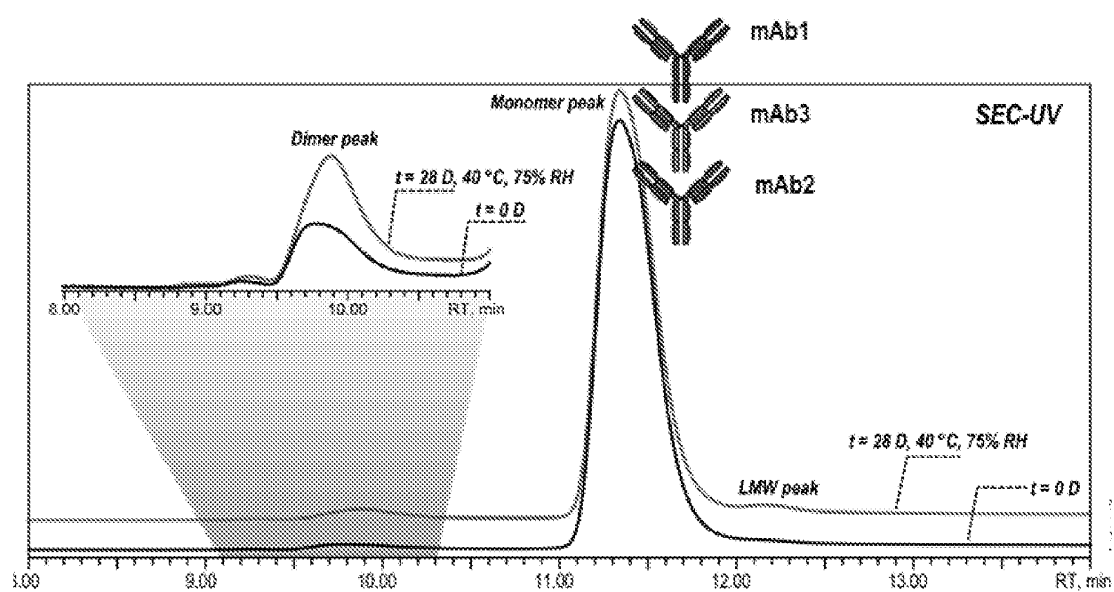


FIG. 6



Samples were deglycosylated under native conditions to remove heterogeneity introduced by Fc N-glycans.

FIG. 7

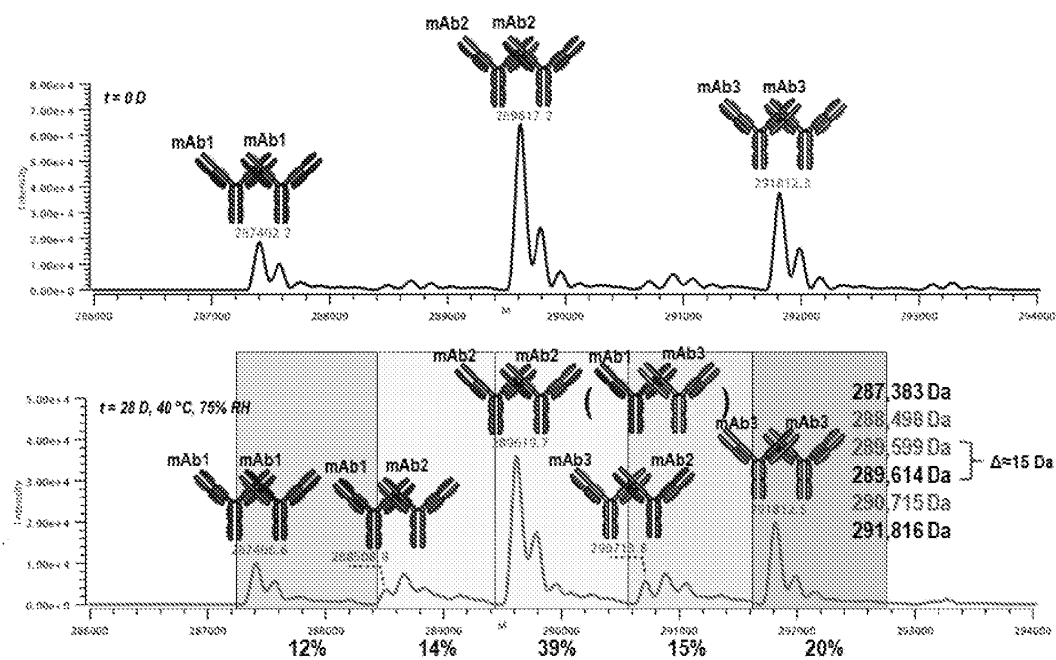


FIG. 8

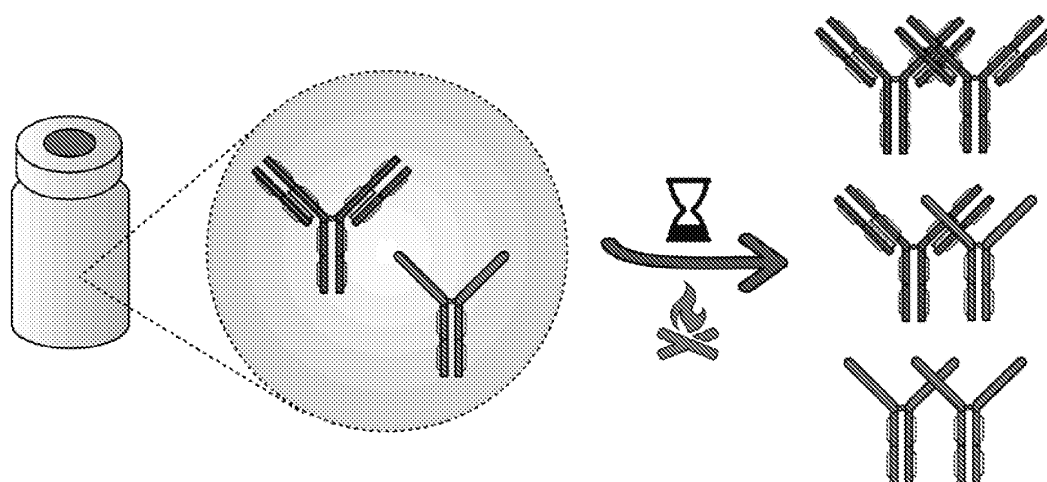


FIG. 9

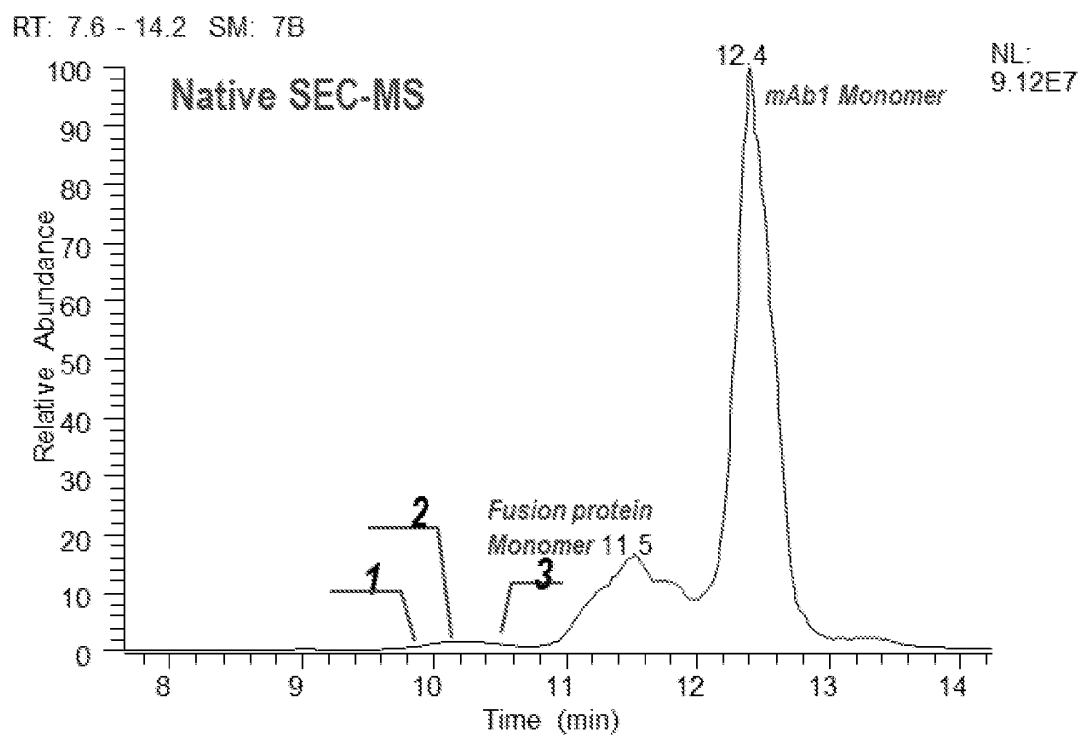


FIG. 10

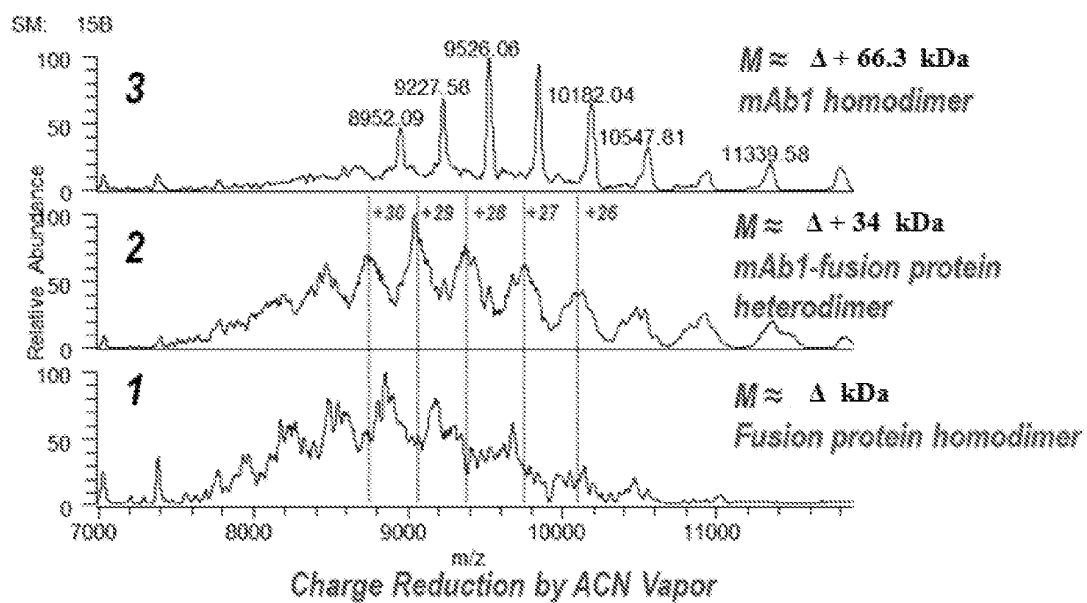
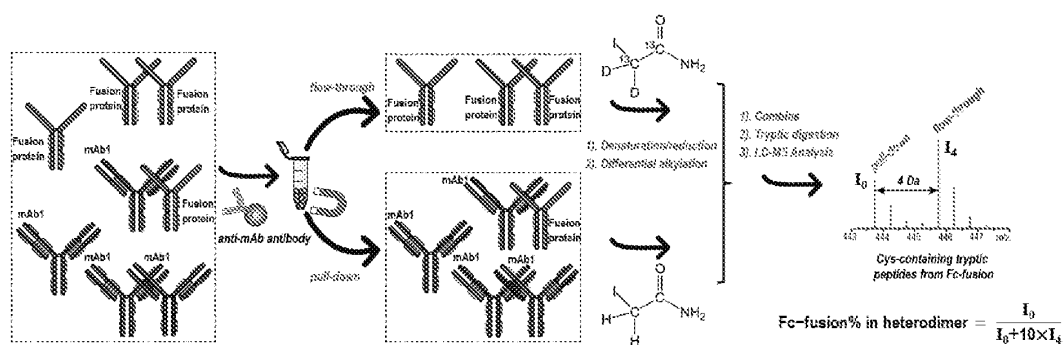


FIG. 11

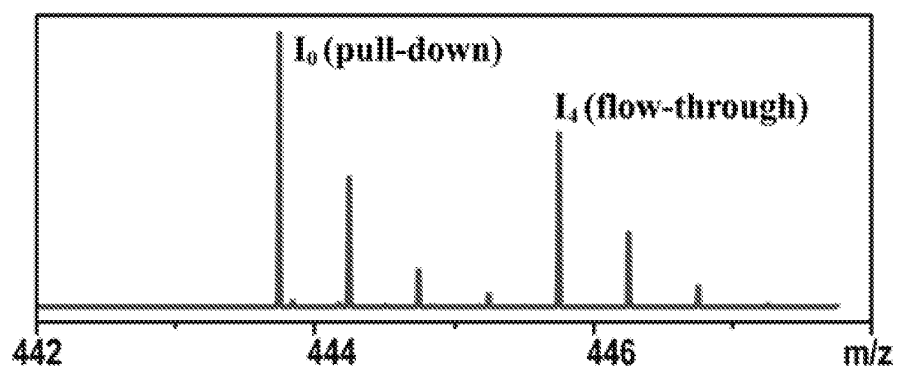


- ❖ The pull-down fraction contains Fc-fusion protein present in heterodimer
- ❖ The flow-through fraction contains Fc-fusion protein present in monomer and homodimer (only 1/10 of the flow-through fraction was used)

FIG. 12

Sample 8

CW-stressed coformulated protein drug:



Sample 7

*Negative control: mix individually CW-stressed proteins
right before analysis:*

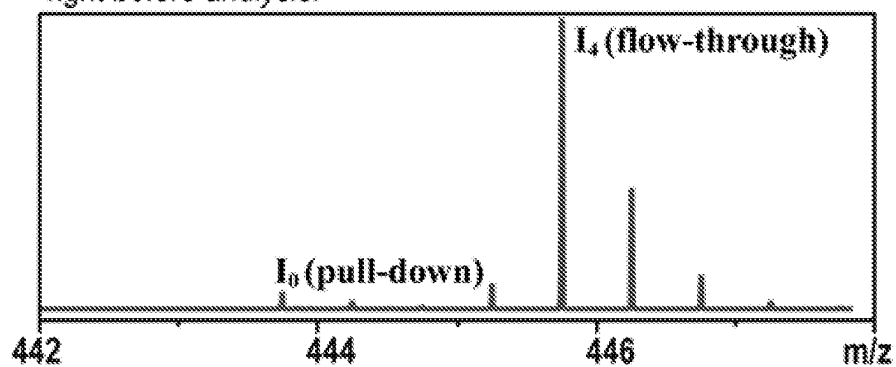


FIG. 13

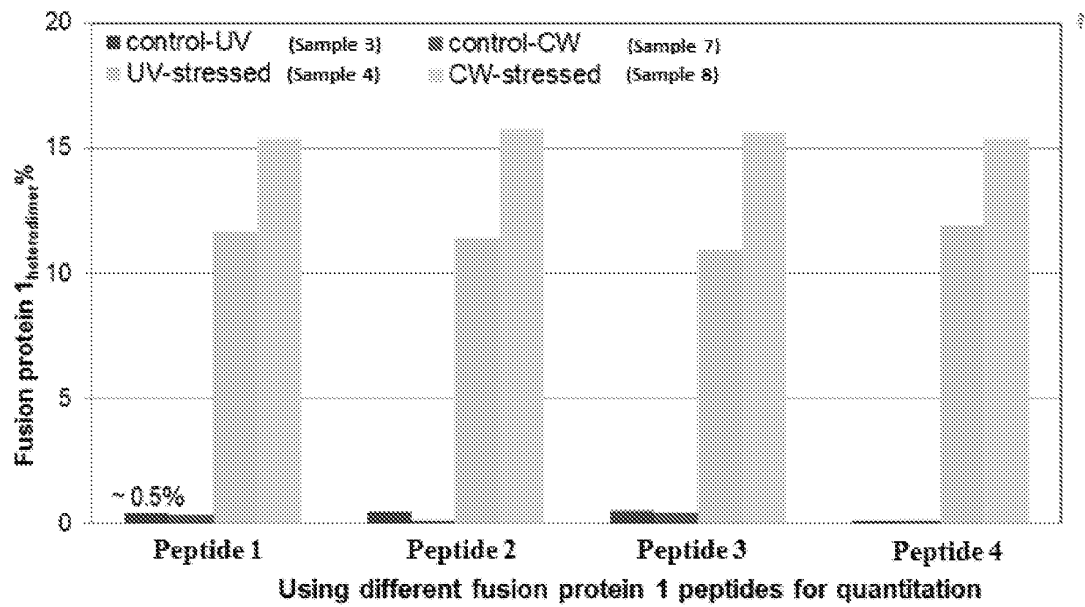
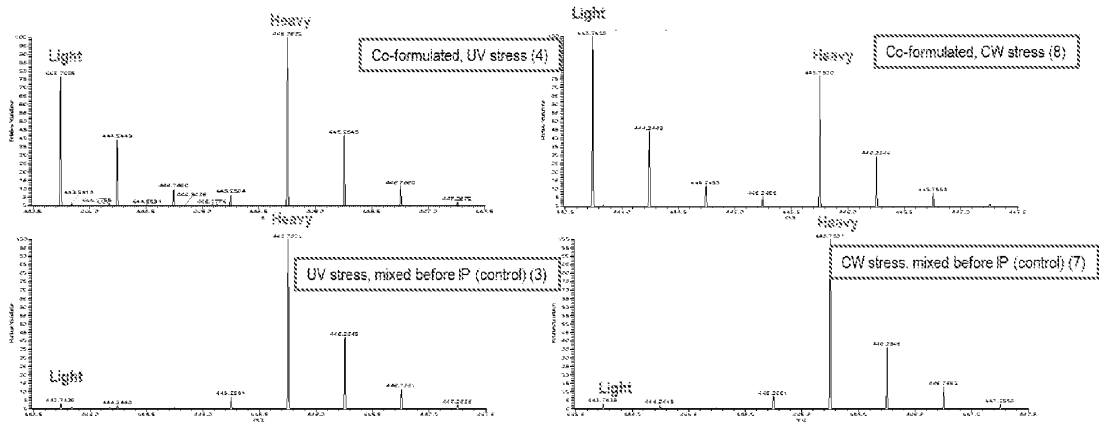


FIG. 14

Peptide 1

Heterodimer quantitation

Condition	UV stress	CW stress
Co-formulated	11.69	15.35
Negative Control	0.43	0.38



* This peptide is partially deamidated; quant is done here using the native peptide.

Strain method	DAI (%)	CAI (%)
Co-formulated	11.37	15.73
Negative Control	0.47	0.16

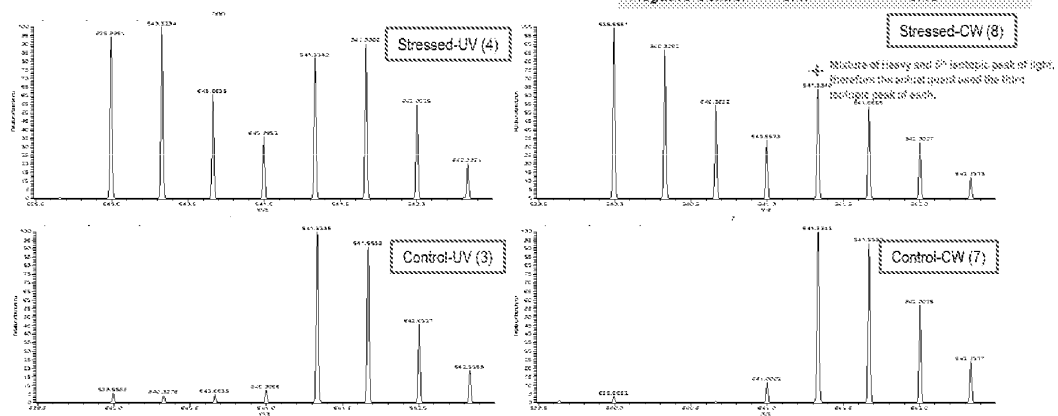


FIG. 16

Peptide 3

* This peptide is fully deamidated; quant is done here using the deamidated peptide.

Stressor	UV (%)	CW (%)
Co-formulated	10.93	15.63
Negative Control	0.52	0.41

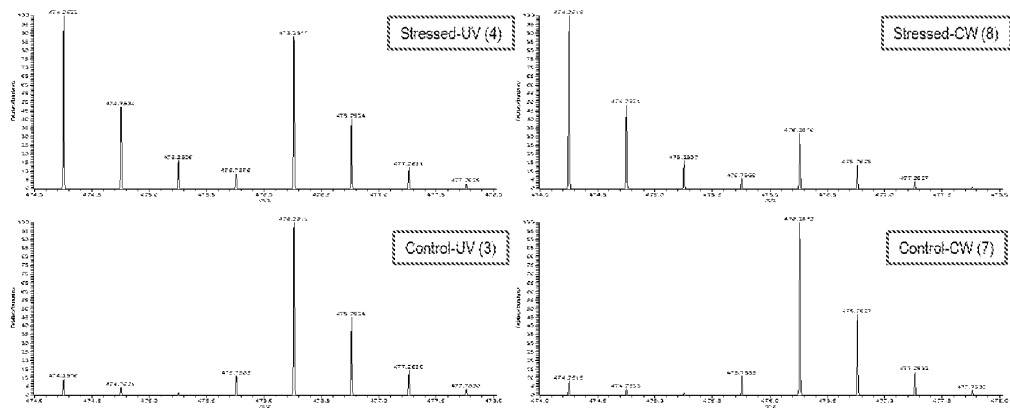


FIG. 17

Peptide 4

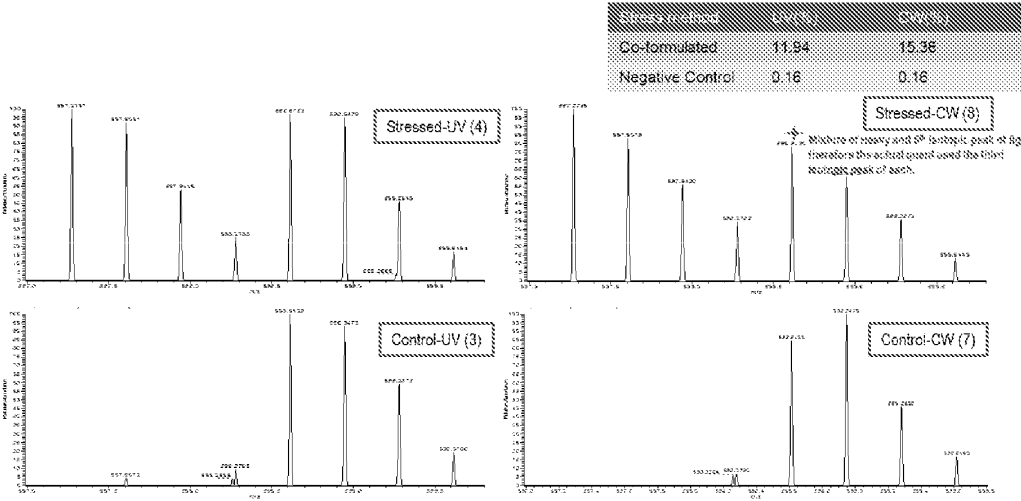


FIG. 18

Converting fusion protein 1_{heterodimer} % to Heterodimer% in UV Peak Areas

What is known:

- 40mg fusion protein 1 and 120mg mAb1
- M.W. of fusion protein 1 (w/ glycans): 117 kDa
- CoE of fusion protein 1: 1.15
- M.W. of mAb1(w/ glycans): 148 kDa
- CoE of mAb1: 1.37
- M.W. of heterodimer (w/ glycans): 117+148 = 265 kDa
- CoE of heterodimer: $(1.15 \times 97.2 + 1.37 \times 145) / (97.2 + 145) = 1.28$ [don't consider glycan]
- The percentage of fusion protein 1 found in heterodimer as determined by MS: a%

Then, the calculated percentage of heterodimer in UV peaks:

$$AU \propto (CoE \times Conc)$$

$$\frac{\left(\frac{40mg}{117kDa} \times a\% \times 265kDa\right) \times 1.28}{(40mg \times 1.15 + 120mg \times 1.37)} \approx 0.55a\%$$

FIG. 19

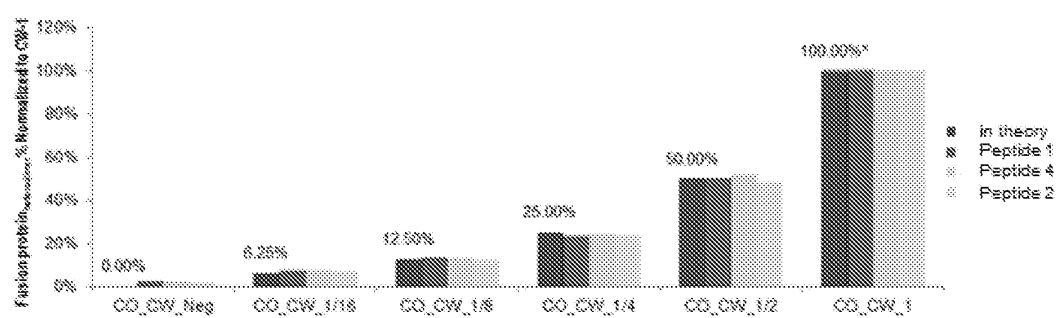


FIG. 20

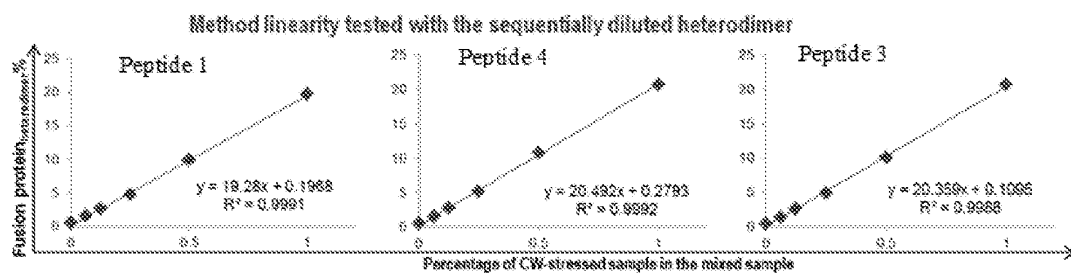


FIG. 21

