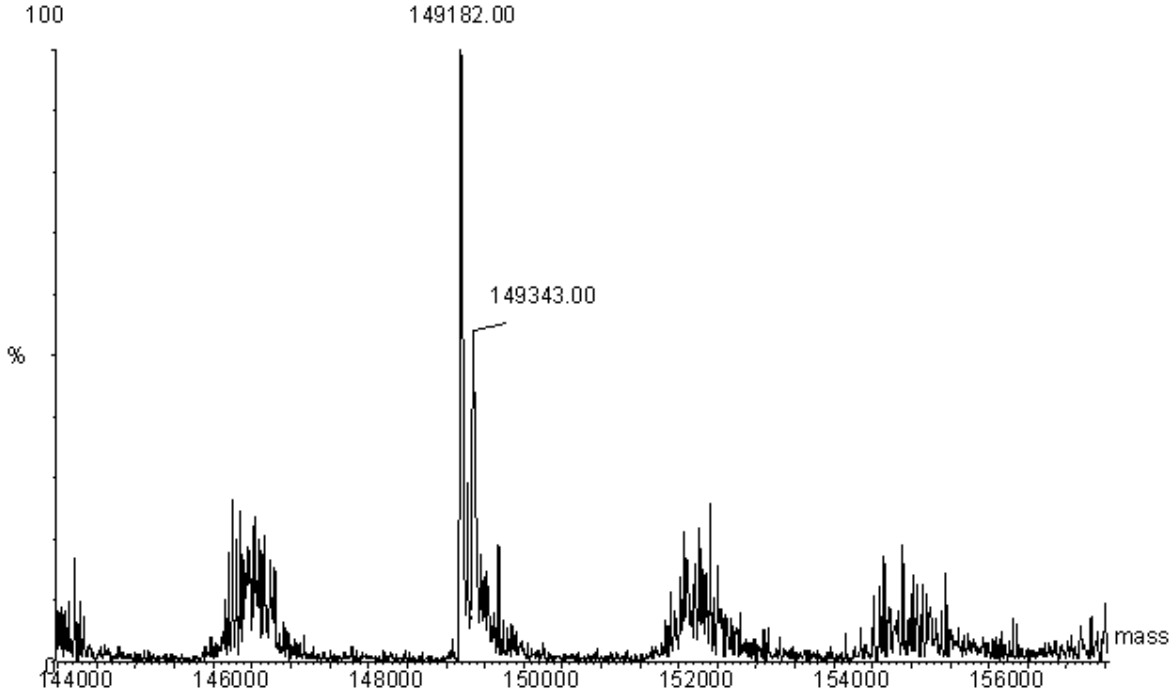
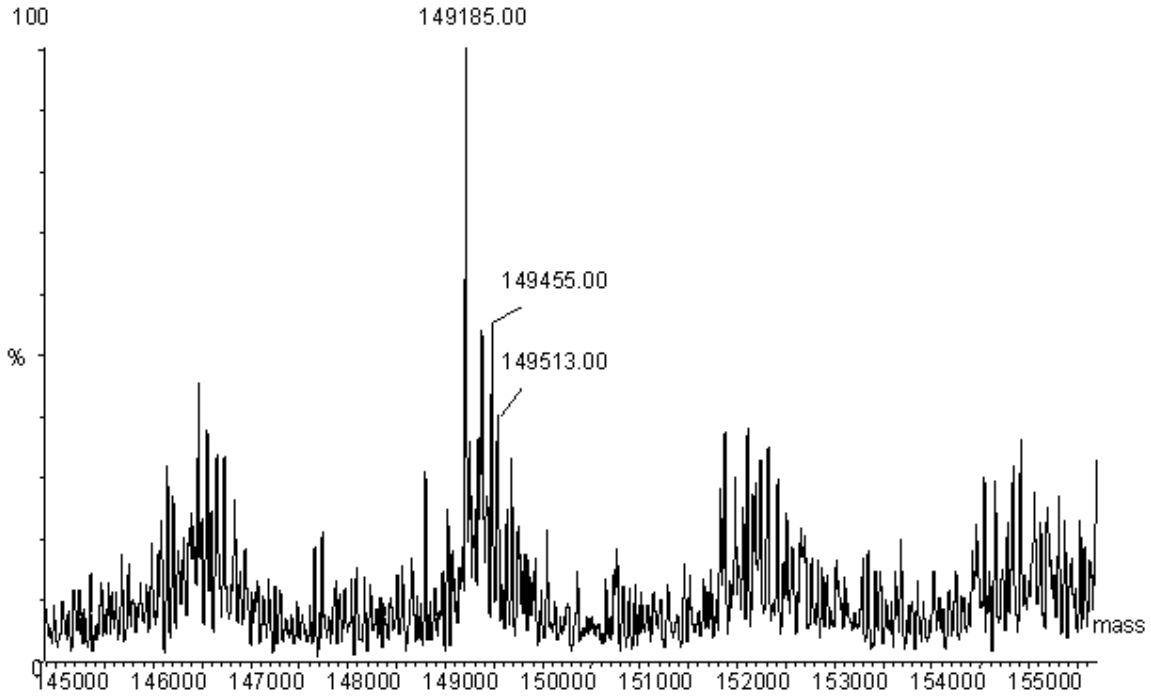
Supporting information

Figure S1. Mass spectra of *ex in vivo* Bevacizumab samples after incubation in vitreous humour from (a) day 0, (b) day 7 to (c) day 14. Structural heterogeneity peaks were found along with time.

(a)



(b)


100
149185.00
149455.00
149513.00
%
0
mass
145000
146000
147000
148000
149000
150000
151000
152000
153000
154000
155000

(c)

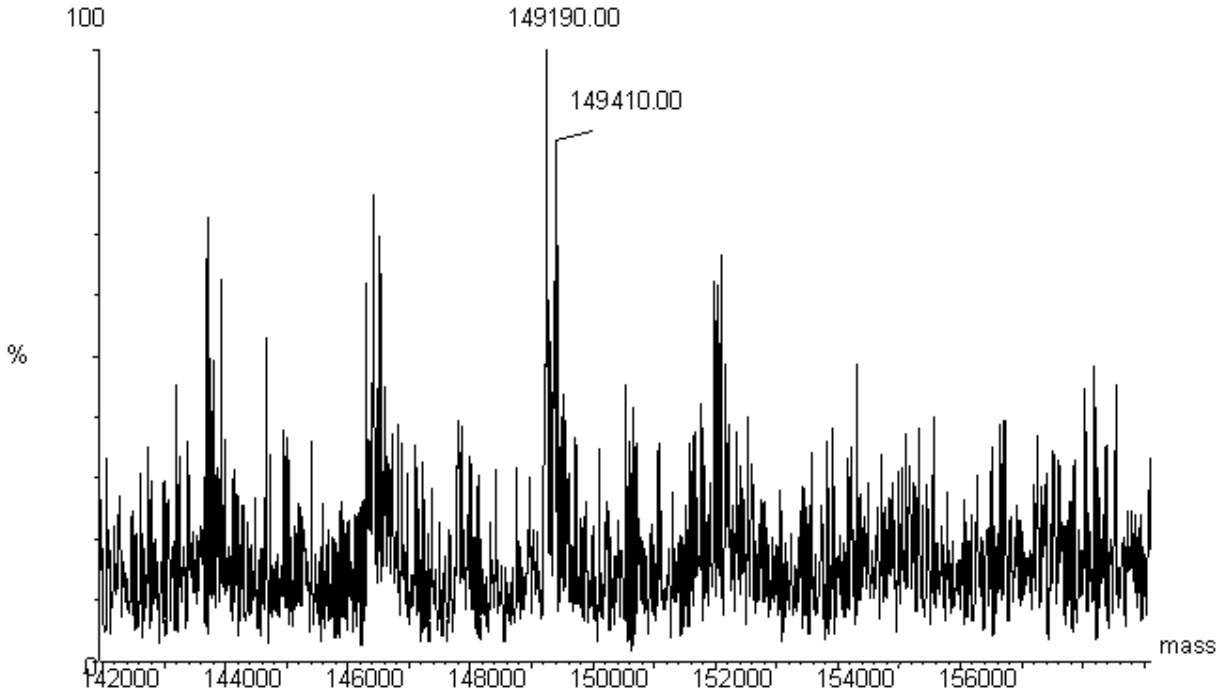
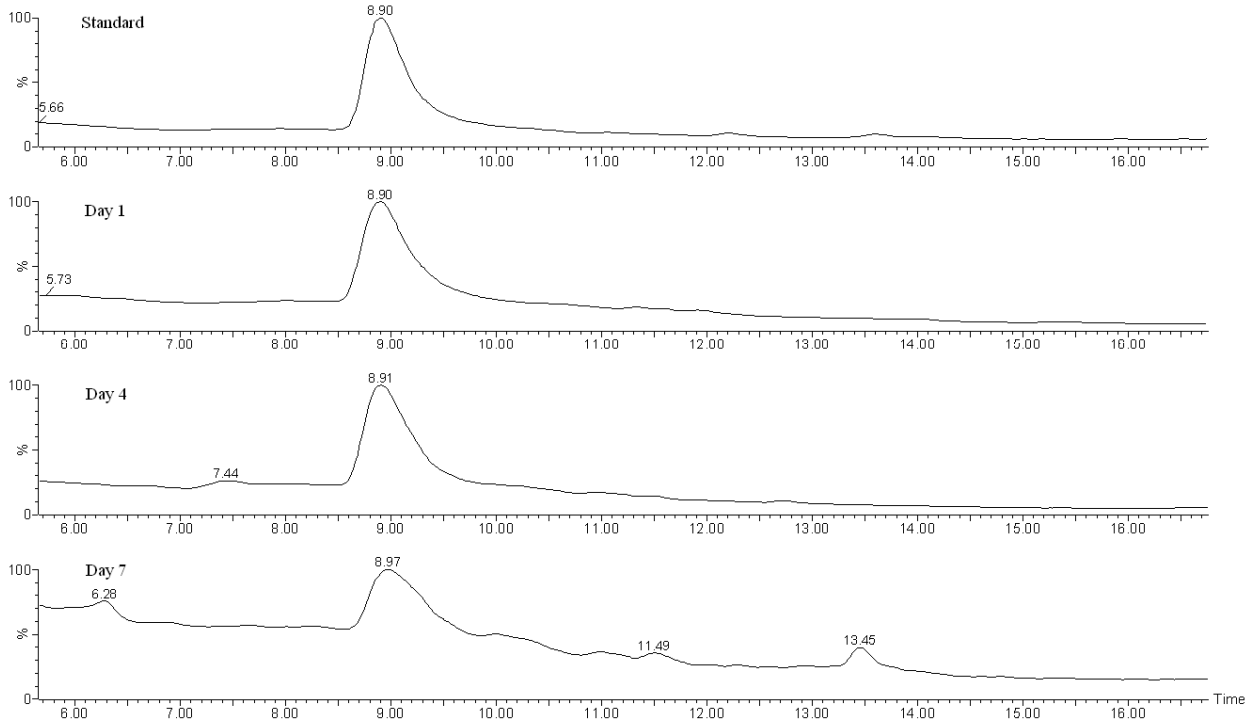
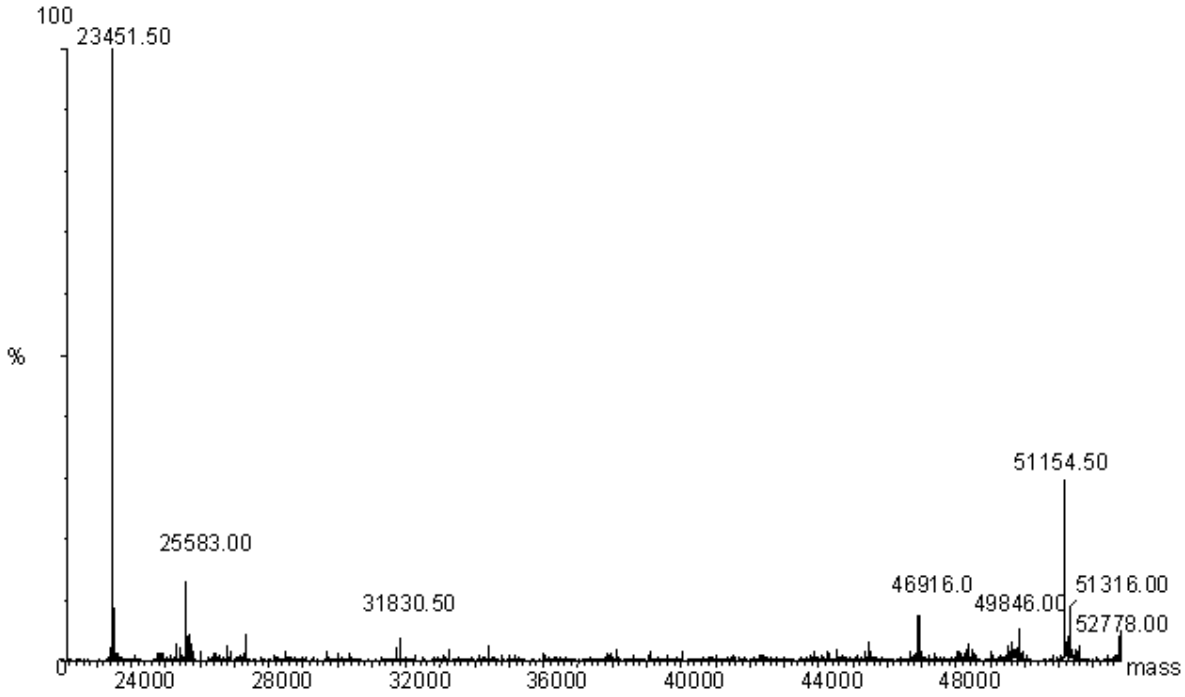

100
149190.00
149410.00
%
0
142000
144000
146000
148000
150000
152000
154000
156000
mass

Figure S2. Chromatograms (a) and mass spectra of standard Bevacizumab spiked in vitreous humor and vitreous samples from 1, 4 and 7 days after intra-vitreal injection following isolation and DTT reduction. The heavy and light chains were co-eluted. (b-e) They showed the respective mass spectrum of the Bevacizumab from spiked standard, day 1 to day 7. (b) It showed mass 23451.5 m/z was the mass of light chain, the mass of 51154.5 m/z was the mass of heavy chain and the mass 51316 m/z was the mass of heavy chain with an additional galactose on the glycan chain. (c) It showed similar mass spectrum whereas the heavy was becoming heterogeneous from (d – e).

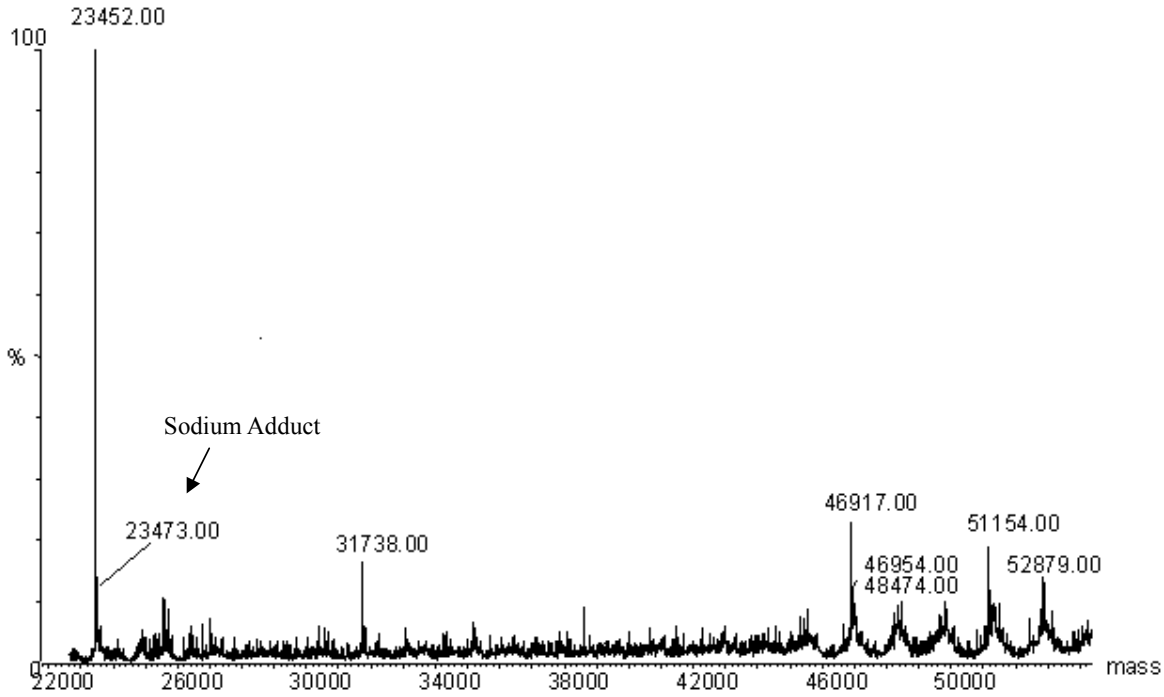
(a)



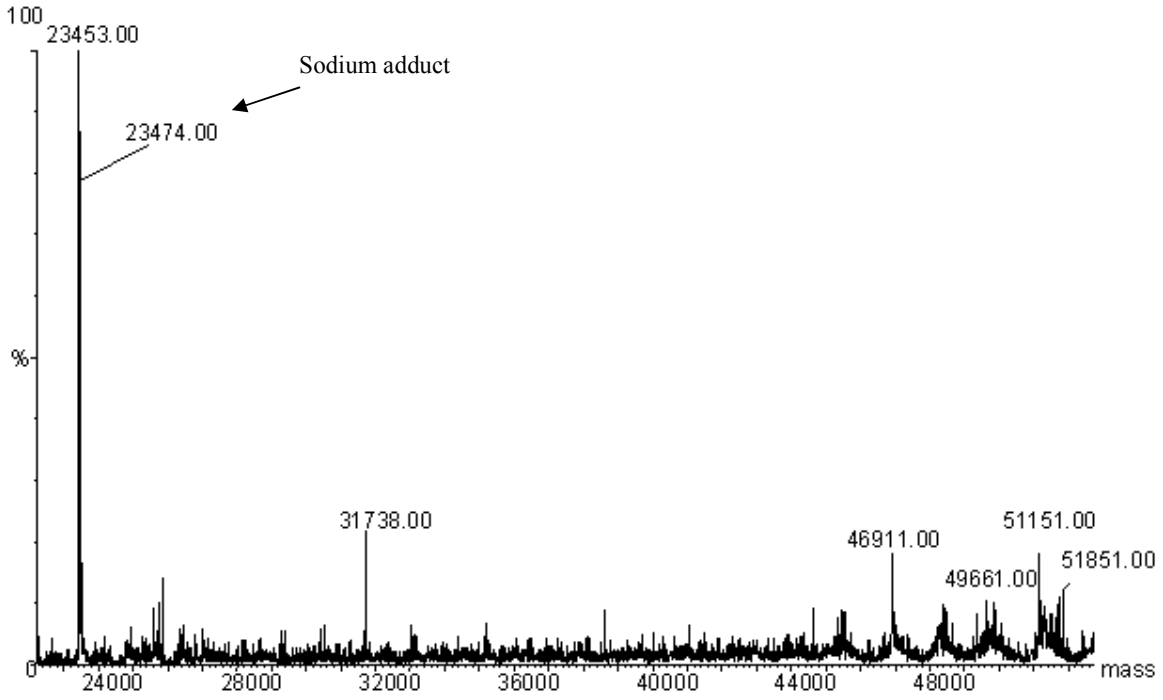
(b)


100
23451.50
%
25583.00
31830.50
46916.0
49846.00
51154.50
51316.00
52778.00
0
24000
28000
32000
36000
40000
44000
48000
mass

(c)


23452.00
100
%
Sodium Adduct
23473.00
31738.00
46917.00
46954.00
48474.00
51154.00
52879.00
0
22000
26000
30000
34000
38000
42000
46000
50000
mass

(d)


100
23453.00
Sodium adduct
23474.00
%
31738.00
46911.00
51151.00
51851.00
49661.00
0
24000
28000
32000
36000
40000
44000
48000
mass

(e)

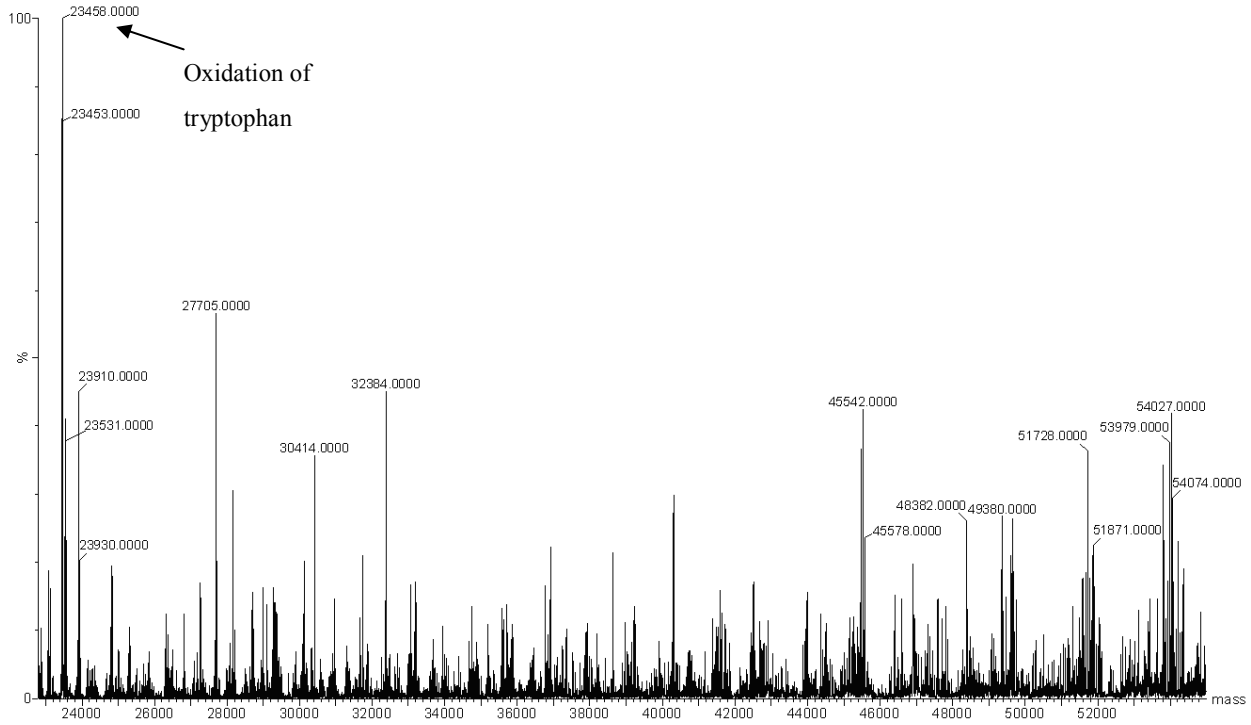

23458.0000
Oxidation of
tryptophan
23453.0000
27705.0000
23910.0000
32384.0000
45542.0000
54027.0000
53979.0000
51728.0000
23531.0000
30414.0000
54074.0000
48382.0000
49380.0000
45578.0000
51871.0000
23930.0000
100
%
0
24000
26000
28000
30000
32000
34000
36000
38000
40000
42000
44000
46000
48000
50000
52000
mass

Figure S3. LCMS chromatograms of spiked Bevacizumab in vitreous humor and 1, 4 and 7 days after intravitreal injection following Poros G/20 isolation, deglycosylated and DTT reduction.

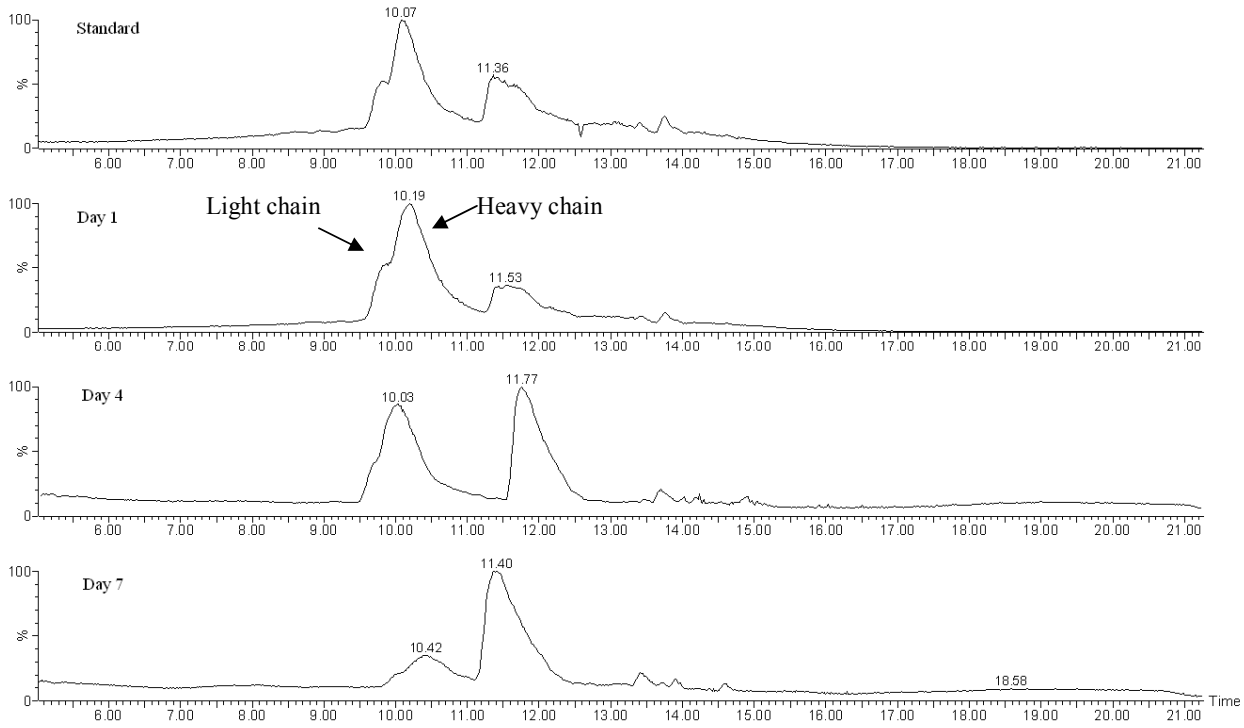


Figure S4. Mass spectrum of spiked Bevacizumab in vitreous humor after deglycosylation by PNGase F. The mass was the mass of intact Bevacizumab minus two times of Go glycan.

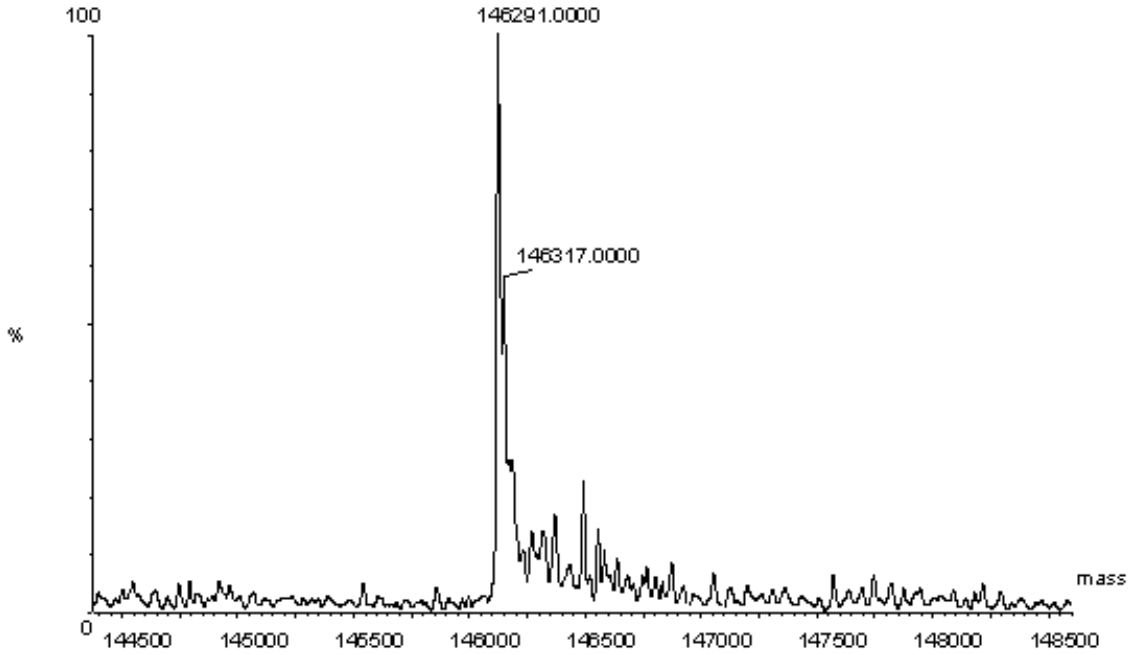


Figure S5. Difference of relative Ka at day 1, 7, and 21 respected to the relative Ka at 4 hours after intra-vitreal injection of three rabbit eyes. The symbol * shows significant difference between the results of day 21 to day 1 and day 7 calculated by ANOVA post hoc Dunnett test ($p<0.05$).

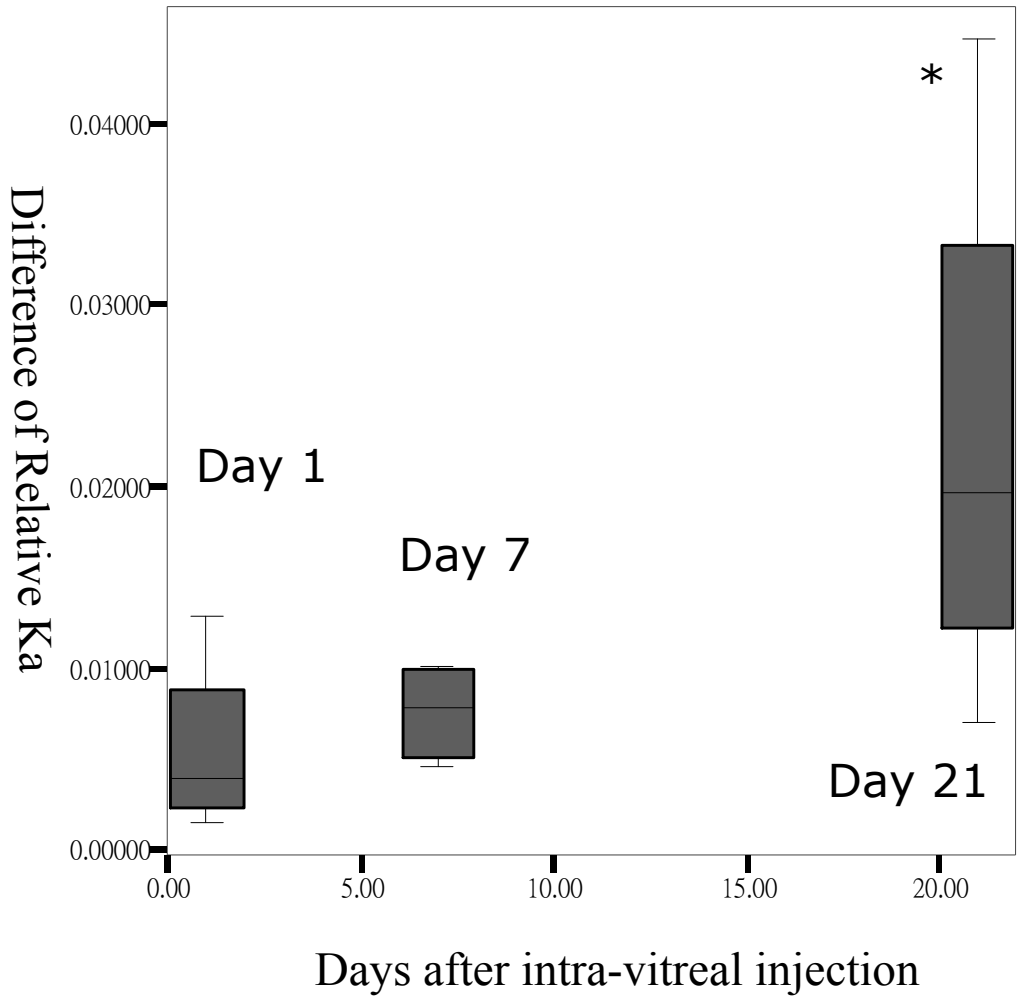


Figure S6. LCMS chromatograms of Bevacizumab after purified by Poros G/20 affinity column and trypsin digestion treatment. The samples were Bevacizumab standards and vitreous samples after different days of intra-vitreal injection.