Supplementary section

Characterization of bispecific antigen-binding biotherapeutic fragmentation sites using microfluidic capillary electrophoresis coupled to mass spectrometry (mCZE-MS)

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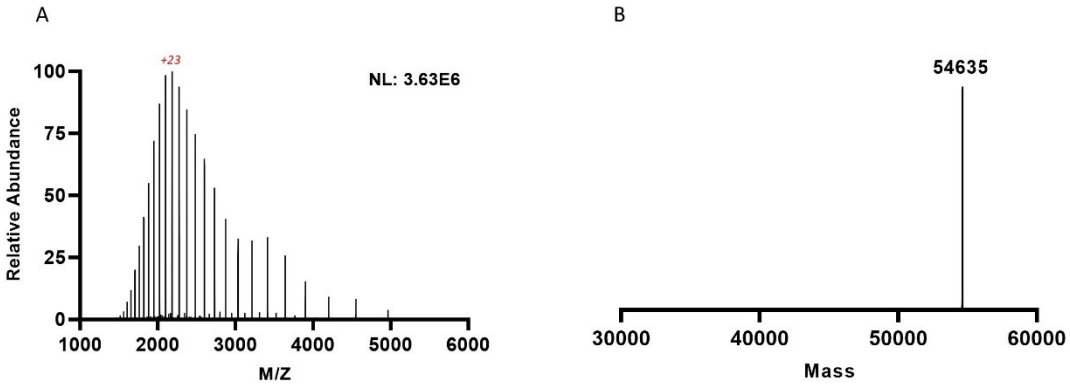

Figure S1: (A) raw and (B) deconvoluted mass spectrum for the unstressed BABB drug substance.

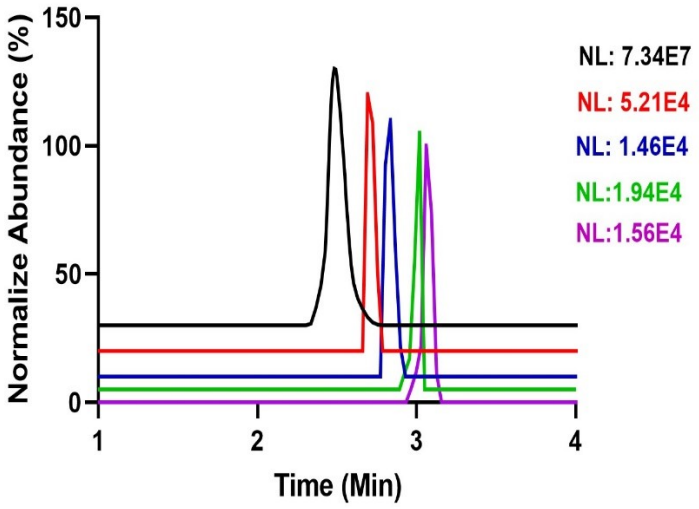

Figure S2: Extracted ion chromatogram (XIC) for mCZE-MS for thermally stressed BABB drug substance for 5 days. XIC for species 1, as observed in figure 2a (black trace). XIC for species 2 and 3, as observed in figure 2b (Red trace). XIC of species 4, as observed in figure 2b (Blue trace). XIC for species 5 and 6 from figure 2b (Green and Purple trace, respectively).

The calculation for LOD for mCZE-MS experiment:

The noise level observed for a blank injection at the migration time corresponding to BABB in the mCZE-MS experiment:

N_{blk}= 3,870 AU.

The limit of detection was estimated as the noise level multiplied by 3:

LOD= 3 X $N_{blk,}$ = 11,610 AU

The lowest peak height determined for qualitative analysis was observed at 55,635 AU for the antigen-binding domain fragment in the affinity purified sample, which is over 14-fold higher than the determined noise level.

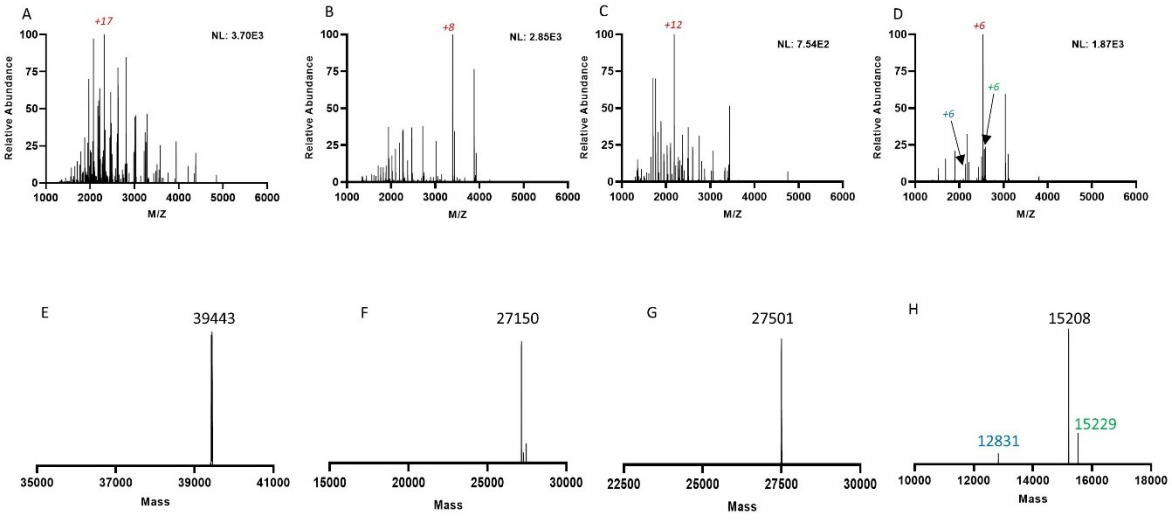

Figure S3: Raw and deconvoluted mass spectra by dSEC-MS analysis for the thermally stressed BABB drug substance for 5 days. Mass spectra related to fragments observed in (A), (B), (C), (D). Deconvoluted mass for fragments observed in (E), (F), (G) and (H), respectively.

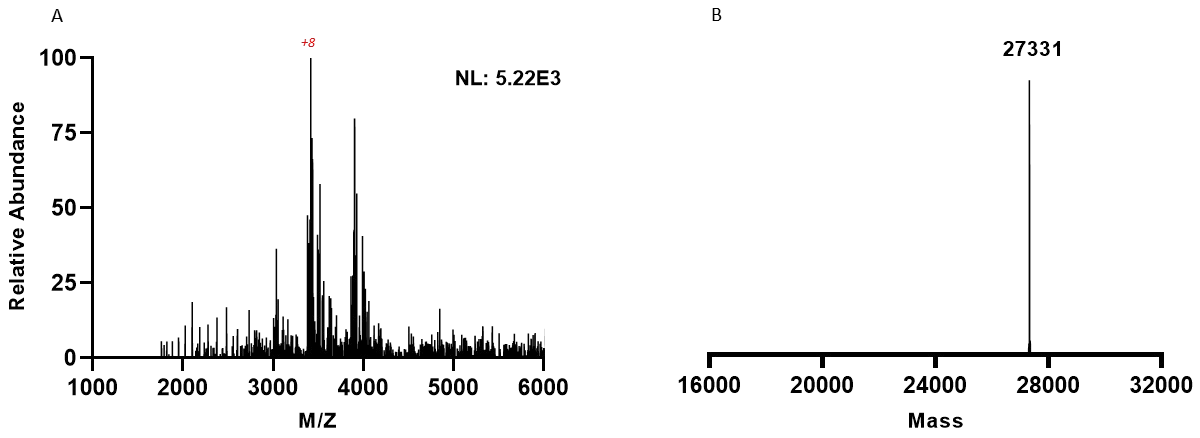

Figure S4: (A) Raw mass spectrum for antigen binding domain and (B) deconvoluted mass spectrum for antigen binding domain fragment, by dSEC-MS analysis for the affinity-purified BABB.

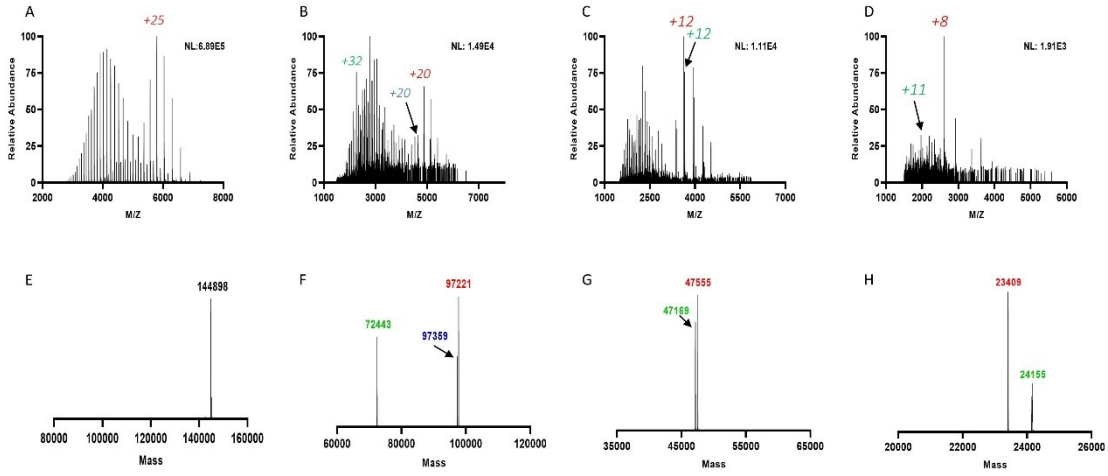

Figure S5: Raw and deconvoluted mass spectra by ZipChip®/CZE-MS analysis for the thermally stressed aglycosylated monoclonal antibody (AmAb). (A), (B), (C), and (D) represent mass spectra related to fragments observed. Deconvoluted masses for the fragments observed are shown in (E), (F), (G), and (H), respectively.

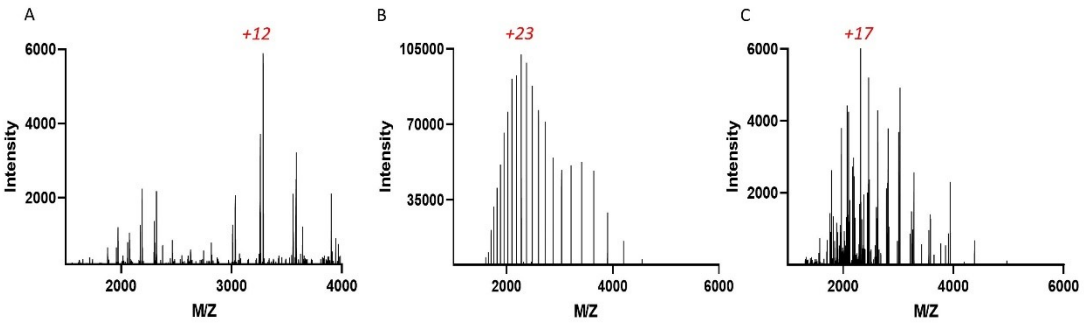

Figure S6: Comparison between mass spectra obtained in the mCZE-MS and dSEC-MS experiments. (A) mass spectrum related to the 39k fragment of the thermally stressed BABB drug substance from mCZE-MS experiment. B) mass spectrum for the 39K clips from dSEC-MS experiment showing abundant peaks related to intact BABB drug substance C) Peaks related to the 39K fragment observed after background subtraction to remove ions related to the intact BABB.

Table S1: Masses observed and predicted species for the thermally stressed AmAb from mCZE-MS experiment along with mass error and predicted species.

Mass observed (Da)*	CV (%)	Theoretical mass (Da)	Mass error (PPM)	Predicted species
144898	3	144899	7	
72443	2	72444.7	23	
97221	4	97221.6	7	
97359	4	97358.8	2	
47555	4	47555.3	6	
47169	3	47170.6	34	
23407	3	23408	43	
24155	4	24155.6	25	

**Indicates average of triplicate measurements*