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Titer and charge-based heterogeneity multiattribute monitoring of mAbs in cell culture harvest using 2D ProA CEX MS

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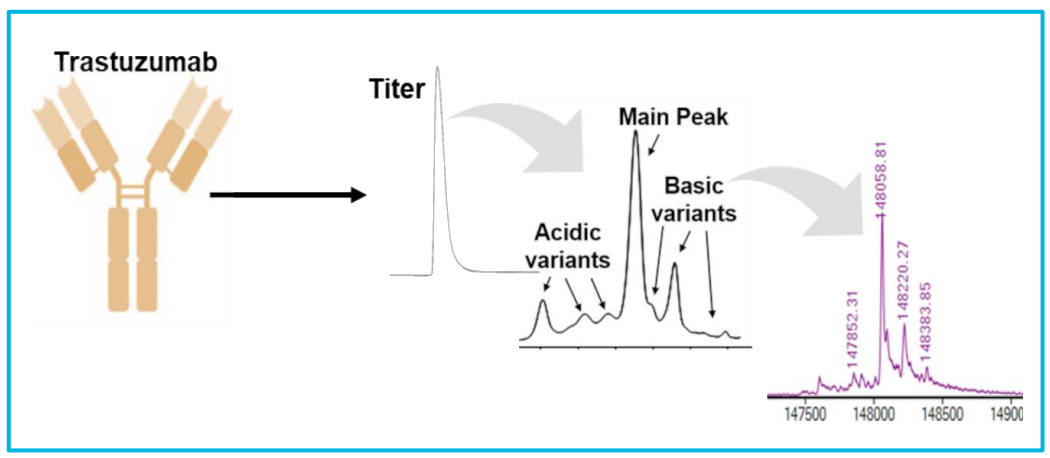
Introduction

Robust monitoring of heterogeneity in biopharmaceutical development is crucial for producing safe and efficacious biotherapeutic products. Multiattribute monitoring (MAM) has emerged as an efficient tool for monitoring of mAb heterogeneities like deamidation, sialylation, glycosylation, and oxidation. Conventional biopharma analysis during mAb development relies on use of one-dimensional methods for monitoring titer and charge-based heterogeneity using non-volatile solvents without direct coupling with mass spectrometry (MS). This approach requires analysis of mAb harvest by ProA for titer estimation followed by separate cation exchange chromatography (CEX) analysis of the purified sample for estimating charge-based heterogeneity. This can take up to 60–90 min due to the required fraction collection and buffer exchange steps. In this work, a native two-dimensional liquid chromatography (2D-LC) mass spectrometry method has been developed with Protein A chromatography in the first dimension for titer estimation and cation exchange chromatography (CEX) in the second dimension for charge variant analysis. The method uses volatile salts for both dimensions and enables easy coupling to MS. The proposed 2D-LC method exhibits a charge variant profile that is similar to that observed via the traditional methods and takes only 15 min for mass identification of each variant. A total of six charge variants were separated by the CEX analysis after titer estimation, including linearity assessment from 5 µg to 160 µg of injected mAb sample. The proposed method successfully estimated charge variants for the mAb innovator and 4 of its biosimilars, showcasing its applicability for biosimilarity exercises. Hence, the 2D ProA CEX MS method allows direct titer and charge variant estimation of mAbs in a single workflow.



Schematic representation of the multiattribute method for titer and charge heterogeneity estimation of mAbs. The peak from first dimension are transferred to the second dimension and then to a mass spectrometer.

Experimental

The 2D-LC system used Protein-A column in the first dimension and a cation-exchange column in the second dimension coupled to a mass spectrometer (additional details can be found in reference 4).

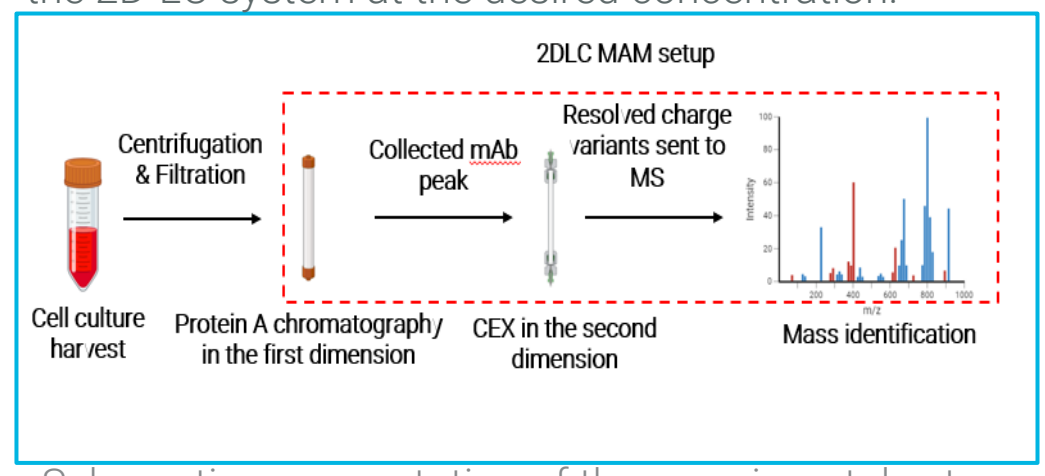
Titer estimation of the samples was done using Bio-Monolith recombinant Protein A (rProtein A) column (4.95 x 5.2 mm) from Agilent Technologies, USA.

CEX analysis of mAbs was performed using an Agilent Bio SCX, NP3, SS column (4.6 x 50 mm, 3 µm) from Agilent Technologies, USA.

The study used a 1290 Infinity II 2D-LC system from Agilent Technologies, USA, equipped with an autosampler, two binary pumps, two multicolumn thermostats, and two diode array detectors. The 2D-LC system was coupled to a 6545XT AdvanceBio LC/Q-TOF system from Agilent Technologies, USA.

The trastuzumab cell culture harvest was centrifuged, filtered and directly injected in the first dimension Protein A column.

Trastuzumab reference product and its biosimilars-Biceltis, Canmab, Trasturel and Vivitra were injected to the 2D-LC system at the desired concentration.



Schematic representation of the experimental set-up

Interconnection of the two dimensions was facilitated by a Multiple Heart-Cutting Valves serving as the multiple heart-cutting (MHC) interface, along with two Multiple Heart-Cutting Valves and an active solvent modulation (ASM) capillary.

Pro A chromatography in the first dimension (^{1}D) with a flowrate of 1.0 ml/min (2.5 min linear gradient of mobile phase B (0%–100 % B)). The elution peak from ^{1}D selected using a typical ^{2}D-heart-cut method through the 2D-LC ASM valve.

The selected peak was then analyzed in the second dimension (^{2}D) using CEX method (5-min linear gradient of mobile phase B (10%–60 % B) at a flow rate of 1.0 ml/min and column temperature of 25 °C).

The Q-TOF parameters were drying gas temperature at 350 °C, drying gas flow 13 L/min, nebulizer pressure 60 psig, sheath gas temperature 300 °C, sheath gas flow 12 L/min, nozzle voltage 5500 V, and fragmentor voltage 175 V.

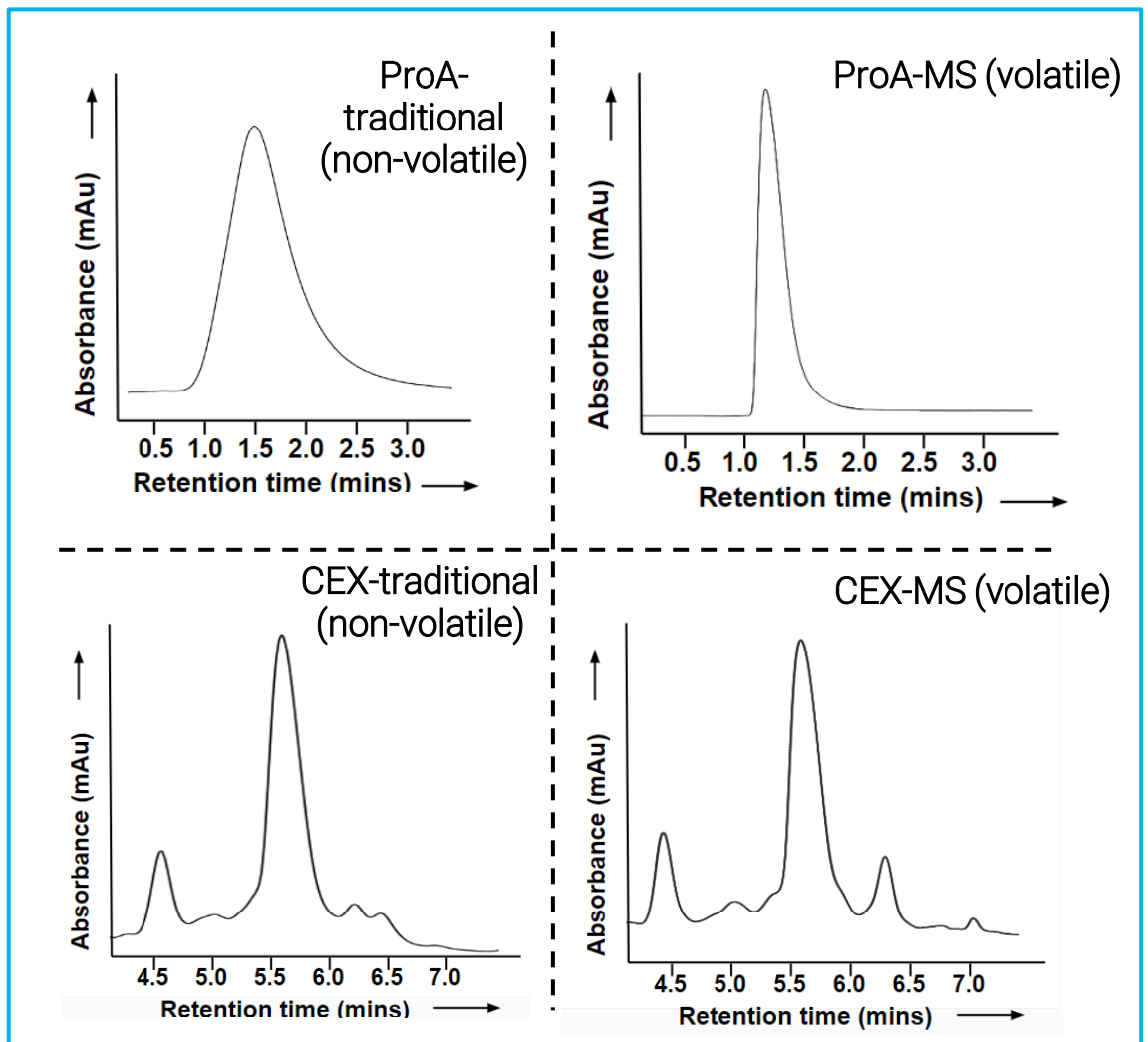
Results and Discussion

Comparison of the conventional ProA and CEX methods with the developed 2D ProA-CEX-MS method

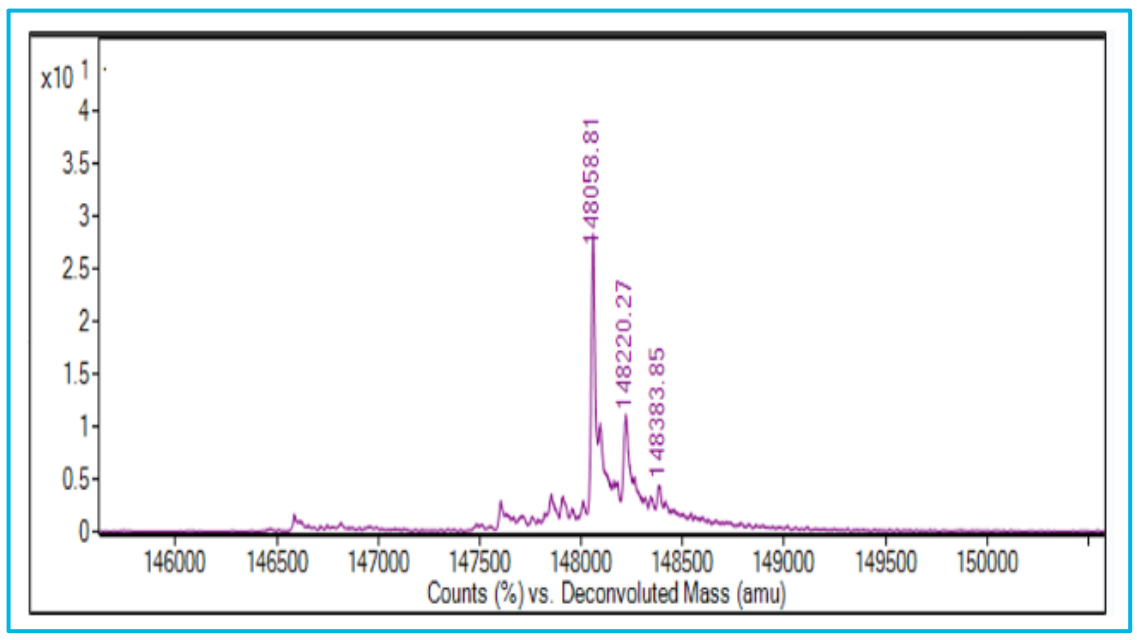
The developed ProA-MS and CEX-MS method profile were comparable to the traditional methods.

Easy mass identification of trastuzumab was accomplished.

Better resolution and peak shape was obtained for the MAM methods



Chromatograms obtained from the conventional and developed methods. The traditional methods using non-volatile buffers (phosphate) that are not compatible with the mass spectrometer system. In contrast, the 2D-LC methods use volatile solvents that are easily mass compatible.



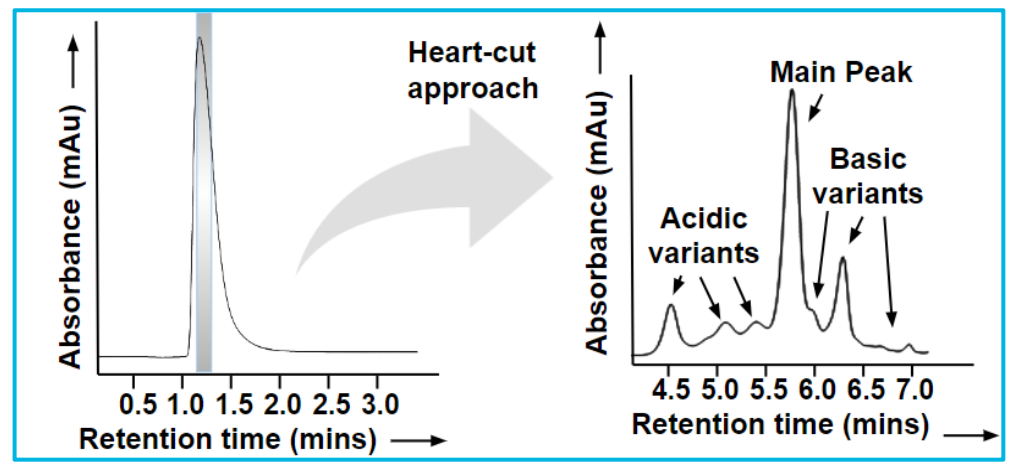
Deconvoluted mass of trastuzumab main peak

Results of the 2D ProA-CEX-MS method for cell-culture sampes and biosimilars of trastuzumab.

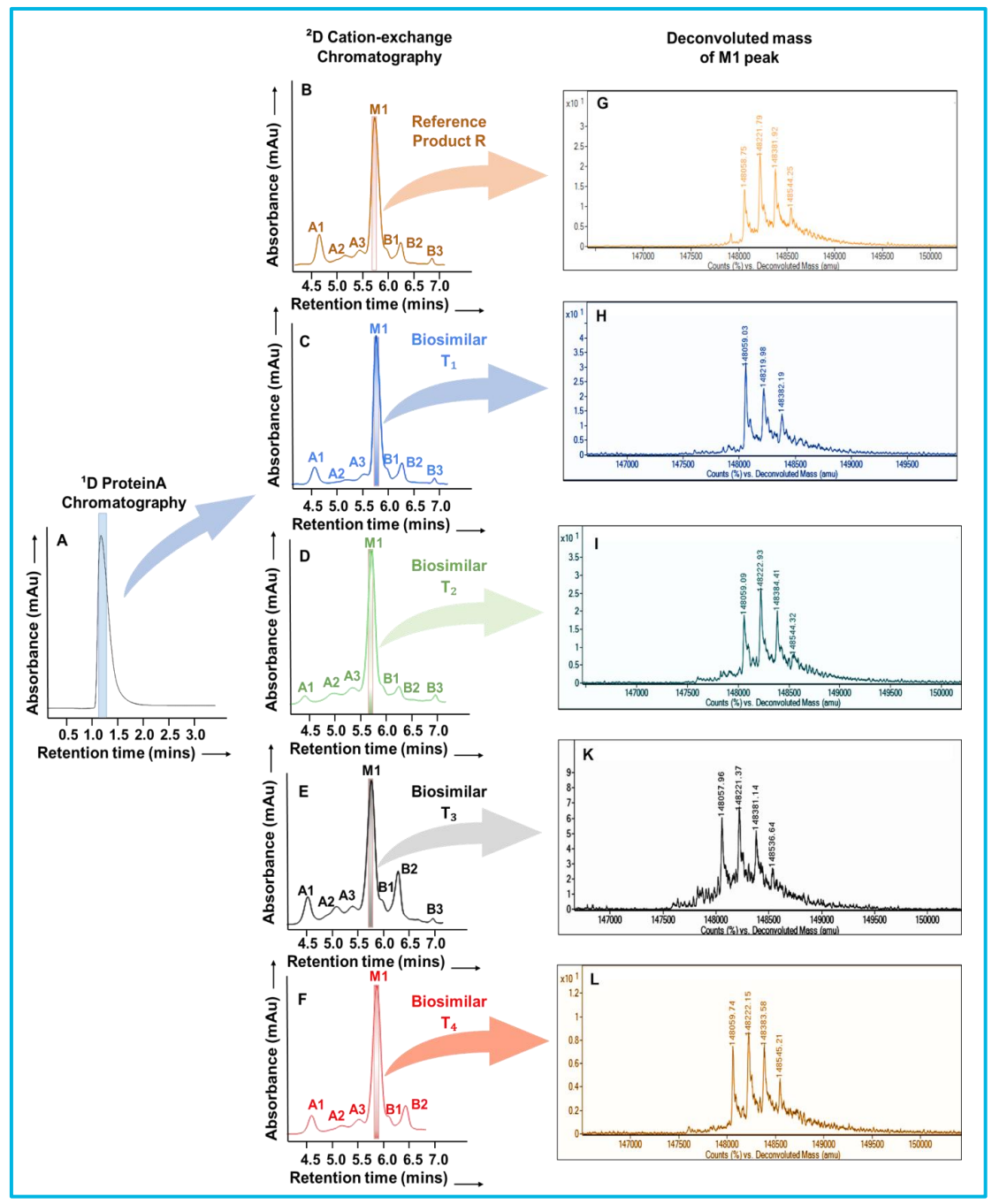
The 2D ProA-CEX-MS method was successful in estimating both titer and charge heterogeneity of cell-culture samples.

The method also successfully resolved the charge variants for trastuzumab biosimilars.

Major charge species resolved and identified in just 15 minutes, faster than any currently available method.



2D ProA-CEX-MS method chromatogram for cell culture sample



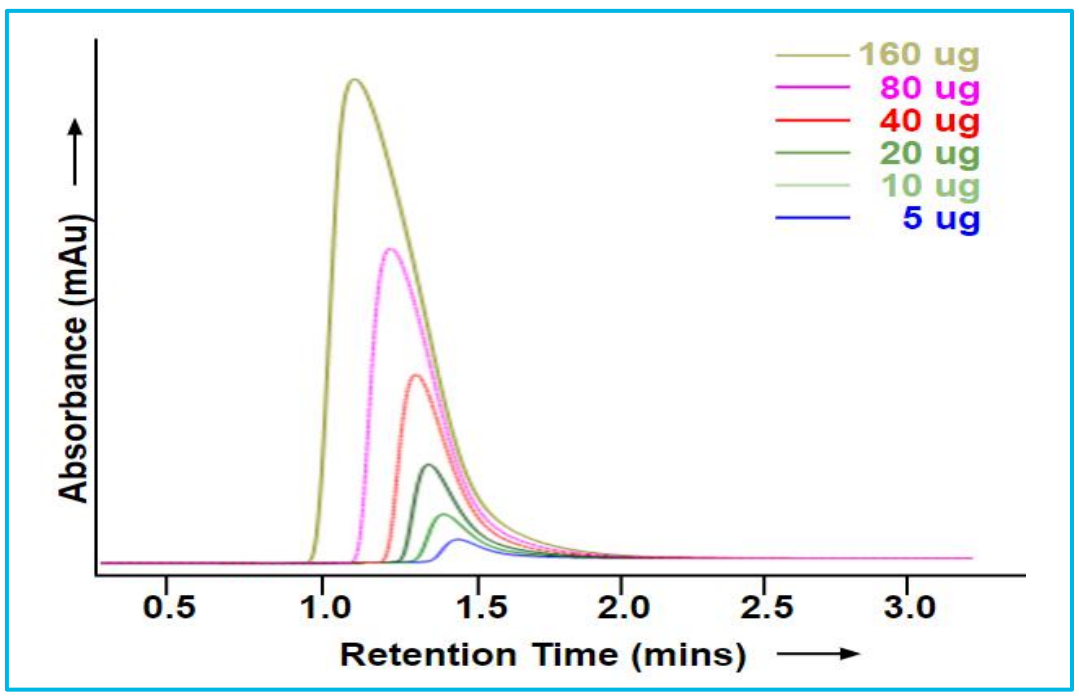
2D-ProA-CEX-MS method chromatograms for trastuzumab biosimilars

Results and Discussion

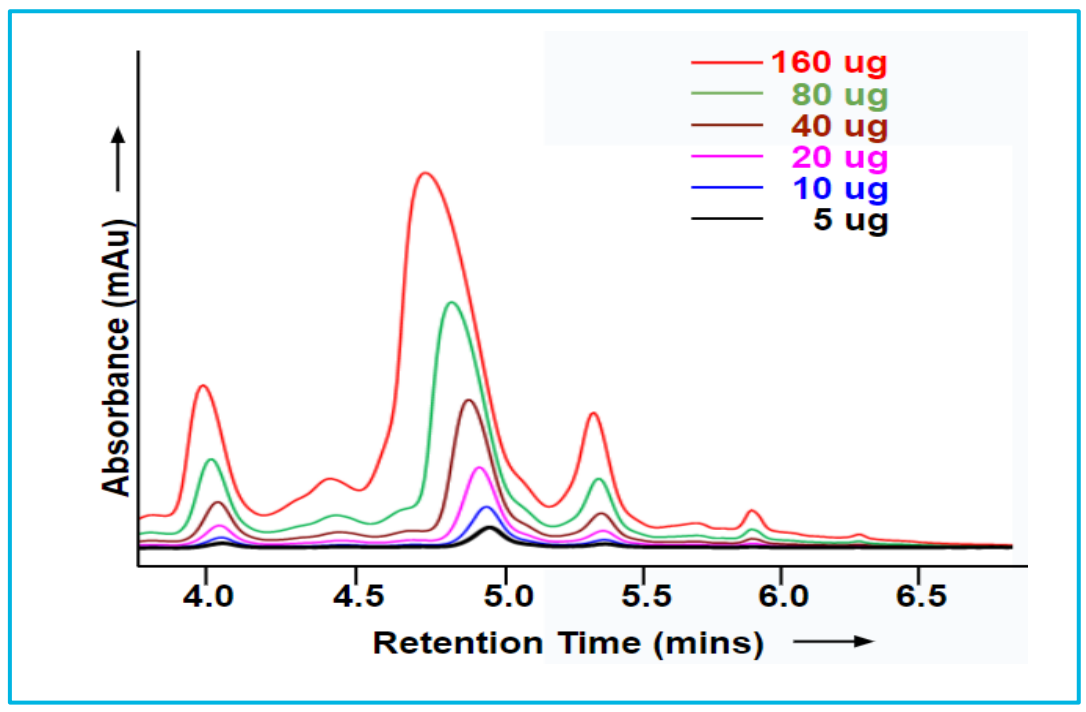
Linearity assessment of the 2D ProA-CEX-MS method

Sample amount from 5 µg to 160 µg were injected and analyzed with the 2D ProA-CEX-MS method.

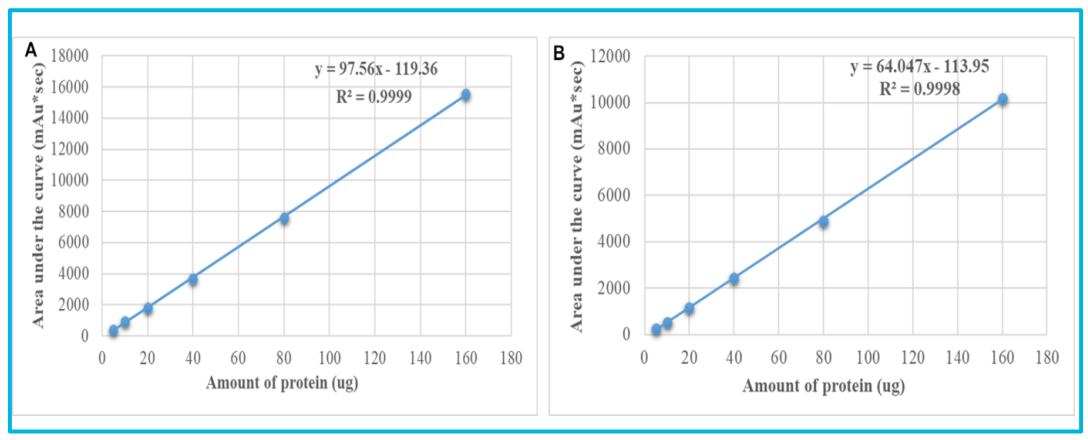
The different amounts of injected trastuzumab revealed R2 values of 0.9999 and 0.9998 for the 2D ProA and CEX methods.



Chromatogram obtained from the ProA method during linearity assessment



Chromatogram obtained from the CEX method during linearity assessment



Graphical representation of the linearity assessment of the 2D ProA-CEX-MS method

Charge variants resolved in the trastuzumab reference and biosimilar products by the 2D ProA-CEX-MS method

Product Name	Deconvoluted mass of acidic variants (Da)			Deconvoluted mass of basic variants (Da)		
	A1	A2	A3	B1	B2	B3
Reference product Herclon (R)	148225	148224	148223	148222	148222	148204
	Deamidation	Deamidation	Deamidation	Isomerization	Isomerization	Succinimide
Biosimilar Biceltis (T_1)	148064	148063	148060	148059	148059	148042
	Deamidation	Deamidation	Deamidation	Isomerization	Isomerization	Succinimide
Biosimilar Canmab (T_2)	148228	148227	148223	148222	148222	148205
	Deamidation	Deamidation	Deamidation	Isomerization	Isomerization	Succinimide
Biosimilar Trasturel (T_3)	148224	148223	148222	148221	148221	148203
	Deamidation	Deamidation	Deamidation	Isomerization	Isomerization	Succinimide
Biosimilar Vivitra (T_4)	148232	148231	148225	148222	148222	-
	Deamidation	Deamidation	Deamidation	Isomerization	Isomerization	

Conclusions

- The 2D-LC method offers a single workflow for simultaneous titer and charge variant estimation of cell culture harvest of mAbs.
- The proposed method is comparable to and is faster than the traditional methods.
- The method takes only 8 minutes without mass and 15 minutes with mass determination.
- The linearity assessment of the 2D-LC method at injected amounts of 5-160 ug of mAb show correlation values of 0.9999 and 0.9998.
- The proposed method can be successfully used to establish biosimilarity in mAbs.
- Major charge species resolved and identified in just 15 minutes, using the intact MAM method.

References

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