ANALYTICAL CHARACTERIZATION AND COMPARABILITY ASSESSMENT OF BIOTHERAPEUTIC PROTEINS

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ANALYTICAL CHARACTERIZATION AND COMPARABILITY ASSESSMENT OF BIOTHERAPEUTIC PROTEINS

by

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Dedicated to my family

Certificate

This is to certify that the thesis entitled “ANALYTICAL CHARACTERIZATION AND COMPARABILITY ASSESSMENT OF BIOTHERAPEUTIC PROTEINS” being submitted by NEH NUPUR to the Indian Institute of Technology Delhi for the award of the degree of Doctor of Philosophy is a record of the original bonafide research work carried out by her under my guidance and supervision. The results contained in this thesis have not been submitted in part or in full to any other University or Institute for the award of any degree or diploma.

I certify that she has pursued the prescribed course of research.

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NEH NUPUR

Abstract

The ever-increasing global prevalence of the various human diseases, the advancements in treatment methods and the demand for targeted therapies have fueled rapid commercialization of biotherapeutic proteins. Despite their enormous potential in providing an efficient model of healthcare, the associated quality, safety, and efficacy issues are a major concern. Following the patent cliff of certain innovator products, biosimilar development has gained momentum and has resulted in several biosimilar approvals across the globe. Owing to structural complexities of biotherapeutic proteins, regulatory agencies mandate thorough analytical characterization of these products, including the microheterogeneity, batch-to-batch variability, stability studies, and impurity profiling as per International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q6B guidelines. Analytical characterization and comparability assessment of critical quality attributes (CQAs) of these proteins consist of extensive physicochemical and functional characterization using appropriate, validated, and orthogonal analytical methods. A major hurdle in this entire exercise is to accurately quantify the various CQAs as well as our limited understanding of the CQAs and their inter-attribute relationships. Another challenge is to assess the impact of each CQA on the product's bioactivity. Hence, an analytical characterization platform should consist of modality and/ or product-specific, tailor-made methods that have been optimized as per the product under consideration.

The first objective presents the comprehensive comparability exercise performed on biotherapeutic products namely insulin glargine, filgrastim (two microbial products) and rituximab (one mammalian product) by means of a wide array of state-of-the-art, validated, and orthogonal analytical methods in accordance with the regulatory guidelines on Similar Biologics. It offers robust evidence of physicochemical and functional similarity of the biosimilars approved in the Indian market with the pertinent innovator products. It also provides significant analytical evidence to help reduce reliance on non-clinical, preclinical, and clinical studies and increase biosimilar acceptance among patients for affordable healthcare. For insulin glargine, an overall analytical comparability was observed across biosimilars with respect to Lantus®, low amounts of product-related variants were observed in Biosimilar 4 with glutamine deamidation, which was found to impact product stability. Also, % aggregation at 14 days exhibited statistical correlation with % aggregation at 0 day and the number of months from expiry. For filgrastim, the biosimilars were found to be comparable to Neupogen®, but

an anomaly was also reported, where a conformational variant was identified in the shoulder peak of Biosimilar 3 using reverse phase chromatography with fluorescence detection and electrospray ionization-mass spectrometry initially recognized as oxidized variant. For rituximab, the biosimilars exhibited analytical comparability to Ristova® with respect to structure and function, but significant charge and glycan-based heterogeneities were also observed, which resulted in effect on bioactivity from the in-vitro assays.

The second objective was to elucidate charge and size-based heterogeneities in monoclonal antibody (mAb)-based therapeutic proteins. Product instabilities are a major concern during manufacturing, formulation development, storage, filling, and shipping. Both size-based (fragments and aggregates) and charge-based (basic and acidic variants) heterogeneities of two products, bevacizumab, and trastuzumab were assessed upon application of different stresses i.e., thermal, mechanical, and hypertonic and low pH stresses. Both mAbs exhibited wide variability in charge variant distribution and behaved differently concerning product stability under different stress conditions. However, the presence of certain charge variants dominated mAb degradation. It was observed that thermal stress had the most significant impact, low pH generated a higher proportion of soluble aggregates while salt and thermal stresses induced precipitation. It was concluded that deamidation-induced aggregation was the most significant contributor to product degradation in bevacizumab, and high acidification causing significant loss of the main variant along with deamidation-induced aggregation were observed in trastuzumab.

The third objective was to elucidate the relationship between charge and glycan-based heterogeneities in mAb-based products. By virtue of their origin, mAbs are inherently complex molecules with a range of heterogeneities with respect to charge and glycan in the final formulation, likely to impact the bioactivity, efficacy, and stability of the product. Charge variants are critical in affirming the need for identification and characterization of these modifications. With respect to glycosylation, different charge variants exhibited significant differences in their glycoform distribution, but certain glycoforms dominated certain charge variants. While the acidic variant, A1, consisted of deamidation and pyroglutamate forms, basic variants, B1 and B2, were lysine variants, and B3, contained methionine oxidation. Statistical models were developed to study the impact of glycan distribution on complement-dependent cytotoxicity activity and charge distribution. It was found that while bioactivity statistically correlated with G1F, galactosylated, high mannose, and afucosylated glycans (linearly), it

correlated with G1F/G2F and [G1F/G2]SA (non-linearly). Also, galactosylated glycans showed increasing dependence (non-linearly) for main and basic fractions, whereas sialylated glycans showed increasing dependence (linearly) for acidic fractions.

The fourth objective was to elucidate disulfide heterogeneities in mAb-based products. Reduced and non-reduced peptide mapping by mass spectrometry analysis is a commonly used method for characterization of post translational modifications (PTMs) and disulfide bridging to assess structural integrity, and heterogeneity of the therapeutic proteins. However, non-enzymatic artifacts induced during sample preparation are often observed when basic pH is used during denaturation and digestion. A reduced and non-reduced peptide mapping method has been proposed for mAb's primary structure characterization with low concentration of reducing and alkylating agent at high temperature under acidic pH. Two rituximab products, commercial and in-house, were used as model proteins to develop and optimize the method. Using this method, PTMs and disulfide scrambled peptides were significantly accessed and found to be less compared to traditional methods at alkaline pH. Results demonstrated that the method is robust, identifies and minimizes artifacts produced during the sample preparation.

Overall, the work presented in the thesis aims to establish robust analytical platforms for characterization and comparability studies of biotherapeutic proteins and provide an insight into quality attributes and the cofactors affecting stability and bioactivity. This information is of significant interest to biopharma manufacturers as well as academic researchers working on development of biotherapeutic products.

सार

विभिन्न मानव रोगों के बढ़ते वैश्विक प्रसार, उपचार विधियों में प्रगति और लक्षित उपचारों की मांग ने जैव-चिकित्सीय प्रोटीन के तेजी से व्यावसायीकरण को जन्म दिया है । स्वास्थ्य देखभाल का एक कुशल मॉडल प्रदान करने में उनकी विशाल क्षमता के बावजूद, गुणवत्ता, सुरक्षा और प्रभावकारिता के मुद्दे एक प्रमुख चिंता का विषय हैं । कुछ नवोन्मेषी उत्पादों की पेटेंट समाप्ति के बाद, बायोसिमिलर विकास ने गति प्राप्त की है और इसके परिणामस्वरूप दुनिया भर में कई बायोसिमिलर अनुमोदन प्राप्त हुए हैं । जैव-चिकित्सीय की संरचनात्मक जटिलताओं के कारण आईसीएच क्यू6बी दिशानिर्देशों के अनुसार सूक्ष्म विषमता, बैच-टू-बैच परिवर्तनशीलता, स्थिरता अध्ययन और अशुद्धि प्रोफाइलिंग सहित नियामक एजेंसियों के संपूर्ण विश्लेषणात्मक लक्षण वर्णन को अनिवार्य करता है । इन प्रोटीनों की महत्वपूर्ण गुणवत्ता विशेषताओं (सीक्यूए) के विश्लेषणात्मक लक्षण वर्णन और तुलनात्मक मूल्यांकन में उपयुक्त, मान्य, ऑर्थोगोनल विश्लेषणात्मक विधियों का उपयोग करके व्यापक भौतिक रासायनिक और कार्यात्मक लक्षण वर्णन शामिल है । संपूर्ण अभ्यास में एक बड़ी बाधा विभिन्न सीक्यूए को साथ-साथ और सटीक रूप से निर्धारित करना और सीक्यूए के अंतर-विशेषता संबंधों के बारे में हमारी सीमित समझ है । एक अन्य चुनौती उत्पाद की जैवसक्रियता पर प्रत्येक सीक्यूए के प्रभाव का आकलन करना है, इसलिए एक विश्लेषणात्मक लक्षण वर्णन मंच में एक सुसंगत दवा उत्पाद देने के लिए उत्पाद विकास रणनीतियों के अनुसार सर्वोत्तम रूप से अनुकूलित तौर-तरीके और उत्पाद-विशिष्ट, दर्जी-निर्मित तरीके शामिल होने चाहिए ।

पहला उद्देश्य जैव-चिकित्सीय उत्पादों जैसे इंसुलिन ग्लार्गिन, फिल्ग्रास्टिम (दो माइक्रोबियल उत्पाद) और रीटक्सिमैब (एक स्तनधारी उत्पाद) पर अत्याधुनिक, मान्य और ऑर्थोगोनल विश्लेषणात्मक तरीकों की एक विस्तृत श्रृंखला के माध्यम से व्यापक तुलनात्मक अभ्यास समान जीवविज्ञान पर नियामक दिशानिर्देशों के अनुसार प्रस्तुत करता है। यह प्रासंगिक इनोवेटर उत्पादों के साथ भारतीय बाजार में स्वीकृत बायोसिमिलर की भौतिक-रासायनिक और कार्यात्मक समानता का मजबूत प्रमाण प्रस्तुत करता है । यह गैर-नैदानिक, प्रीक्लिनिकल और क्लिनिकल अध्ययनों पर निर्भरता को कम करने और सस्ती स्वास्थ्य देखभाल के लिए रोगियों के बीच बायोसिमिलर स्वीकृति बढ़ाने में मदद करने के लिए महत्वपूर्ण विश्लेषणात्मक साक्ष्य प्रदान करता है । इंसुलिन ग्लार्गिन के लिए, लैंटस के संबंध में बायोसिमिलर में एक समग्र विश्लेषणात्मक तुलना देखी गई है, लेकिन बायोसिमिलर 4 में ग्लूटामाइन डीमिडेशन के साथ उत्पाद-संबंधित वेरिएंट की कम मात्रा देखी गई है, जो उत्पाद स्थिरता को प्रभावित करने के लिए पाया

गया है । इसके अलावा, 14 दिनों के % एकत्रीकरण ने 0 दिन के % एकत्रीकरण और समाप्ति से महीनों की संख्या के साथ सांख्यिकीय सहसंबंध प्रदर्शित किया है । फिल्ग्रास्टिम के लिए, बायोसिमिलर को न्यूपोजेन से तुलनीय पाया गया है, लेकिन एक विसंगति भी बताई गई है, जहां आरपीसी-एफएलडी और ईएसआई-एमएस का उपयोग करते हुए बायोसिमिलर 3 के शोल्डर शिखर में एक गठनात्मक संस्करण की पहचान की गई है, जिसे शुरू में ऑक्सीकृत संस्करण के रूप में मान्यता दी गई थी । रीटक्सिमैब के लिए, बायोसिमिलर ने संरचना और कार्य के संबंध में रिस्तोवा के लिए विश्लेषणात्मक तुलनात्मकता प्रदर्शित की, लेकिन महत्वपूर्ण चार्ज और ग्लाइकेन-आधारित विषमताएं देखी गईं है, जिसके परिणामस्वरूप इन-विट्रो परख से बायोएक्टिविटी पर कुछ प्रभाव पड़ा है ।

दूसरा उद्देश्य मोनोक्लोनल एंटीबॉडी आधारित चिकित्सीय प्रोटीन में चार्ज और आकार-आधारित विषमताओं को स्पष्ट करना है । उत्पाद अस्थिरता निर्माण, निर्माण विकास, भंडारण और परिवहण के दौरान एक प्रमुख चिंता का विषय है । दोनों आकार-आधारित (टुकड़े और समुच्चय) और चार्ज-आधारित (मूल और अम्लीय वेरिएंट) दो मोनोक्लोनल एंटीबॉडी उत्पादों, बेवाकिज़ुमैब, और ट्रैस्टुज़ुमैब की विषमताओं का मूल्यांकन विभिन्न तनावों यानी थर्मल, मैकेनिकल और हाइपरटोनिक और निम्न पीएच तनाव के आवेदन पर किया गया है । दोनों मोनोक्लोनल एंटीबॉडी ने चार्ज वैरिएंट वितरण में व्यापक परिवर्तनशीलता का प्रदर्शन किया है और विभिन्न तनाव स्थितियों के तहत उत्पाद स्थिरता के संबंध में अलग-अलग व्यवहार किया है, हालांकि कुछ चार्ज वेरिएंट की उपस्थिति मोनोक्लोनल एंटीबॉडी गिरावट पर हावी रही । यह देखा गया है कि उष्ण तनाव का सबसे महत्वपूर्ण प्रभाव पाया गया है, कम पीएच ने घुलनशील समुच्चय का उच्च अनुपात उत्पन्न किया है जबकि नमक और उष्ण तनाव ने वर्षण को प्रेरित किया है । यह निष्कर्ष निकाला गया है कि बेवाकिज़ुमैब में उत्पाद की गिरावट के लिए डीमिडेशन-प्रेरित एकत्रीकरण सबसे महत्वपूर्ण योगदानकर्ता है और डीमिडेशन-प्रेरित एकत्रीकरण के साथ- साथ उच्च अम्लीकरण और मुख्य प्रकार के नुकसान को ट्रैस्टुज़ुमैब में देखा गया है ।

तीसरा उद्देश्य मोनोक्लोनल एंटीबॉडी उत्पादों में चार्ज और ग्लाइकेन-आधारित विषमताओं के बीच संबंध को स्पष्ट करना है । अपने मूल के आधार पर, मोनोक्लोनल एंटीबॉडी स्वाभाविक रूप से जटिल अणु होते हैं, जिनमें अंतिम सूत्रीकरण में चार्ज और ग्लाइकेन के संबंध में कई प्रकार की विषमताएं होती हैं, जो उत्पाद की जैव-सक्रियता, प्रभावकारिता और स्थिरता को प्रभावित कर सकती हैं। इन संशोधनों की पहचान और लक्षण वर्णन की आवश्यकता की पुष्टि करने में चार्ज वेरिएंट महत्वपूर्ण हैं। ग्लाइकोसिलेशन के संबंध में, विभिन्न चार्ज वेरिएंट ने अपने ग्लाइकोफॉर्म वितरण में महत्वपूर्ण अंतर प्रदर्शित किया है, लेकिन कुछ ग्लाइकोफॉर्म को कुछ चार्ज वेरिएंट पर हावी पाया गया है । मूल रूप से अम्लीय संस्करण,

ए1, में एस्पारागीन डीमिडेशन और पाइरोग्लूटामेट रूप शामिल है, बी1 और बी2, लाइसिन वेरिएंट है, और बी3 में मेथियोनीन ऑक्सीकरण है । सीडीसी परख और चार्ज वितरण पर ग्लाइकेन वितरण के प्रभाव का अध्ययन करने के लिए सांख्यिकीय मॉडल विकसित किया गया है और यह पाया गया कि सीडीसी परख सांख्यिकीय रूप से G1F/G1F, G1F, गैलेक्टोसिलेटेड, उच्च मैनोस, और अफ्यूकोसिलेटेड (रैखिक रूप से), और [G1F/G2]SA (गैर-रैखिक रूप से) रूपों के साथ सहसंबद्ध है। इसके अलावा, गैलेक्टोसिलेटेड रूपों ने मुख्य और बुनियादी अंशों के लिए बढ़ती निर्भरता (गैर-रैखिक रूप से) दिखाई, जबकि सियालेटेड रूपों ने अम्लीय अंशों के लिए बढ़ती निर्भरता (रैखिक रूप से) दिखाई है ।

चौथा उद्देश्य मोनोक्लोनल एंटीबॉडी उत्पादों में डाइसल्फ़ाइड विषमताओं को स्पष्ट करना है । मास स्पेक्ट्रोमेट्री विश्लेषण द्वारा रेड्यूसड और गैर-रेड्यूसड पेप्टाइड मैपिंग संरचनात्मक अखंडता, विविधता और चिकित्सीय प्रोटीन की गुणवत्ता का आकलन करने के लिए पीटीएम और डाइसल्फ़ाइड लिंकेज लक्षण वर्णन के लिए आमतौर पर इस्तेमाल की जाने वाली विधि है । हालांकि, आकलन करने के दौरान विषमताओं को अक्सर तब देखा जाता है जब मूल पीएच विकृतीकरण और पाचनक्रिया के दौरान उपयोग किया जाता है । इन विषमताओं को कम करने के लिए, कई शोधकर्ताओं द्वारा विभिन्न अम्लीय पीएच स्थितियों का उपयोग करने वाले तरीके विकसित किए गए हैं । हालांकि, अम्लीय पीएच छूटी हुई और गैर-विशिष्ट दरारों को बढ़ाता है, जो विश्लेषण करता है । इस अध्ययन में, हमने मोनोक्लोनल एंटीबॉडी के लक्षण वर्णन के लिए एक रेड्यूसड और गैर-रेड्यूसड पेप्टाइड मैपिंग विधि का उपयोग किया है जो टीसीईपी के समावेश के साथ- साथ अल्काइलेटिंग एजेंट की कम सांद्रता, उच्च तापमान और अम्लीय पीएच पर विषमताओं को कम करता है । दो उत्पादों, एक स्वीकृत और एक लैब निर्मित रीटक्सिमैब का उपयोग विधि को विकसित और अनुकूलित करने के लिए मॉडल प्रोटीन के रूप में किया गया है । इस पद्धति का उपयोग करते हुए, पीटीएम और गैर डाइसल्फ़ाइड बॉन्ड पेप्टाइड्स को महत्वपूर्ण रूप से एक्सेस किया गया और क्षारीय पीएच पर पारंपरिक तरीकों की तुलना में कम पाया गया है । इस अध्ययन ने इस बात पर परिणाम प्रदर्शित किया है कि यह विधि मजबूत है और पेप्टाइड मैपिंग विधि में नमूना तैयार करने के दौरान उत्पादित विषमताओं को कम करता है।

कुल मिलाकर, इस थीसिस कार्य का उद्देश्य जैव-चिकित्सीय प्रोटीन के लक्षण वर्णन और तुलनात्मक अध्ययन के लिए मजबूत विश्लेषणात्मक प्लेटफॉर्म स्थापित करना है और गुणवत्ता विशेषताओं, स्थिरता और जैव-सक्रियता को प्रभावित करने वाले सहकारकों में एक अंतर्दृष्टि प्रदान करना है। जानकारी का यह टुकड़ा निर्माताओं को कुछ प्रमुख चुनौतियों का समाधान करने में मदद कर सकता है जो कि उत्पाद की गुणवत्ता प्रोफ़ाइल को प्राप्त करने के लिए प्रक्रिया और उत्पाद विकास के दौरान सामना करना पड़ता है ।

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List of Abbreviations

2AB	*2-Aminobenzamide*
2D-LC	*Two-Dimensional Liquid Chromatography*
AAS	*Atomic Absorption Spectroscopy*
ABC	*Ammonium Bicarbonate*
ACN	*Acetonitrile*
ADCC	*Antibody-Dependent Cellular Cytotoxicity*
AF	*Afucosylated Glycans*
AF4	*Asymmetric Field-Flow Fractionation*
APS	*Ammonium Persulfate*
Arg	*Arginine*
Asn	*Asparagine*
Asp	*Aspartate*
CD	*Circular Dichroism Spectroscopy*
CDC	*Complement-Dependent Cytotoxicity*
CDR	*Complementarity-Determining Regions*
CDSCO	*Central Drugs Standard Control Organisation*
CE	*Capillary Electrophoresis*
CEX	*Cation Exchange Chromatography*
CGE	*Capillary Gel Electrophoresis*
CHO	*Chinese Hamster Ovary*
CID	*Collision-Induced Dissociation*
CIEF	*Capillary Isoelectric Focussing*
CM	*Carboxy-Methyl*
cm	*Centimeter*
CpB	*Carboxypeptidase B*
CQA	*Critical Quality Attribute*
CSD	*Comparative Signature Diagram*
Cys	*Cysteine*
CZE	*Capillary Zone Electrophoresis*
Da	*Dalton*
DAD	*Diode Array Detector*
DBT	*Department of Biotechnology*
DLS	*Dynamic Light Scattering*
DMB	*1, 2-Diamino-4, 5-Methylenedioxy-Benzenedihydrochloride*
DMEM	*Dulbecco's Modified Eagle's Medium*
DP	*Drug Product*
DSC	*Differential Scanning Calorimetry*
DTT	*Dithiothreitol*
EC_{50}	*Half Maximal Effective Concentration*
EDTA	*Ethylenediaminetetraacetic Acid*
ELISA	*Enzyme-Linked Immunosorbent Assay*
EMA	*European Medicines Agency*
ESI	*Electrospray Ionization*
ETD	*Electron-Transfer Dissociation*
EU	*European Union*
FA	*Formic acid*
FACS	*Fluorescence-Activated Cell Sorting*

FBS	*Fetal Bovine Serum*
FFF	*Field Flow Fractionation*
FLD	*Fluorescence Detection*
FLR	*Fluorescence Spectroscopy*
FTIR	*Fourier Transform Infrared Spectroscopy*
GAL	*Galactosylated Glycans*
gCQA	*Glycosylation-Related Critical Quality Attribute*
G-CSF	*Granulocyte-Colony Stimulating Factor*
GI	*Glycosimilarity Index*
Gln	*Glutamine*
Gn-HCl	*Guanidine Hydrochloride*
HC	*Heavy Chain*
HCD	*Host Cell DNA*
HCP	*Host Cell Protein*
HDX	*Hydrogen Deuterium Exchange*
HECD	*Higher-Energy C-trap Dissociation*
HEPES	*4-(2-Hydroxy-Ethyl)-1-Piperazine-Ethane-Sulfonic acid*
HILIC	*Hydrophilic Interaction Chromatography*
HM	*High Mannose*
HMWs	*High Molecular Weight Species*
HOS	*Higher Order Structure*
HPAEC	*High Performance Anion Exchange Chromatography*
HSQC	*Heteronuclear Single Quantum Coherence*
IAM	*Iodoacetamide*
ICH	*International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use*
IgG	*Immunoglobulin G*
IM-MS	*Ion Mobility-Mass Spectrometry*
IU	*International Units*
KQA	*Key-Quality Attributes*
LC	*Light Chain*
LDH	*Lactate Dehydrogenase*
LIF	*Laser-Induced Fluorescence*
LMWs	*Low Molecular Weight Species*
LO	*Light Obscuration*
Lys	*Lysine*
m/z	*Mass-to-Charge ratio*
mAB	*Monoclonal Antibody*
MALS	*Multi-Angle Light Scattering*
MAM	*Multi-Attribute Methods*
mAU	*Milli Absorbance Units*
MaxEnt	*Maximum Entropy*
mdeg	*Millidegree*
Met	*Methionine*
MFDS	*Ministry of Food and Drug Safety*
MFE	*Molecular Feature Extraction*
MFI	*Micro Flow Imaging / Mean Fluorescent Intensity*
mg	*Milligram*
min	*Minutes*

mM	*Millimolar*
MRE	*Mean Residue Ellipticity*
MS	*Mass Spectrometry*
MVDA	*Multivariate Data Analysis*
MWCO	*Molecular Weight Cut-Off*
NaCl	*Sodium Chloride*
NAGA	*N-Glycolyl-Neuraminic Acid*
nm	*Nanometer*
NaN_3	*Sodium Azide*
NANA	*N-Acetyl-Neuraminic Acid*
NanoDSF	*Nano Differential Scanning Fluorimetry*
NK	*Natural Killer Cells*
NMR	*Nuclear Magnetic Resonance Spectroscopy*
NPRA	*National Pharmaceutical Regulatory Agency*
NTA	*Nanoparticle Tracking Analysis*
OD	*Optical Density*
PAD	*Pulsed Amperometric Detection*
PBMC	*Peripheral Blood Mononuclear Cells*
PBS	*Phosphate-Buffered Saline*
PCA	*Principal Component Analysis / Procainamide*
PCDL	*Personal Compound Database Library*
PCR	*Polymerase Chain Reaction*
PD	*Pharmacodynamics*
Phe	*Phenylalanine*
pI	*Isoelectric Point*
PK	*Pharmacokinetics*
PLS	*Partial Least Square*
PMDA	*Pharmaceuticals and Medical Devices Agency*
pMod	*Peak Modeling*
ppm	*Parts per Million*
Pro	*Proline*
PSD	*Particle Size Distribution*
PTM	*Post Translational Modifications*
pyroGlu	*Pyroglutamate*
QTOF	*Quadrupole Time-of-Flight*
RPC	*Reverse Phase Chromatography*
RT	*Retention Time*
RRT	*Relative Retention Time*
SA	*Sialylated Glycans*
SBP	*Similar Biotherapeutic Product*
SD	*Standard Deviation*
SDS-PAGE	*Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis*
SEC	*Size Exclusion Chromatography*
SH	*Sulfhydryl group*
SPE	*Solid Phase Extraction*
SPR	*Surface Plasmon Resonance*
SV-AUC	*Sedimentation Velocity-Analytical Ultra-Centrifugation*
T	*Absolute Temperature*
TCC	*Total Compound Chromatogram*

TCEP-HCl	*Tris(2-Carboxy-Ethyl) Phosphine Hydrochloride*
TCSPC	*Time-Correlated Single-Photon Counting*
TEM	*Transmission Electron Microscopy*
TEMED	*Tetramethylethylenediamine*
TFA	*Trifluoroacetic Acid*
TIC	*Total Ion Chromatogram*
TOF	*Time-of-Flight*
Tris-HCl	*Tris Hydrochloride*
Try	*Tryptophan*
Tyr	*Tyrosine*
UATR	*Universal Attenuated Total Reflectance*
USFDA	*United States Food and Drug Administration*
UV-Vis	*Ultraviolet/ Visible*
V_{cap}	*Capillary Voltage*
V_{frag}	*Fragmentor Voltage*
WHO	*World Health Organisation*
XRC	*X-Ray Crystallography*
λ_{max}	*Maximum wavelength*
µL	*Microliter*
°C	*Degree Celsius*