USE OF MULTIATTRIBUTE METHODS FOR CHARACTERIZATION AND STABILITY ASSESSMENT OF RECOMBINANT MONOCLONAL ANTIBODIES

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by

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Certificate

This is to certify that the thesis entitled **"USE OF MULTIATTRIBUTE METHODS FOR CHARACTERIZATION AND STABILITY ASSESSMENT OF RECOMBINANT MONOCLONAL ANTIBODIES"** being submitted by **DEEPIKA SARIN** 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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Abstract

Biopharmaceutical products, specifically monoclonal antibodies (mAbs), have transformed the healthcare industry and have emerged as effective therapies for managing complex and otherwise challenging-to-manage diseases, such as cancer, rheumatism, and autoimmune disorders. However, the manufacturing of mAbs is a complex process involving multiple bioprocessing steps of production and purification that give rise to mAb heterogeneities such as post-translational modifications (PTMs) and degradations. Modifications that specifically impact mAb activity, efficacy, and safety are often referred to as critical quality attributes (CQAs) and require constant monitoring and characterization to ensure consistent product quality. Besides the continuous need for characterization, mAb products are also susceptible to degradation during storage and distribution. For instance, exposure to extreme pH, temperature, or light can impact the mAb's chemical and physical stability. Similarly, oxidizing/reducing agents and mechanical or interfacial stress factors like agitation and freeze-thaw can affect mAb stability. Even mAb excipients, generally used to preserve stability, can harm mAb structure and function under certain conditions. Therefore, monitoring of product quality is critical for maintaining mAb safety and efficacy. This thesis explores two significant issues that are pertinent to delivering safe and efficacious biotherapeutic products. First is creation of cutting-edge approaches for characterization of these products. Second is approach for enhancing stability of biotherapeutic products.

In Chapter 3, the effect of repeated freezing and thawing on mAb oxidation has been evaluated. Freeze-thaw operations during manufacturing, storage, and distribution are known to substantially influence the mAb's chemical and physical stability. Most of the investigations thus far have focused on aggregation arising from freeze-thaw is primarily known to affect the efficacy and immunogenicity of mAbs, while the effects of chemical degradation like oxidation are not explored much. This study (Chapter 3) aims to evaluate the impact of repeated freeze-thaw on the oxidation of two mAbs (trastuzumab and rituximab) in different buffer systems (formulation buffer, phosphate-buffer saline and histidine buffer). An increase in oxidation by 3X is observed for trastuzumab samples in the formulation buffer, while the increase is less (1X oxidation) for rituximab samples. Oxidation of the two mAbs in histidine buffer alone was also assessed, and a significant increase of 7X and 8X oxidation was observed, respectively. The presence of oxidized and charged species was also confirmed with intact mass and peptide mapping analysis. The results highlight the effect of histidine-polysorbate 20 buffer in

promoting mAb oxidation and the stabilizing effects of citrate-polysorbate 80 buffer upon repeated freeze-thaw. Maximum formation of high molecular weight (HMW) species (15.94%) was observed in the phosphate-buffer saline samples of trastuzumab. An increase in acidic (19.54% to 23.22%) and basic variants (5.23% to 9.44%) was observed for rituximab, whereas a decrease in acidic variants (32.60% to 26.63%) was observed for trastuzumab samples.

Stability of complex biotherapeutics like monoclonal antibodies is paramount for their safe and efficacious use. In Chapter 4, impact of higher concentrations of trehalose dihydrate, a commonly used osmolyte as mAb excipient, on mAb stability has been explored. Excipients are inactive ingredients that are added to the purified product so as to offer it a stable environment. Trehalose dihydrate is a non-reducing sugar that is commonly used as a stabilizing agent in biotherapeutic formulations under liquid and frozen states. The stabilizing effect of trehalose against aggregation in protein formulations is well known. The present study (Chapter 4) aims to offer insights into the stability effects of higher trehalose concentration (230 mM) on liquid trastuzumab under different forced stress conditions including thermal, light with and without hydrogen peroxide (H_2O_2), and humidity stresses. Under thermal stress, while HMW accounted for 38.80% in the trastuzumab sample without trehalose, it was 4.89% at high trehalose concentration. Similarly, under light stress with H_2O_2, the trastuzumab sample without trehalose had >80% more HMW than at high trehalose concentration. Two other IgG1 mAbs (rituximab and bevacizumab) were also evaluated for stability at higher trehalose concentrations (230 mM). Similar to trastuzumab, stabilization was observed under thermal stress for rituximab and bevacizumab at higher trehalose concentration compared to samples without trehalose (21.90% and 29.90% HMW, respectively). Likewise, accelerated (under humidity stress) stress induced secondary and tertiary structure disruptions were reduced at higher trehalose concentration. Overall, mAb stability under forced stress conditions improved significantly at higher trehalose concentrations. While higher trehalose concentration (>200 mM) is used in mAb formulations and is known to minimise aggregation under thermal stress, however, the current study aims to also explore the stability imparted under light (with H_2O_2), and humidity stresses for three different mAbs.

The studies conducted in Chapter 3 and 4 reflect the stability concerns for mAb products that alter its structure and induce physical/chemical heterogeneity. Thus, the manufacture of safe and efficacious mAb products is contingent upon the implementation of comprehensive monitoring of heterogeneity in biopharmaceutical development. However, extensive increase in mAb approvals combined with limitations of current analytical methods has given rise to the

need for innovative analytical methods for mAb characterization. Multi-attribute 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 protein A chromatography (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 minutes due to the required fraction collection and buffer exchange steps. In this work (Chapter 5), a native two-dimensional liquid chromatography (2DLC) mass spectrometry method has been developed with Protein A chromatography in the first dimension for titer estimation and 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 2DLC method exhibits a charge variant profile that is similar to that observed *via* the traditional methods and takes only 15 minutes 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 ug to 160 ug 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.

Charged heterogeneity of mAb products is regarded as a CQA depending on its impact on the safety and efficacy profile of the product. Hence, manufacturers are expected to perform a comprehensive characterization of the charge heterogeneity to ensure that the manufactured product meets its specifications. Further, monitoring is also expected during the product lifecycle to demonstrate consistency in product quality. However, conventional analytical methods for characterization of hydrophobic and charge variants are non-volatile salt-based and require manual fraction collection and desalting steps before analysis through mass spectrometry can be performed. In Chapter 6, a workflow of a two-dimensional liquid chromatography method using MS-compatible buffers coupled with native mass spectrometry was performed to characterize hydrophobic variants in the first dimension and charge variants in the second dimension without any need of manual fractionation. This novel 2D HIC-WCX-MS workflow identified 10 variants in mAb A out of which 2 variants are exclusive to the 2D orthogonal method. Similarly, for mAb B a total of 11 variants are identified including 5

variants to be exclusive to the 2D orthogonal workflow. When compared to stand-alone HIC resolved only 4 variants for both mAbs and WCX resolved 7 variants for mAb A and 6 variants for mAb B. In addition, the proposed method allows direct characterization of hydrophobic/charge variant peaks through native mass spectrometry in a single run workflow.

Though 2DLC methods have gained popularity for resolving complex charged species, capillary electrophoresis (CE) is regarded as a sensitive and faster tool for charged species estimation in biotherapeutics. In Chapter 6, we also aim to combine the separation power of chromatographic and electrophoretic tools (LC-CE) so as to achieve maximum resolution of mAb charge variants. Hydrophobic interaction chromatography (HIC) is the preferred LC mode with CE for achieving successful separation of both charge and hydrophobic variants for two of the mAbs (trastuzumab: mAb A and rituximab: mAb B). The standalone HIC and CZE methods separated 4 hydrophobic variants and 7 charge variants for each mAb, while the 2DLC method separated 10 variants for mAb A and 11 variants mAb B. On the other hand, the HIC-CZE-UV method resolved 29 variants and 23 variants in mAb A and mAb B, respectively. The reproducibility of the HIC-CZE-UV method was demonstrated by %change in values of RT and peak area as < 5% (mAb A) < 3% (mAb B) and < 12% (for both mAbs), respectively. Thus, the utility of the proposed LC-CE method for characterization of mAb charge variants has been displayed.

Overall, the studies presented in this thesis focus on understanding the stability concerns in mAbs and developing advanced MAM methods to improve characterization and monitoring of mAb CQAs.

सारांश

बायोफार्मास्युटिकल उत्पाद, विशेष रूप से मोनोक्लोनल एंटीबॉडी (एमएबीएस), ने हेल्थकेयर उद्योग को बदल दिया है और कैंसर, गठियावाद और ऑटोइम्यून विकारों जैसे जटिल और अन्यथा चुनौतीपूर्ण-प्रबंधन रोगों के प्रबंधन के लिए प्रभावी उपचार के रूप में उभरा है। हालांकि, एमएबीएस का निर्माण एक जटिल प्रक्रिया है जिसमें उत्पादन और शुद्धि के कई बायोप्रोसेसिंग चरण शामिल हैं जो एमएबी विषमताओं जैसे कि पोस्ट-ट्रांसलेशनल संशोधनों (पीटीएम) और गिरावट को जन्म देते हैं। संशोधनों जो विशेष रूप से एमएबी गतिविधि, प्रभावकारिता और सुरक्षा को प्रभावित करते हैं, उन्हें अक्सर महत्वपूर्ण गुणवत्ता विशेषताओं (सीक्यूए) के रूप में संदर्भित किया जाता है और लगातार उत्पाद की गुणवत्ता सुनिश्चित करने के लिए निरंतर निगरानी और लक्षण वर्णन की आवश्यकता होती है। लक्षण वर्णन की निरंतर आवश्यकता के अलावा, एमएबी उत्पाद भी भंडारण और वितरण के दौरान गिरावट के लिए अतिसंवेदनशील होते हैं। उदाहरण के लिए, चरम पीएच, तापमान या प्रकाश के संपर्क में एमएबी के रासायनिक और भौतिक स्थिरता को प्रभावित कर सकते हैं। इसी तरह, एजेंटों और यांत्रिक या इंटरफेसियल तनाव कारकों जैसे आंदोलन और फ्रीज-थाव को ऑक्सीकरण/कम करना MAB स्थिरता को प्रभावित कर सकता है। यहां तक कि mAb excipients, आमतौर पर स्थिरता को संरक्षित करने के लिए उपयोग किया जाता है, कुछ शर्तों के तहत MAB संरचना और कार्य को नुकसान पहुंचा सकता है। इसलिए, एमएबी सुरक्षा और प्रभावकारिता को बनाए रखने के लिए उत्पाद की गुणवत्ता की निगरानी महत्वपूर्ण है। पहले इन उत्पादों के लक्षण वर्णन के लिए अत्याधुनिक दृष्टिकोण का निर्माण है। दूसरा बायोथेरेप्यूटिक उत्पादों की स्थिरता को बढ़ाने के लिए दृष्टिकोण है।

अध्याय 3 में, एमएबी ऑक्सीकरण पर बार -बार ठंड और विगलन के प्रभाव का मूल्यांकन किया गया है। विनिर्माण, भंडारण और वितरण के दौरान फ्रीज-थाव संचालन को एमएबी के रासायनिक और भौतिक स्थिरता को काफी प्रभावित करने के लिए जाना जाता है। इस प्रकार अब तक की अधिकांश जांच फ्रीज-पिघलने से उत्पन्न होने वाले एकत्रीकरण पर ध्यान केंद्रित करती हैं, मुख्य रूप से mAbs की प्रभावकारिता और प्रतिरक्षा को प्रभावित करने के लिए जाना जाता है, जबकि ऑक्सीकरण जैसे रासायनिक गिरावट के प्रभावों को ज्यादा नहीं खोजा जाता है। इस अध्ययन (अध्याय 3) का उद्देश्य विभिन्न बफर सिस्टम (फॉर्मूलेशन बफर, फॉस्फेट-बफर खारा और हिस्टिडीन बफर) में दो एमएबीएस के ऑक्सीकरण पर बार-बार फ्रीज-थाव के प्रभाव का मूल्यांकन करना है। फॉर्मूलेशन बफर में ट्रैस्टुजुमाब नमूनों के लिए 3x द्वारा ऑक्सीकरण में वृद्धि देखी जाती है, जबकि रीटक्सिमैब नमूनों के लिए वृद्धि कम (1x ऑक्सीकरण)

है। अकेले हिस्टिडीन बफर में दो mAbs के ऑक्सीकरण का भी मूल्यांकन किया गया था, और क्रमशः 7x और 8x ऑक्सीकरण की उल्लेखनीय वृद्धि देखी गई थी। ऑक्सीकृत और चार्ज प्रजातियों की उपस्थिति को भी बरकरार द्रव्यमान और पेप्टाइड मैपिंग विश्लेषण के साथ पुष्टि की गई थी। परिणाम एमएबी ऑक्सीकरण को बढ़ावा देने में हिस्टिडीन-पॉलीसोर्बेट 20 बफर के प्रभाव को उजागर करते हैं और बार-बार फ्रीज-थॉ पर साइट्रेट-पॉलीसोर्बेट 80 बफर के स्थिर प्रभाव को बढ़ाते हैं। उच्च आणविक भार (एचएमडब्ल्यू) प्रजातियों (15.94%) का अधिकतम गठन ट्रैस्टुजुमाब के फॉस्फेट-बफर खारा नमूनों में देखा गया था। अम्लीय में वृद्धि (19.54% से 23.22%) और बुनियादी वेरिएंट (5.23% से 9.44%) रितक्सिमैब के लिए देखी गई, जबकि ट्रैस्टुज़ुमैब नमूनों के लिए अम्लीय वेरिएंट (32.60% से 26.63%) में कमी देखी गई।

मोनोक्लोनल एंटीबॉडी जैसे जटिल बायोथेरेप्यूटिक्स की स्थिरता उनके सुरक्षित और प्रभावकारी उपयोग के लिए सर्वोपरि है। अध्याय 4 में, Trehalose dihydrate के उच्च सांद्रता का प्रभाव, mAb स्थिरता पर mAb excipient के रूप में आमतौर पर इस्तेमाल किया जाने वाला ऑस्मोल्टे का पता लगाया गया है। Excipients निष्क्रिय तत्व हैं जिन्हें शुद्ध उत्पाद में जोड़ा जाता है ताकि यह एक स्थिर वातावरण की पेशकश की जा सके। Trehalose dihydrate एक गैर-कम करने वाली चीनी है जिसे आमतौर पर तरल और जमे हुए राज्यों के तहत बायोथेरेप्यूटिक योगों में एक स्थिर एजेंट के रूप में उपयोग किया जाता है। प्रोटीन योगों में एकत्रीकरण के खिलाफ ट्रेहलोज का स्थिर प्रभाव अच्छी तरह से जाना जाता है। वर्तमान अध्ययन (अध्याय 4) का उद्देश्य थर्मल, हाइड्रोजन पेरोक्साइड (H_2O_2), और आर्द्रता तनावों के साथ थर्मल, प्रकाश के साथ और बिना अलग -अलग जबरन तनाव स्थितियों के तहत तरल ट्रैस्टुज़ुमैब पर उच्च ट्रेलोज एकाग्रता (230 मिमी) के स्थिरता प्रभावों में अंतर्दृष्टि प्रदान करना है। थर्मल तनाव के तहत, जबकि एचएमडब्ल्यू ने ट्रेहलोज के बिना ट्रैस्टुज़ुमाब नमूने में 38.80% का हिसाब लगाया, यह उच्च ट्रेलोज एकाग्रता में 4.89% था। इसी तरह, H_2O_2 के साथ हल्के तनाव के तहत, trahalose के बिना trastuzumab नमूना उच्च trehalose एकाग्रता की तुलना में 80% अधिक HMW था। दो अन्य IgG1 mAbs (rituximab और bevacizumab) का मूल्यांकन भी उच्च ट्रेलोज सांद्रता (230 मिमी) पर स्थिरता के लिए किया गया था। Trastuzumab के समान, स्थिरीकरण को Trehalose (21.90% और 29.90% HMW, क्रमशः) के बिना नमूनों की तुलना में उच्च ट्रेलोस एकाग्रता में रितक्सिमैब और बेवाकिज़ुमाब के लिए थर्मल तनाव के तहत देखा गया था। इसी तरह, त्वरित (आर्द्रता तनाव के तहत) तनाव प्रेरित माध्यमिक और तृतीयक संरचना में व्यवधान उच्च ट्रेलोज एकाग्रता में कम हो गए थे। कुल मिलाकर, जबरन तनाव की स्थिति के तहत एमएबी स्थिरता ने उच्च ट्रेलोज सांद्रता में काफी सुधार किया। जबकि उच्च ट्रेलोज एकाग्रता (> 200 मिमी) का उपयोग एमएबी योगों में किया जाता है और इसे थर्मल तनाव के तहत एकत्रीकरण को कम

करने के लिए जाना जाता है, हालांकि, वर्तमान अध्ययन का उद्देश्य प्रकाश के तहत प्रदान की गई स्थिरता (एच 2 ओ 2 के साथ), और तीन अलग -अलग एमएबी के लिए आर्द्रता तनाव का भी पता लगाना है।

अध्याय 3 और 4 में किए गए अध्ययन एमएबी उत्पादों के लिए स्थिरता की चिंताओं को दर्शाते हैं जो इसकी संरचना को बदलते हैं और भौतिक/रासायनिक विषमता को प्रेरित करते हैं। इस प्रकार, सुरक्षित और प्रभावशाली एमएबी उत्पादों का निर्माण बायोफार्मास्यूटिकल विकास में विषमता की व्यापक निगरानी के कार्यान्वयन पर आकस्मिक है। हालांकि, वर्तमान विश्लेषणात्मक तरीकों की सीमाओं के साथ संयुक्त एमएबी अनुमोदन में व्यापक वृद्धि ने एमएबी लक्षण वर्णन के लिए अभिनव विश्लेषणात्मक तरीकों की आवश्यकता को जन्म दिया है। मल्टी-एट्रीब्यूट मॉनिटरिंग (MAM) Deamidation, Sialylation, Glycosylation और Oxidation जैसे mAb विषमताओं की निगरानी के लिए एक कुशल उपकरण के रूप में उभरा है। एमएबी विकास के दौरान परंपरागत बायोफार्मा विश्लेषण मास स्पेक्ट्रोमेट्री (एमएस) के साथ प्रत्यक्ष युग्मन के बिना गैर-वाष्पशील सॉल्वैंट्स का उपयोग करके टिटर और चार्ज-आधारित विषमता की निगरानी के लिए एक आयामी तरीकों के उपयोग पर निर्भर करता है। इस दृष्टिकोण को चार्ज-आधारित विषमता का आकलन करने के लिए शुद्ध नमूना के अलग-अलग कटियन एक्सचेंज क्रोमैटोग्राफी (CEX) विश्लेषण के बाद टिटर अनुमान के लिए प्रोटीन ए क्रोमैटोग्राफी (PROA) द्वारा MAB फसल के विश्लेषण की आवश्यकता होती है। यह आवश्यक अंश संग्रह और बफर एक्सचेंज चरणों के कारण 60-90 मिनट तक का समय लग सकता है। इस काम में (अध्याय 5), एक देशी दो-आयामी तरल क्रोमैटोग्राफी (2DLC) मास स्पेक्ट्रोमेट्री विधि को प्रोटीन के साथ एक क्रोमैटोग्राफी के साथ विकसित किया गया है, जो चार्ज वेरिएंट विश्लेषण के लिए दूसरे आयाम में टिटर अनुमान और CEX के लिए पहले आयाम में क्रोमैटोग्राफी है। विधि दोनों आयामों के लिए वाष्पशील लवण का उपयोग करती है और एमएस के लिए आसान युग्मन को सक्षम करती है। प्रस्तावित 2DLC विधि एक चार्ज वेरिएंट प्रोफ़ाइल प्रदर्शित करती है जो पारंपरिक तरीकों के माध्यम से देखी गई समान है और प्रत्येक संस्करण की जन पहचान के लिए केवल 15 मिनट लगती है। टिटर अनुमान के बाद CEX विश्लेषण द्वारा कुल छह चार्ज वेरिएंट को अलग कर दिया गया था, जिसमें 5 कुरूपता के लिए 5 कुरूपता का आकलन शामिल है। प्रस्तावित विधि ने एमएबी इनोवेटर और 4 इसके बायोसिमिलर के 4 के लिए चार्ज वेरिएंट का सफलतापूर्वक अनुमान लगाया, बायोसिमिलरिटी एक्सरसाइज के लिए इसकी प्रयोज्यता को प्रदर्शित किया। इसलिए, 2D PROA CEX MS विधि एक ही वर्कफ़्लो में MABs के प्रत्यक्ष टिटर और चार्ज वेरिएंट अनुमान की अनुमति देती है।

MAB उत्पादों की चार्ज विषमता को उत्पाद की सुरक्षा और प्रभावकारिता प्रोफ़ाइल पर इसके प्रभाव के आधार पर CQA के रूप में माना जाता है। इसलिए, निर्माताओं से अपेक्षा की जाती है कि वे यह सुनिश्चित करें कि निर्मित उत्पाद अपने विनिर्देशों को पूरा करता है, यह सुनिश्चित करने के लिए चार्ज विषमता का एक व्यापक लक्षण वर्णन करता है। इसके अलावा, उत्पाद की गुणवत्ता में स्थिरता प्रदर्शित करने के लिए उत्पाद जीवनचक्र के दौरान भी निगरानी की उम्मीद है। हालांकि, हाइड्रोफोबिक और चार्ज वेरिएंट के लक्षण वर्णन के लिए पारंपरिक विश्लेषणात्मक तरीके गैर-वाष्पशील नमक-आधारित हैं और मास स्पेक्ट्रोमेट्री के माध्यम से विश्लेषण से पहले मैनुअल अंश संग्रह और डिसालिंग चरणों की आवश्यकता होती है। अध्याय 6 में, देशी द्रव्यमान स्पेक्ट्रोमेट्री के साथ मिलकर एमएस-संगत बफ़र्स का उपयोग करके दो-आयामी तरल क्रोमैटोग्राफी विधि का एक वर्कफ़्लो पहले आयाम में हाइड्रोफोबिक वेरिएंट को चिह्नित करने के लिए किया गया था और मैनुअल अंशांकन की आवश्यकता के बिना दूसरे आयाम में वेरिएंट को चार्ज किया गया था। इस उपन्यास 2 डी एचआईसी-डब्ल्यूसीएक्स-एमएस वर्कफ़्लो ने एमएबी में 10 वेरिएंट की पहचान की, जिसमें से 2 वेरिएंट 2 डी ऑर्थोगोनल विधि के लिए अनन्य हैं। इसी तरह, mAb b के लिए कुल 11 वेरिएंट की पहचान की जाती है, जिसमें 5 वेरिएंट शामिल होते हैं, जो 2 डी ऑर्थोगोनल वर्कफ़्लो के लिए अनन्य होते हैं। जब स्टैंड-अलोन एचआईसी की तुलना में एमएबीएस और डब्ल्यूसीएक्स दोनों के लिए केवल 4 वेरिएंट हल किए गए, तो एमएबी ए के लिए 7 वेरिएंट और एमएबी बी के लिए 6 वेरिएंट को हल किया, इसके अलावा, प्रस्तावित विधि एक एकल रन वर्कफ़्लो में देशी मास स्पेक्ट्रोमेट्री के माध्यम से हाइड्रोफोबिक/चार्ज वेरिएंट चोटियों के प्रत्यक्ष लक्षण वर्णन की अनुमति देती है।

हालांकि 2DLC तरीकों ने जटिल चार्ज प्रजातियों को हल करने के लिए लोकप्रियता हासिल की है, केशिका वैद्युतकणसंचलन (CE) को बायोथेरेप्यूटिक्स में चार्ज किए गए प्रजातियों के अनुमान के लिए एक संवेदनशील और तेज उपकरण माना जाता है। अध्याय 6 में, हम क्रोमैटोग्राफिक और इलेक्ट्रोफोरेटिक टूल्स (एलसी-सीई) की पृथक्करण शक्ति को संयोजित करने का लक्ष्य रखते हैं ताकि एमएबी चार्ज वेरिएंट के अधिकतम रिज़ॉल्यूशन को प्राप्त किया जा सके। हाइड्रोफोबिक इंटरैक्शन क्रोमैटोग्राफी (एचआईसी) दो mAbs (trastuzumab: mAb a और rituximab: mAb B) के लिए चार्ज और हाइड्रोफोबिक वेरिएंट दोनों के सफल पृथक्करण को प्राप्त करने के लिए सीई के साथ पसंदीदा एलसी मोड है। स्टैंडअलोन एचआईसी और CZE विधियों ने प्रत्येक mAb के लिए 4 हाइड्रोफोबिक वेरिएंट और 7 चार्ज वेरिएंट को अलग कर दिया, जबकि 2DLC विधि ने mAb A और 11 वेरिएंट MAb B के लिए 10 वेरिएंट को अलग कर दिया। दूसरी ओर, HIC-CZE-UV विधि ने क्रमशः MAB A और MAB B में 29 वेरिएंट और 23 वेरिएंट को हल किया। HIC-CZE-UV विधि की प्रजनन क्षमता को क्रमशः RT और Peak क्षेत्र के मूल्यों में% परिवर्तन द्वारा <5% (MAB A) <3% (MAB B) और <12% (दोनों MABs के लिए) के रूप में

प्रदर्शित किया गया था। इस प्रकार, MAB चार्ज वेरिएंट के लक्षण वर्णन के लिए प्रस्तावित LC-CE विधि की उपयोगिता प्रदर्शित की गई है।

कुल मिलाकर, इस थीसिस में प्रस्तुत किए गए अध्ययन mAbs में स्थिरता की चिंताओं को समझने और MAB CQAs के लक्षण वर्णन और निगरानी में सुधार करने के लिए उन्नत MAM तरीकों को विकसित करने पर ध्यान केंद्रित करते हैं।

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