simAbs

White paper

Improving the quality of a therapeutic antibody by continuous manufacturing

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Abstract

The simAbs continuous production platform has been designed to consistently produce high-quality biotherapeutics. In this white paper, we demonstrate the comparability and consistency in terms of product quality of a monoclonal antibody produced by continuous manufacturing with its originator molecule. This side-by-side assessment focusses on structural identity, purity, integrity and product quality. We show that continuous manufacturing of a biosimilar shows consistent and improved critical quality attributes compared to its fed-batch originator antibody.

Introduction

Over the last decade, the pharmaceutical industry has been facing an increasing demand for the production of highly advanced biologicals. Progress in the understanding of complex diseases enabled researchers to design breakthrough therapies to treat some of the most devastating diseases we are currently facing. These highly expensive treatments have not only become a burden on the patients, but also on the healthcare system. Finding a more cost-efficient production platform is therefore needed to broaden current production capabilities but more importantly, to reduce the total production costs[1]. The manufacturing of biotherapeutics, including monoclonal antibodies (mAbs), is a complex procedure and is associated with high production and operating costs. To address these drawbacks, simAbs designed an innovative continuous manufacturing platform that allows for the production of biotherapeutics at a low-cost while still achieving high quality standards. The platform relies on continuous bioproduction and product purification. The structure and biological activity of mAbs, as well as other biologicals, is highly sensitive to subtle changes in the manufacturing conditions. Therefore, it is of utmost importance to prove the robustness and consistency of the manufactured product. In order to obtain this crucial information, we performed a case study for the commercially available Trastuzumab (Herceptin®), where we assessed the quality profile of the product manufactured using our continuous production platform and benchmarked this to the originator molecule.

This report discusses a follow-up study of the work that has been described in a previously published white paper *"The simAbs continuous production platform ensures high product quality and biological activity of biotherapeutics"*. Here, we will specifically focus on product identity, integrity and quality by employing a broad panel of analytical techniques.

Materials and Method

Continuous manufacturing

A stable CHO suspension cell line was developed for the production of Trastuzumab (IgG1) by making use of multiple transduction rounds in combination with single cell expansion. More specifically, a codon optimized vector was generated and used for the lentiviral transduction of a CHO suspension cell line. After passing internal quality control checks, a seed train using this stable cell line was initiated for the inoculation of a perfusion bioreactor. A constant viable cell density of $25x10^6$ cells/ml was maintained throughout the run at a perfusion rate of 1.5 vessel volume per day. The expression vector was designed in such a way that the produced mAbs

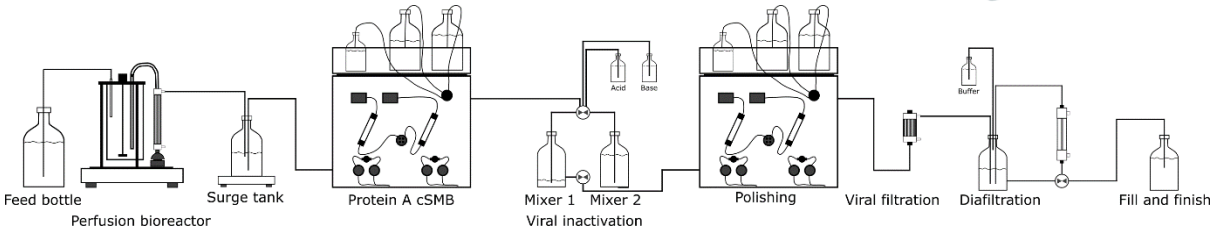

Figure 1. Overview of all unit operations composing the simAbs continuous manufacturing platform.

were secreted in the culture media. To separate the mAbs from the cells, an ATF (Alternating Tangential Flow) cell retention system was used. Further purification was performed by protein A chromatography followed by viral inactivation, ion exchange chromatography, viral filtration, tangential flow filtration and a final sterile filtration (**Figure 1**).

Product quality assessment

In-depth analyses on product quality were performed by employing a panel of complementary tests where drug substance samples were compared to the originator molecule Herceptin (Roche).

CEX-HPLC

Charge variants were analysed using a preparative ion exchange column. Here we opted to analyse the samples using cation exchange chromatography (CEX)-HPLC. Samples were diluted to 2.5 mg/mL in mobile phase prior to separation on a CEX column with a NaCl salt gradient using an Agilent Technologies 1260 HPLC system. The final column load was 25 µg.

SEC-HPLC

Sample purity, protein aggregation and protein fragmentation under native conditions were studied using size-exclusion chromatography (SEC)-HPLC. Samples were diluted to 2 mg/mL in mobile phase prior to analysis on a SEC column with a pore size of 200 Å using an Agilent Technologies 1260 HPLC system. The final column load for all samples was 50 µg.

RPLC-MS (non-reduced)

Post-translational modifications are typically analysed using reversed phase liquid chromatography (RPLC). When coupled to a mass spectrometer (MS), this platform is able to accurately analyse complex biologics. Samples were desalted using a polymeric reversed phase cartridge and 5 µg sample was loaded. Data were collected on an Agilent Technologies 1290 Infinity UHPLC system combined with a 6540 Q-TOF (Quadrupole Time of Flight) MS (Agilent) operated in the extended mass range mode.

RPLC-UV-MS (reduced)

Samples were denatured using guanidine hydrochloride (GuHCL) and reduced with dichlorodiphenyltrichloroethane (DDT). Reduced samples (10 µg load) were separated by reversed phase liquid chromatography on an Agilent Technologies 1290 Infinity UHPLC system using a C8 column with trifluoroacetic acid (TFA) as mobile phase. Eluting peaks were detected by UV-detection and analysed using a 6530 Q-TOF MS (Agilent) system.

RPLC-MS / peptide mapping (reduced)

Peptide mapping was performed using guHCl as denaturant. Afterwards, samples were reduced using DDT followed by a subsequent alkylation with iodoacetamide. Samples were digested for 4h using trypsin. RPLC-MS analysis was performed on a C18 column using TFA as solvent additive to both water and acetonitrile. A column load of 2.5 µg per sample was used and data was acquired on an Agilent Technologies 1290 Infinity UHPLC system combined with a 6530 Q TOF MS (Agilent) system.

Results and discussion

Purity

In our previously published white paper, we reported a high product purity for the drug substance produced using our continuous manufacturing platform. This was associated with similar biological activity and antigen affinity when compared to the originator molecule Herceptin. Here, we performed more in-depth analyses to critically assess the product quality. To achieve structural information about charge modifications, we performed a CEX-HPLC analysis. Herceptin together with the drug substance samples, that

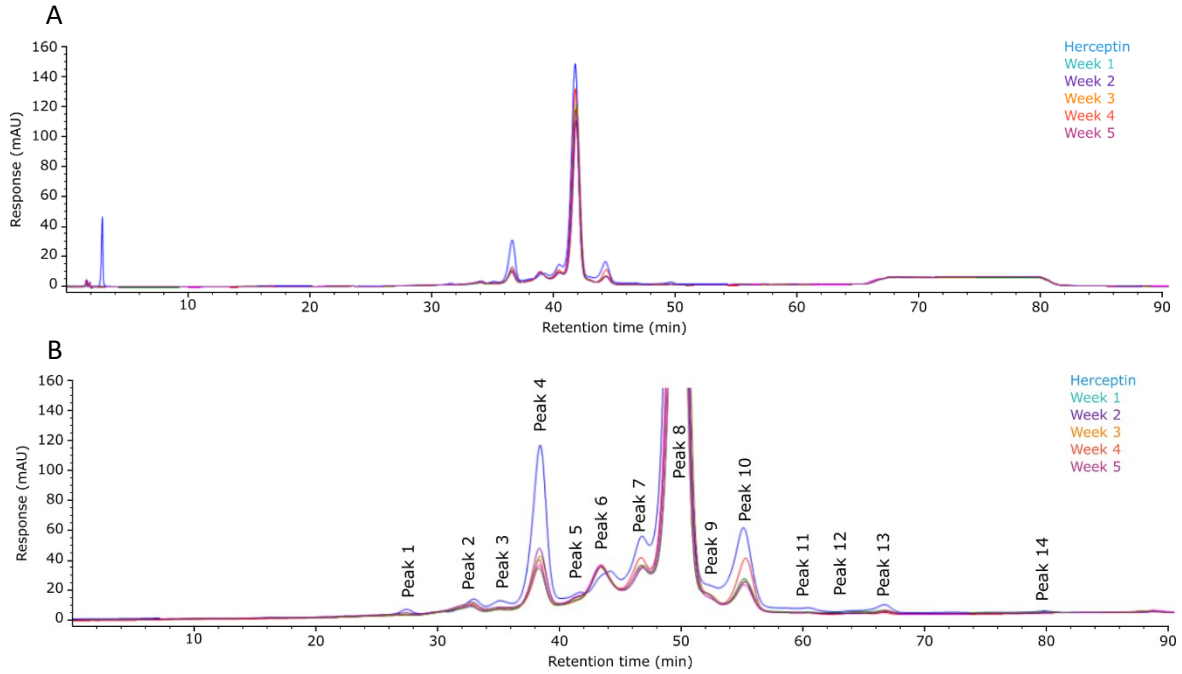

Figure 2. Product purity determined by CEX-HPLC. Elution profiles of Herceptin and weekly obtained samples are shown overlayed (**A**). Zoomed in region with peak annotation as used in Table 1 (**B**).

were taken along the 5-week-during production run, were analysed using identical operating conditions. All drug substance samples showed highly comparable purity profiles when compared to Herceptin (**Figure 2**). Interestingly, these five samples all have a lower presence of basic and acidic variants (**Table 1**). It has been reported that an increased degree of basic variants can be caused by aspartate isomerization (isoaspartate formation) in the complementary determining region (CDR) of a mAb[2]. This conversion is a spontaneous degradation process that negatively affects mAb stability and activity[3]. In addition, the lower presence of acidic variants points to a possible decreased deamidation of asparagine residues[4]. Both the decreased presence of basic and acidic variants can be seen as an advantageous effect for antigen binding of the produced mAb.

Along with CEX-HPLC, the samples were analysed using SEC-HPLC to assess for product purity and to obtain more information about low- and high-molecular weight species. As can be appreciated from **Figure 3**, overall product purity was similar for all samples but a small increase in high molecular weight (HMW) and low molecular weight (LMW) variants was detected in the drug substance samples (**Table 2**). These percentages are considered as very small as they are barely above the system's detection limit. Deviations in the ratio between HMW/Monomer/LMW are most likely attributed to mAb stability which is dependent on the formulation of the buffer used to store the mAb. In this case, the buffer formulation and pH of the commercially available Herceptin is different from the PBS buffer used for the simAbs samples, possible explaining the small variations observed in this study.

Table 1. Table showing the relative area percentages of peaks annotated to the elution profile in Figure 2B. The total sum of the acidic and basic variants are highlighted in bold for all analysed samples.

Reference	Relative area%					
	Herceptin	Week 1	Week 2	Week 3	Week 4	Week 5
Peak 1	1.7	1.3	1.5	1.7	1.8	2.4
Peak 2	1.5	2.5	2.3	2.6	2.7	3.1
Peak 3	1.2	0.9	1.0	1.0	1.3	1.3
Peak 4	11.4	5.8	5.5	6.1	6.4	7.6
Peak 5	1.8	1.6	1.8	1.7	1.9	2.0
Peak 6	4.4	6.6	7.2	7.8	7.0	7.3
Peak 7	6.0	6.1	5.8	5.6	5.6	5.9
Peak 8	60.3	66.2	65.9	66.9	65.7	62.8
Peak 9	1.3	1.7	1.7	1.4	1.4	1.3
Peak 10	8.1	6.3	4.6	4.0	4.0	4.3
Peak 11	0.6	0.2	0.6	0.4	0.7	0.6
Peak 12	0.3	0.3	0.5	0.2	0.3	0.3
Peak 13	1.1	0.1	1.2	0.3	0.9	1.0
Peak 14	0.3	0.2	0.3	0.4	0.2	0.2
Sum acidic	**28.1**	**24.9**	**25.2**	**26.5**	**26.7**	**29.4**
Sum basic	**11.6**	**8.9**	**8.9**	**6.6**	**7.6**	**7.7**
Total	100.0	100.0	100.0	100.0	100.0	100.0
Total Area	52225	38719	35233	35213	37223	35464

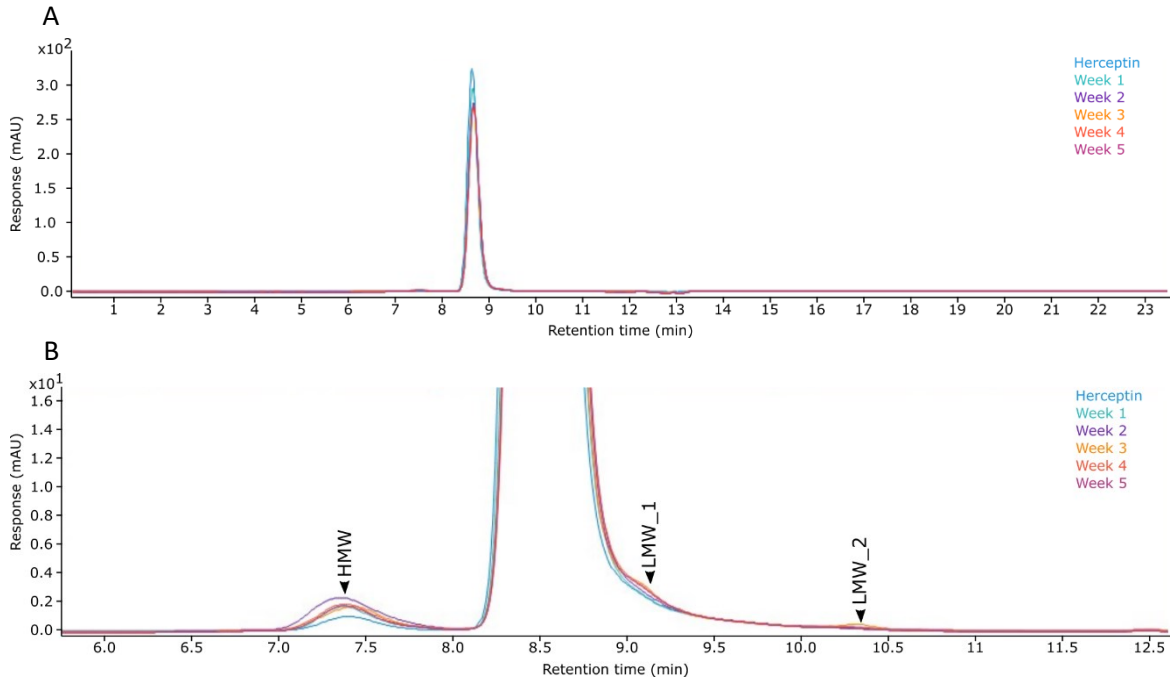

Figure 3. Product purity determined by SEC-HPLC. Elution profiles of Herceptin and weekly obtained samples are shown overlayed (**A**). Zoomed in region with peak annotation for HMW (high molecular weight) and LMW (low molecular weight) as used in Table 1 (**B**).

Table 2. Table showing the relative area percentages of the high molecular weight (HMW), Monomer and low molecular weight (LMW) species for the analysed product.

Reference	Relative area (%)			Total Area (mAU*s)
	%HMW	Monomer	%LMW	
Herceptin	0.5	99.5	0.00	5169.9
Week 1	1.0	99.0	0.01	4879.2
Week 2	1.5	98.5	0.01	4669.3
Week 3	1.1	98.7	0.15	4531.0
Week 4	1.0	98.9	0.04	4635.1
Week 5	1.1	98.9	0.02	4643.1

Glycoprofiling

Depending on the purpose of analysis, LC-MS can investigate antibody properties under reducing and non-reducing conditions. These different levels of protein analysis are typically performed in parallel to provide orthogonal and more comprehensive information. In this case study, we performed first RPLC-MS, under non-reducing conditions, aiming to confirm product identity and structural integrity. A monoclonal antibody is typically decorated with N-type glycans, such as G0F, G1F and G2F, on both antibody heavy chains. As depicted in **Table 3,** the overall glycosylation of Herceptin and the five drug substance samples were similar from a qualitative perspective but differ from a quantitative perspective. More specifically, a higher galactosylation degree (G1F and G2F) can be found on the product generated by simAbs.

This is a typical effect of the different culture conditions used during bioproduction. The originator Herceptin has been manufactured using fed-batch conditions, while the drug substance samples were manufactured using a perfusion set-up. The observed variation lies within the acceptable range and thus does not pose any issues regarding product safety or biological activity. In contrast, a lower degree of mannose N-glycan (Man5) and G0 was detected on the simAbs samples compared to Herceptin. It has been reported that a lower degree of Man5 decoration on mAbs is favourable because of its slower clearance from the human body by the immune system. Furthermore, it is generally accepted that a low system clearance is one of the prominent favourable features of IgG1-based therapeutical mAbs[5]. Overall, we can conclude that the glycosylation profile of the drug substance generated by continuous manufacturing is comparable to the originator molecule and thus lies in the acceptable product quality range approved by regulatory authorities. Antibody glycation is an important protein modification that could potentially affect bioactivity (antigen binding) and molecular stability. Glycation levels are best kept low and are as such seen as a potential critical quality attribute (CQA)[6]. Therefore, the glycation profile of therapeutic proteins should be well characterized.

We performed a RPLC-MS analysis under reducing conditions to quantitate the total glycation levels (**Figure 4** and **5**).

Table 3. Table showing the relative abundances of the detected glycoforms for the analysed samples.

Relative abundance (%)						
Glycoform	**Herceptin**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**
No glycosylation	0.5	0.7	0.9	1.2	1.2	1.2
Man5/Man5	1.5	0.0	0.0	0.0	0.3	0.3
1xG0F	0.5	0.3	0.2	0.2	0.3	0.2
1xG1F	0.5	0.4	0.3	0.3	0.4	0.3
G0/G0	1.8	0.0	0.0	0.0	0.0	0.0
G0F/G0	4.8	0.7	0.8	0.8	1.0	0.9
G0F/G0F	20.8	12.3	11.5	13.1	13.1	14.9
G0F/G1F	30.0	29.3	27.6	29.6	29.5	29.8
G1F/G1F (or G0F/G2F)	23.9	31.8	32.3	31.4	31.1	29.6
G1F/G2F	11.5	17.4	18.7	16.5	16.3	15.7
G2F/G2F	4.2	7.1	7.6	6.8	6.9	7.0

These data are summarized in **Table 4** for the mAb heavy chain (HC) and in **Table 5** for the mAb light chain (LC). We can conclude from these data that the HC shows a very low glycation degree while the LC shows an absolute absence. The total mAb glycation level of the drug substance samples are clearly less than the originator molecule. It has been reported that certain sugar residues present in formulation buffers used for mAbs can have an effect on the glycation degree[7]. This could be a plausible explanation for our observations as the formulation buffer of Herceptin is different from the PBS buffer we used for the drug substance material.

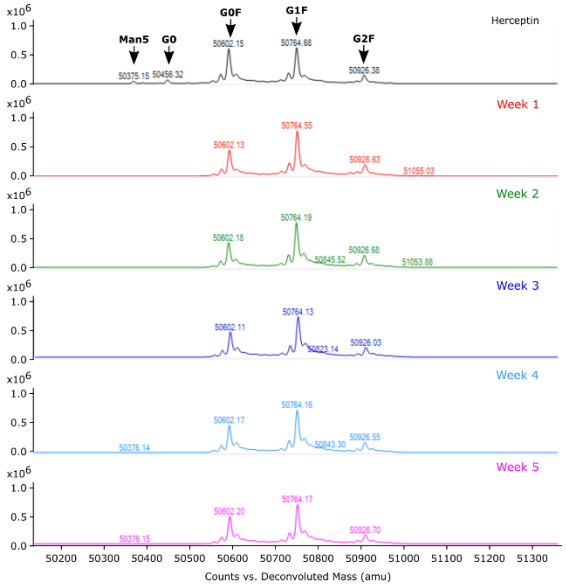

Figure 4. Elution profiles for the heavy chain area (15-17.6 min) obtained during RPLC-UV-MS run under reducing conditions.

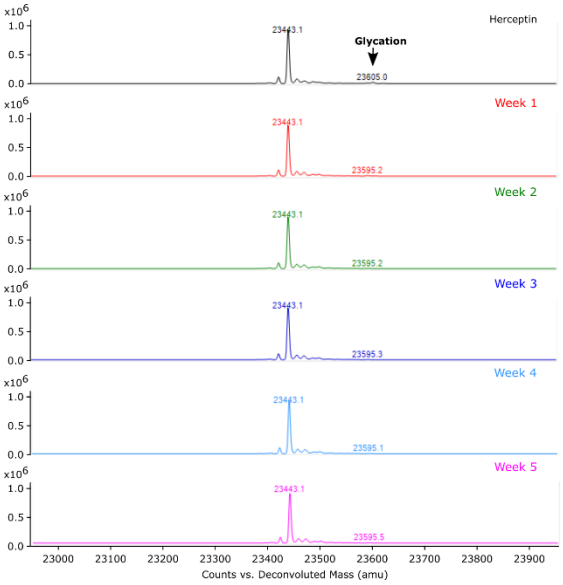

Figure 5. Elution profiles for the light chain area (10.5-14.3 min) obtained during RPLC-UV-MS run under reducing conditions.

Table 4. Relative N-glycan abundances for the glycation levels obtained for the mAb heavy chain (HC).

	Abundance N-glycans (%)					
	Herceptin	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**
No glycation	0.8	0.5	0.8	0.7	1.4	1.1
HC (Man5)	2.6	0.0	0.0	0.0	0.1	0.1
HC (G0)	4.4	0.0	0.0	0.0	0.0	0.0
HC (G0F)	40.5	31.5	30.2	33.9	33.7	35.7
HC (G1F)	41.7	54.5	54.4	52.6	52.3	51.3
HC (G2F)	9.9	13.6	14.7	12.8	12.6	11.8

Table 5. Relative N-glycan abundances for the glycation levels obtained for the mAb light chain (LC).

	Abundance N-glycans (%)					
	Herceptin	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**
LC	97.9	100.0	100.0	100.0	100.0	100.0
Glycation	2.1	0.0	0.0	0.0	0.0	0.0

Peptide mapping

Peptide mapping enables the profiling of a protein's primary structure as well as its post-translational modifications (PTMs) such as oxidation, deamidation and glycosylation. An accurate characterization of therapeutic proteins is required as it can yield both qualitative and quantitative information. Minimizing protein degradation, such as amino acid oxidation and deamidation, is crucial in obtaining an accurate representation of the therapeutic protein[8]. Peptide mapping has become an important part of the suite of acceptance criteria used in the evaluation of biological products as outlined in the ICH-Q6B (International Council for

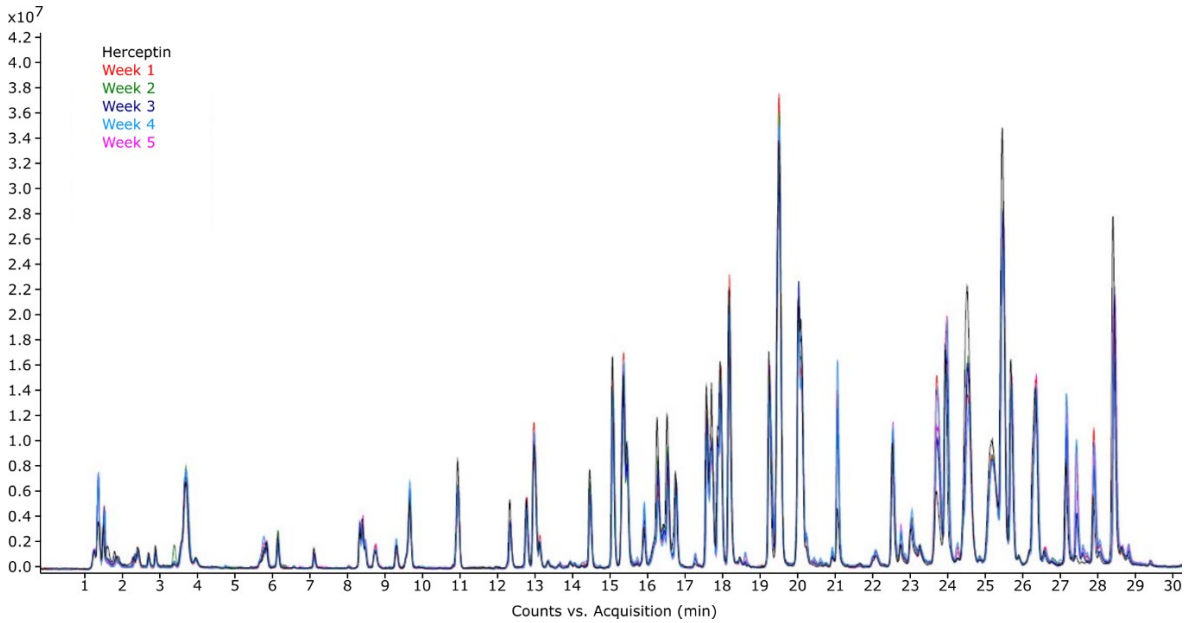

Figure 6. Peptide map of all analysed samples shown in overlay. The trace represents the total ion chromatogram. Each peak was mapped to peptides identified using mass spectrometry data.

Table 6. Selected overview of all post-translational modifications that show variations in between the originator molecule Herceptin and the drug substance samples.

		Abundance (%)					
Peptide sequence	**Modification**	**Herceptin**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**
SLSLSPGK	Lysine clipping	2.7	4.1	1.6	0.8	0.8	0.8
SLSLSPG		96.6	95.4	97.7	98.7	98.7	98.9
SLSLSP		0.7	0.5	0.7	0.5	0.5	0.3
WGGDGFYAMDYWGQGTLVTVSSASTK	Iso-aspartate formation	4.5	2.2	2.3	2.0	2.3	2.6
WGGDGFYAMDYWGQGTLVTVSSASTK		95.5	97.8	97.7	98.0	97.7	97.4
ASQDVNTAVAWYQQKPGK	Deamidation	93.0	98.0	97.8	97.7	97.1	96.5
ASQDVNTAVAWYQQKPGK		7.0	2.0	2.2	2.3	2.9	3.5

Harmonization-Q6B) guidelines. Here, we analysed Herceptin together with the five drug substance samples on a RPLC-MS platform. For all samples a complete sequence coverage was obtained in combination with a 100% amino acid identity overlap (**Figure 6**). An increased lysine clipping was observed on the C-terminal peptide for all drug substance samples. Although the difference is significant compared to Herceptin, C-terminal lysine cleavage is a common PTM for mAbs and has no disadvantageous effect on biological activity[9]. When taking into account aspartate isomerisation, we noticed a significant lower degree for all drug substance samples. It has been widely accepted that the mAb isomerization degree in the CDRs should be kept as low as possible to maintain high antigen affinity[10]. Interestingly, a small variation in deamidation levels could be observed for the peptide ASQDVNTAVAWYQQKPGK.

More specifically, all drug substance samples showed a reduced level of deamidation compared to its originator molecule Herceptin (**Table 6**). Deamidation typically occurs on asparagine residues and is a form of chemical degradation that adversely impacts antigen binding efficacy. It is therefore important to maintain deamidation levels at a very low degree. No differences in oxidation levels were observed between all the analysed samples (data not shown).

Conclusion

In this case study, we ran a continuous production process for five weeks during which the produced antibody was directly harvested and processed by a series of multiple purification steps. By applying a constant feed of fresh culture media, a stable CHO suspension culture was maintained, characterized by a high cell viability and stable cell density. This way, we were able to achieve a consistent product

quality where purity and biological activity were indistinguishable from each other when comparing early and late product material. In all analyses performed, the antibody generated using the continuous platform had a comparable quality profile during the entire production run. Our in-depth analyses revealed that for a number of criteria, such as deamidation, iso-aspartate formation, glycation, presence of acidic and basic variants, the drug substance produced by continuous manufacturing even outperformed Herceptin. We can conclude that our multi-step production line is able to meet the current standards in bioprocessing. We have created a continuous manufacturing platform that offers increased flexibility and consistent product quality while still maintaining very high yield and product purity.

About the simAbs team

The simAbs team consists of knowledgeable process engineers that are combining their complementary expertise in bioprocessing to develop a cost-efficient continuous manufacturing platform. We are bringing together a variety of expertise covering all aspects of the antibody production chain. Our ingenuity is constantly taken to the next level by collaborating with leading knowledge institutes and seasoned product and software developers. Meet our experienced team at www.simabs.com.

Acknowledgements

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More information?

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