Turk J Pharm Sci 2023;20(1):48-57
DOI: 10.4274/tjps.galenos.2022.47690

ORIGINAL ARTICLE

Stability Evaluation of the Biosimilar Monoclonal Antibody Using Analytical Techniques

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ABSTRACT

Objectives: Determination of the drug substance (DS) and drug product (DP) stability is especially important for biosimilar monoclonal antibodies since it can affect the quality, efficacy, and safety of the drugs. The main objective of this study was to determine the stability of the biosimilar candidate (TUR01) using state-of-the-art (current) analytical techniques.

Materials and Methods: Analytical techniques used in this study were isoelectric focusing on capillary electrophoresis, capillary electrophoresis-sodium dodecyl sulfate, size exclusion chromatography-ultra-high performance liquid chromatography, binding affinity, and physicochemical and microbiological tests. DS was kept in polyethylene terephthalate copolyester, glycol modified (PETG) bottles at ≤-65.0˚C and 5.0 ± 3.0˚C for 18 months, where the pre-filled syringe stability study was conducted at 5.0 ± 3.0˚C for 24 months and 25.0 ± 2.0˚C/60% ± 5 relative humidity (RH) for 6 months. The accelerated condition for DS was accepted as 5.0 ± 3.0˚C, while it was 25.0 ± 2.0˚C for the DP.

Results: The results indicated that TUR01 DS was stable when it was stored under long-term storage conditions at ≤-65˚C and at 5 ± 3˚C at least 18 months. Also, TUR01 DP was stable at 5 ± 3˚C for 24 months and at 25 ± 2˚C with 60.5% RH for 2 months without any significant changes.

Conclusion: State-of-the-art analytical techniques proved to be invaluable tools for evaluate the stability of the TUR01 DS and drug product.

Key words: Biosimilar monoclonal antibody, drug substance and drug product stability, analytical techniques, and stability indicating methods

INTRODUCTION

Biosimilars are a fast licensure pathway, which provides access for patients to reach life-saving medications through lower healthcare costs. In this study, a biosimilar monoclonal antibody (mAb) candidate (TUR01), which can serve as a tumor necrosis factor alpha (TNF-α) inhibitor, was developed. TUR01 is a fully human mAb, immunoglobulin isotype G subclass 1 molecule produced by Chinese hamster ovary cells. The proven mechanism of TUR01 is through blocking pro-inflammatory activity. By neutralizing soluble TNFα, it can inhibit the inflammatory response. The possible indications for this biosimilar are psoriasis, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, hidradenitis suppurativa, uveitis, and juvenile idiopathic arthritis.[1,2]

For marketing authorization, the stability of biosimilars must be demonstrated due to the International Conference on Harmonisation of Technical Requirements (ICH) for registration of pharmaceuticals for human use, especially based on ICHQ1A, ICHQ5C, ICHQ5E, and ICHQ6B.[3] The ICHQ5C (stability testing of biotechnological/biological products) specifically focuses on the biotechnological drugs considering their distinguishing properties. Based on this guidance, the stability protocol must include the testing to judge the potency, purity, molecular characterization, and product characteristics during the stability period. The stability testing needs to be conducted both for drug substance (DS) and drug product (DP). Although most of the mAbs are in the same formulation buffer in DS or DP forms, their packaging material and storage temperatures can be different. DS can be kept in bottles or bags before primary packaging, which differs depending on the route of administration. Most of the proteins can be stable at very low temperatures such as -80˚C for very long periods (5 years). At lower temperatures such as -80˚C, the drugs can be kept in plastic material rather than glass material due to the glass

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Received: 30.01.2022, Accepted: 28.04.2022

breakage at low temperatures. For this reason, it is more cost-effective for manufacturers to keep the DS as long as possible until sending it to the primary packaging. For both DS and DP, the stability analysis was conducted at two different temperatures for stability, one normal and one accelarated condition (≤-65.0˚C and 5.0 ± 3.0˚C for DS and 5.0 ± 3.0˚C and 25.0 ± 2.0˚C/60% ± 5 relative humidity (RH) for DP) due to ICH guidelines. Accelerated studies can enlighten us for longer periods before conducting a prolonged study.

During the shipping and storage of the mAbs, many stress factors can cause physical or chemical instability. For this reason, the stability period of biosimilars at different temperatures and storage conditions must be demonstrated. Physical instability can cause adsorption to the surface, which can lead to unfolding and aggregation. Chemical instability can cause degradation through asparagine deamidation, oxidation, and aspartic acid isomerization, and so on.[3] In addition to the physical and chemical stability, biological assessment has high importance in providing the potential efficacy of mAbs. Based on the mechanism of action, binding and/or any other biological assessment needs to be conducted throughout the shelf life of the study.[4]

Physicochemical and functional analyses with orthogonal analytical techniques need to be applied to understand whether there is any change in the primary, secondary, and higher-order structure during the extended periods for any developed biosimilars to determine the life span of the drug and storage conditions.[4,5] Determination of the stability period is extremely important since drug instability can affect the quality, efficacy, and safety of the monoclonal antibody.[6,7] Additionally, stability data become very valuable in the incidents, where the cold chain is broken[7,8] throughout the warehouse storage, distribution, and usage periods. Any changes that can impact the quality need to be monitored during the stability period.[9-11]

The main objective of this study was to determine the stability of TUR01 using state-of-the-art analytical techniques including isoelectric focusing capillary electrophoresis (icIEF), capillary electrophoresis-sodium dodecyl sulfate (CE-SDS), size exclusion chromatography-ultra-high performance liquid chromatography (SEC-UPLC), binding affinity, physicochemical and microbiological tests, as demonstrated in Figure 1. The physicochemical and microbiological tests used here are based on the pharmacopeia methods, whereas others are based on the in-house developed product-specific monographs. For microbiological tests, endotoxin and bioburden were followed during the stability study. In terms of physicochemical tests, appearance, color, opalescence, pH, osmolality, sub-visible particulates, and extractable volume determination were used during the stability period. The stability study was conducted in the same formulation buffer but in two different containers, both in polyethylene terephthalate copolyester, glycol modified (PETG) Nalgene bottles and glass type I pre-filled syringes as DS and DP forms, respectively. The bottles were kept at ≤-65.0˚C and 5.0 ± 3.0˚C for 18 months, whether the pre-filled syringe stability study was conducted at 5.0 ± 3.0˚C for 24 months and 25.0 ± 2.0˚C/60% ± 5 RH for 6 months. The accelerated condition for DS is accepted as 5.0 ± 3.0˚C whether it is 25.0 ± 2.0˚C for the DP. This study was designed to demonstrate if any subvisible particles, degradation or aggregation occur during the stability period affecting the safety and integrity of the product. Furthermore, biological assessment with SPR was conducted to measure the binding of the biosimilar to TNF-α, which directly shows the changes in the efficacy of the drug.

MATERIALS AND METHODS

Appearance

The appearance was evaluated by visual inspection according to the pharmacopeia methods, Ph. Eur. 2.9.20.[12]

pH

pH was measured by potentiometric determination according to the Ph. Eur. 2.2.3.[12]

Color

Visual inspection according to the method described in Ph. Eur. 2.2.2. was used to determine the degree of coloration.[12]

Opalescence

A turbidimeter was used to evaluate the clarity and degree of opalescence according to the method described in Ph. Eur. 2.2.1.[12]

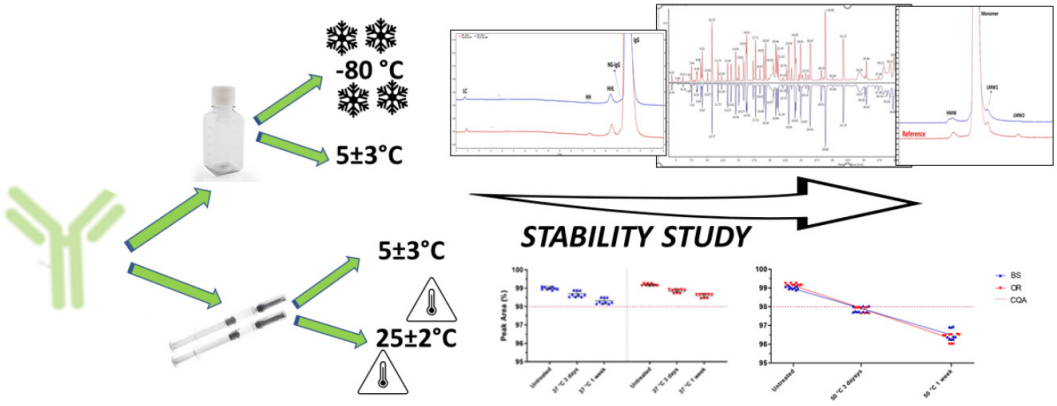

Figure 1. Overview of the conducted stability study for biosimilar TNF-α inhibitor

Osmolality

The osmolality is evaluated by an osmometer (Advanced Instruments, OsmoTECH) according to the method described in Ph. Eur. 2.2.35.[12]

Sub-visible particulates

The light obscuration particle count test was used to evaluate the sub-visible particles according to the Ph. Eur. 2.9.19 method.[12]

Extractable volume

The gravimetric volume determination method was used to measure the extractable volume due to Ph. Eur. 2.9.17.[12]

Bioburden

Bioburden was evaluated by the total aerobic microbial count method using membrane filtration due to Ph. Eur. 2.6.12.[12]

Bacterial endotoxin

Bacterial endotoxin was determined using *Limulus amebocyte* lysate kinetic turbidimetric technique, in accordance with Ph. Eur. 2.6.14 (equivalent to USP ‹85›). The results are expressed as EU/mL.[12]

Sterility

Sterility was evaluated by membrane filtration test according to the method described in the Ph. Eur. 2.6.1.[12]

Protein content

Protein content was measured in a nanodrop (Thermo Scientific, Nanodrop One) using optical densitometry method. Firstly, blank measurement was done with 2.2 mL formulation buffer. Then, protein sample was mixed and 2.2 mL of the mixed sample was measured by a nanodrop. This procedure was repeated thrice and the average value was taken. The absorption of the samples was determined at 280 nm. Results were reported as mg/mL.

Isoform profile and isoform abundance measurement

Isoform profile and isoform abundance were measured by capillary isoelectric focusing (protein simple, iCE3) using ultraviolet (UV) detection at 280 nm. Both the reference and samples were diluted to 1 mg/mL in deionized water, with a final volume of at least 20 µL. Master mix was prepared due to the suppliers' protocol and added to both reference and samples before vortexing for 3 s. All the samples were centrifuged at 10,000 rpm for 3 min before transferring to the vials for analysis on iCE3. Pre-focusing was done at 1.5 kV for 1 min and focusing was at 3 kV for 6 min. Pharmalyte ampholytes at pH 3-10 and pH 8-10.5 were used for generation of a pH gradient. The pI markers (7.9 and 10.0) were used to determine the charge variants isoelectric pH (pI) values.

Electrophoretic purity determination

Electrophoretic purity was determined using a PA800 Plus Capillary Electrophoresis System (Sciex, PA800 plus). Species are separated based on their hydrodynamic size while passing through the capillary and detected by a photodiode array detector. Both the reference and samples were diluted with deionized water to a final concentration of 2 mg/mL in a 0.5 mL tube. An immunoglobulin isotype G (IgG) control standard was aliquoted at room temperature and prepared in a 0.5 mL tube. The prepared samples were vortexed approximately 5 s prior to centrifugation for 30 s at 14000 rpm. The samples were incubated at 70°C for about 10 min followed by cooling for about 3 min. Reference and blank samples were transferred to 200 µL volume microvials. All injections were made in triplicate tubes. For non-reduced conditions, a final concentration of 500 mM iodoacetamide (Sigma Aldrich, USA) and for reduced conditions, β-mercaptoethanol (Merck, USA) were used. The analysis was conducted under 15 kV for 40 min and 220 nm was used for the main electropherogram wavelength.

Monomer and aggregate determination

Monomer and aggregate values were measured at 280 nm by SEC (Waters Acquity H-Class Bio UPLC with UV detector). Both reference and samples were diluted to 2.50 mg/mL with formulation buffer followed by vortexing 3 s. Then, the samples were injected into the system. The injection was done into a BEH SEC 200 (4.6 x 30 cm, 200 Å) column with a flow rate of 0.25 mL/min was used for the separation. The mobile phase was at pH 7.4 with 20 mM phosphate (Sigma, Germany) and 188 mM sodium chloride (Merck, Germany).

Biological activity - binding kinetics

The KD value (relates to the concentration of antibody) for binding to the soluble antigen was determined in a single-cycle assay format using surface plasmon resonance (SPR) with Biacore T200 (Cytiva). CM5 S series sensor chipand amine coupling kit (GE Healthcare, Cat ID BR-1000-50) was used. The biosimilar antibody was captured *via* a protein A/G (Pierce, Cat ID 21186) immobilized surface matrix, and several concentrations of antigen were injected consecutively. Before injection, soluble antigen was diluted from 200 nM to 2.5 nM in 1X HBS-EP+ running buffer and mixed thoroughly. Multiple diluents were prepared. Reference and samples were also diluted to 2.5 µg/mL using 1X HBS-EP+ buffer. A vortex was applied after each step. The binding of antigen to TUR01 was measured and fitted using a 1:1 kinetic binding model. A relative binding affinity was calculated by comparing the values obtained for the sample with respect to the reference.

RESULTS

In this study, the stability period and stability-indicating methods were investigated for both DS and DP of a recently developed biosimilar mAb functioning as a TNF-α inhibitor. DS stability was conducted for 18 months in PETG Nalgene bottles both at ≤-65.0°C and 5.0 ± 3.0°C, whereas DP stability was conducted for 24 months in type I glass pre-filled syringes at 5.0 ± 3.0°C and at 25.0 ± 2.0°C/60% ± 5 RH. The determined stability methods according to the European Pharmacopeia for both DS and DP are listed in Table 1. The critical quality attributes of the developed inhibitor were determined according to the physicochemical and functional properties of the 18 commercially available originators. The acceptance criteria

for shelf-life were defined due to these set critical quality attributes, as specified in Table 1.

For this particular molecule, the pH should be between 5 and 5.4 and osmolality needs to be between 285 and 340 mOsm/kg. Isoform profile with icIEF was designated as an identity test. The critical quality range for the protein content was defined as 45-55 mg/mL and the biological activity for the biosimilars in terms of comparative KD needs to be between 80-120%. Monomer amounts including the shelf life of both DS and DP should be over 98% and the IgG percentage should be over 95%.

Drug substance stability

Although most of the mAb are relatively stable at ≤-65.0˚C, there are some circumstances that mAbs can be unstable. To determine the stability of TUR0, appearance, acidic and basic variants, protein content, biological activity, monomers, and

Table 1. Stability test methods, their acceptance criteria, and information regarding the methods used

Test type	Acceptance criteria	Reference method
Physicochemical Tests		
Appearance	Without visible particles	Visual inspection Ph. Eur. 2.9.20
Color	Not more intensely coloured than reference solution B7	Visual inspection Ph. Eur. 2.2.2
Opalescence	Not more opalescent than reference solution IV	Nephelometry Ph. Eur. 2.2.1
pH	5.0 – 5.4	Potentiometric determination Ph. Eur. 2.2.3
Osmolality	285-340 mOsm/kg	Osmometry Ph. Eur. 2.2.35
Sub-visible particulates		Light obscuration particle count test Ph. Eur. 2.9.19
≥10 µm	NMT 6000 particles/container	
≥25 µm	NMT 600 particles/container	
Extractable volume	0.79-0.84 mL	Volume determination (gravimetric) Ph. Eur. 2.9.17
Identity		
Isoform profile	Comparable to Ref. Std. Report pI range and % peak areas	icIEF In -house monograph
Assay		
Protein content	45.0-55.0 mg/mL	Absorbance at 280 nm (OD) In -house monograph
Biological activity: binding by biacore	80-120% (KD of sample/KD of reference material)	TNF-α binding using surface plasmon resonance In -house monograph
PURITY		
Size exclusion (Monomer, HMW species/ aggregates, LMW species	Monomer IgG: ≥98%	SE-UPLC In -house monograph
	HMW/aggregates ≤2%	
	Report value for LMW	
Electrophoretic purity (reducing)	≥ 95.0% IgG (heavy and light chains)	Reducing CE-SDS PAGE In -house monograph
Electrophoretic purity (non-reducing)	Report values for IgG LC, HH, HHL, and NG-IgG	Non-reducing CE-SDS PAGE In -house monograph
Microbiological tests		
Sterility	No growth	Membrane filtration Ph. Eur. 2.6.1
Bacterial endotoxins	≤0.2 EU/mg	LAL test: chromogenic kinetic method Ph. Eur. 2.6.14, USP ‹85›

aggregates, bioburden, and endotoxin were monitored for 18 months. Ultra-high performance liquid chromatography-size exclusion chromatography (SEC-UPLC), the protein contents, and binding activity were defined as the stability- indicating factors for drug substance. Table 2 shows all the data at months 0, 3, 6, 9, 12, and 18 at ≤-65.0˚C.

The stability of the DS was also followed at 5.0 ± 3.0˚C for 18 months and all the specifications were measured at months 0, 1, 2, 3, 6, 9, 12, and 18. Table 3 shows all the measurements for 18 months.

As indicated in Table 2 and Table 3, there was not any change in the appearance of the molecule for 18 months and it has passed. The isoform profile proves the similarity of the DS during 18 months to the reference standard. Figure 1 shows the acidic, basic variants and the main peak of DS at 0 months; at ≤-65.0˚C, 18 months; at 5.0 ± 3.0˚C, 18 months with the reference standard. The lowest main peak was 73.34% at ≤-65.0˚C whereas it was 71.95% at 5.0 ± 3.0˚C. At lower temperature, the monomer amount was 99% the lowest, although it was 98% at 5.0 ± 3.0˚C at the end of stability period. At both temperatures, endotoxin amount was lower than 0,1 EU/mg at the end and 18 months and bioburden was zero.

In all our detailed analyzes, we have demonstrated that our biosimilar DS is stable at ≤-65.0˚C and 5.0 ± 3.0˚C for 18 months in Nalgene PETG bottles.

Drug product stability

Liquid pharmaceuticals are generally being kept at 5.0 ± 3.0˚C due to the ease of reaching 5.0 ± 3.0˚C refrigerators at both pharmacies, hospitals, and houses. For this reason, it is critical to check stability during the shelf-life of the DP at 5.0 ± 3.0˚C. To see the stability of TUR01 drug product, protein content, appearance, color, opalescence, sub-visible particulates, pH, osmolality, extractable volume, acidic and basic variants, protein content, biological activity, monomers and aggregates, low molecular fragments, sterility, and endotoxin were followed for 24 months. Table 4 shows all the data at months 0, 1, 2, 3, 6, 9, 12, 18, and 24 at 5.0 ± 3.0˚C.

In addition to this, DP stability was followed at 25.0 ± 2.0˚C/60% ± 5 RH to see how long TUR01 can stay at room temperature. Similar specifications were checked, which are tabulated in Table 5.

DP stability results at 5.0 ± 3.0˚C for 24 months did not show any critical change, except the minor changes toward the end of 24 months. Most of the tests, including protein content,

Table 2. Drug substance stability results at ≤-65.0˚C for 18 months

Test methods	Months					
	0	3	6	9	12	18
Appearance	Pass	Pass	Pass	Pass	Pass	Pass
Isoform profile icIEF	Similar to reference standard	Similar to reference standard	Similar to reference standard	Similar to reference standard	Similar to reference standard	Similar to reference standard
pI Range	AP 2: 8.66	AP 2: 8.66	AP 2: 8.66	AP 2: 8.68	AP 2: 8.67	AP 2: 8.67
	AP 1: 8.78	AP 1: 8.79	AP 1: 8.77	AP 1: 8.79	AP 1: 8.77	AP 1: 8.78
	MP: 8.88	MP: 8.89	MP: 8.87	MP: 8.90	MP: 8.87	MP: 8.88
	BP 1: 8.96	BP 1: 8.96	BP 1: 8.95	BP 1: 8.97	BP 1: 8.95	BP 1: 8.95
	BP 2: 8.98	BP 2: 8.99	BP 2: 8.97	BP 2: 9.01	BP 2: 8.98	BP 2: 8.98
% Peak areas	AP 2: 5.80	AP 2: 5.73	AP 2: 5.83	AP 2: 5.08	AP 2: 5.66	AP 2: 5.74
	AP 1: 17.12	AP 1: 16.77	AP 1: 18.03	AP 1: 17.43	AP 1: 17.22	AP 1: 17.48
	MP: 74.44	MP: 74.80	MP: 73.34	MP: 75.07	MP: 74.12	MP: 74.00
	BP 1: 1.50	BP 1: 1.71	BP 1: 1.79	BP 1: 1.70	BP 1: 1.98	BP 1: 1.66
	BP 2: 1.13	BP 2: 0.99	BP 2: 1.00	BP 2: 0.72	BP 2: 1.02	BP 2: 1.12
Protein content	48.2	48.3	47.5	47.9	47.7	47.5
Biological activity	91	100	102	100	87	97
SE-UPLC	Monomer 99	99	99	99	99	99
	HMW 1	1	1	1	1	1
	LMW 0.51	0.53	0.49	0.61	0.58	0.44
Bioburden	0	NT	NT	NT	NT	0
Endotoxin	0.01	NT	NT	NT	NT	≤ 0.1 EU/mg

NT: Not tested, AP: Acidic peak, BP: Basic peak, MP: Main peak

opalescence, pH, and osmolality did not show significant change. There was variability in subvisible particulate measurement, which was due to the variability caused from the equipment itself. However, we started to see critical changes at 25.0 ± 2.0˚C after 3 months as expected. Especially, monomer amount decreased to 97.40% and 96.20%, while aggregates and low molecular fragments were increased. Figure 1 demonstrates the SEC analysis results showing the changes from month 0 to month 6.

The non-reduced CE-SDS shown in Figure 2 also confirms the SEC results. As the aging happens at 25.0 ± 2.0˚C, total IgG decreases, and the amount of HC, HH, and HHL increases, which explains the increase in low molecular weight fragments. In addition to non-reduced CE-SDS analysis, reduced CE-SDS analysis was carried out for 6 months, as shown in Figure 3. Although there was not any change at cold temperatures in the amount of total HC and LC, there was a small decrease in the total HC and LC at 25.0 ± 2.0˚C from 99.41% to 98.90% (Figures 4, 5).

As a result, we have shown that our formulated DS is stable at ≤-65.0˚C and 5.0 ± 3.0˚C for at least 18 months and our formulated DP is stable in syringes at 5.0 ± 3.0˚C for 24 months. It was also shown that DP stability at 25.0 ± 2.0˚C/60% ± 5 RH starts decreasing after 2 months.

DISCUSSION

In this study, a deep investigation was conducted to understand the stability of TUR01 DS and drug product. Both accelerated and prolonged stability were studied at different temperatures. In this way, it was aimed to cover a larger range of temperatures for TUR01 to understand its behavior.

Different physical and chemical instabilities can occur for monoclonal antibodies during their storage, transportation, and administration. The approval and marketing of the biosimilars requires an extensive comparability study with the reference products. In addition to the comparability, the stability studies become extensively important since different modifications can be observed on the biosimilar molecule due to the variability of the cell line, cell culture conditions, passage number of the cells, and post-translational modifications. Due to these differences, extensive stability study is required by the regulatory agencies before the approval and marketing of the biosimilars.[13]

Table 3. Drug substance stability results at 5.0 ± 3.0˚C for 18 months

Test methods		Months							
		0	1	2	3	6	9	12	18
Appearance		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
Isoform profile icIEF		Similar to reference standard	Similar to reference standard	Similar to reference standard	Similar to reference standard	Similar to reference standard	Similar to reference standard	Similar to reference standard	Similar to reference standard
pI Range		AP 2: 8.66	AP 2: 8.67	AP 2: 8.67	AP 2: 8.67	AP 2: 8.67	AP 2: 8.68	AP 2: 8.67	AP 2: 8.67
		AP 1: 8.78	AP 1: 8.78	AP 1: 8.77	AP 1: 8.78	AP 1: 8.77	AP 1: 8.79	AP 1: 8.78	AP 1: 8.78
		MP: 8.88	MP: 8.88	MP: 8.88	MP: 8.89	MP: 8.87	MP: 8.90	MP: 8.88	MP: 8.88
		BP 1: 8.96	BP 1: 8.96	BP 1: 8.96	BP 1: 8.96	BP 1: 8.95	BP 1: 8.97	BP 1: 8.95	BP 1: 8.95
		BP 2: 8.98	BP 2: 8.99	BP 2: 8.99	BP 2: 9.00	BP 2: 8.98	BP2: 9.00	BP 2: 8.98	BP 2: 8.98
% Peak area		AP 2: 5.80	AP 2: 5.69	AP 2: 4.90	AP 2: 5.29	AP 2: 5.38	AP 2: 5.32	AP 2: 6.42	AP 2: 5.18
		AP 1: 17.12	AP 1: 17.73	AP 1: 17.56	AP 1: 17.74	AP 1: 17.11	AP 1: 18.01	AP 1: 18.21	AP 1: 18.55
		MP: 74.44	MP: 73.32	MP: 74.15	MP: 74.29	MP: 74.77	MP: 73.80	MP: 71.95	MP: 72.74
		BP 1: 1.50	BP 1: 2.02	BP 1: 2.18	BP 1: 1.82	BP 1: 1.73	BP 1: 2.03	BP 1: 2.20	BP 1: 2.27
		BP 2: 1.13	BP 2: 1.24	BP 2: 1.20	BP 2: 0.86	BP 2: 1.02	BP2: 0.85	BP 2: 1.22	BP 2: 1.27
Protein content		48.2	48.5	49.0	48.5	47.6	48.9	48.0	49.3
Biological activity		91	94	96	105	112	97	99	104
SEC	Monomer	99	98	98	98	98	98	98	98
	HMW	1	1	1	1	1	1	1	1
	LMW	0.51	0.50	0.48	0.57	0.59	0.83	0.75	0.71
Bioburden		0	NT	NT	NT	NT	NT	NT	0
Endotoxin		0.01	NT	NT	NT	NT	NT	NT	≤0.1 EU/mg

NT: Not tested, AP: Acidic peak, BP: Basic peak, MP: Main peak

Temperature changes during processing, storage or transportation can cause perturbations to the monoclonal antibody. Therefore, it is crucial to have information at different temperatures for a certain time.[14] Not only high temperatures, but also low temperatures can also cause changes in protein conformation. In particular, freeze-thaw cycles have shown an impact on the mAb aggregation in the literature.[15-19]

At ≤-65.0˚C, it is proven that TUR01 is stable in Nalgene bottles for 18 months. Any significant changes have not been observed during 18 months in the product characteristics. However, there has been only a slight change in the main peak at 5.0 ± 3.0˚C for 18 months (ICE data). This slight decrease was still in the range of critical quality attributes showing the stability of TUR01 DS.

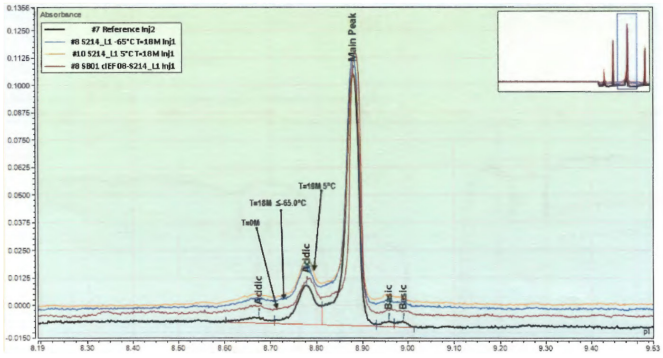

Figure 2. Comparison of DRS to DS at 0 month, DS at 5.0 ± 3.0˚C at 18 months and DS ≤-65.0˚C at 18 months

DS: Drug substance, Drug reference standard

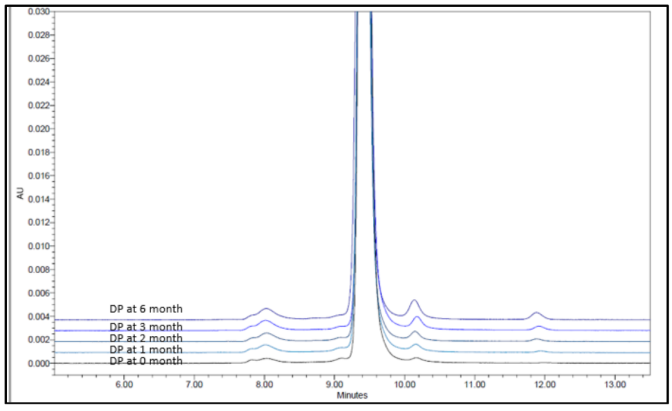

Figure 3. Size exclusion chromatography analysis results of drug product at months 0, 1, 2, 3, and 6 at 25.0 ± 2.0˚C

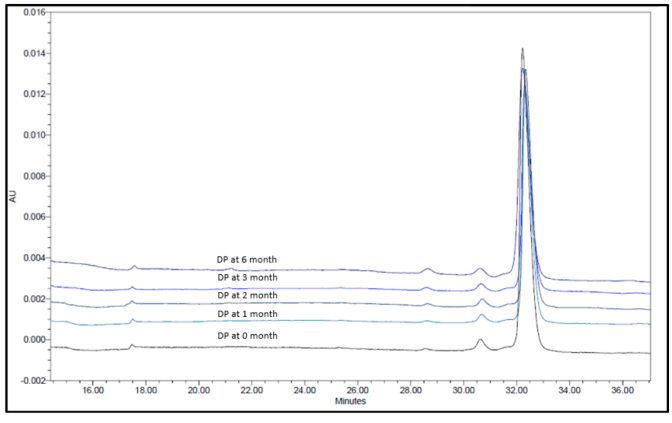

Figure 4. Non-reduced capillary electrophoresis-sodium dodecyl sulfate analysis results of drug products at months 0, 1, 2, 3, and 6 at 25.0 ± 2.0˚C

In the primary packaging, we have also proven the stability of the TUR01 DP at 5.0 ± 3.0˚C for 24 months. There has been a slight decrease in the main peak, while a slight increase was observed in the acidic peak after 6 months. All the ICE data have demonstrated that the product characteristics have been in the range of critical quality attributes. However, there have been also a slight increase in the aggregate and low molecular weight, but all the values remained in the range even after 24 months at +2-8˚C. The stability study of TUR01 DP at 25.0 ± 2.0˚C was conducted for 6 months and it has been proven that the product is stable only for 2 months at room temperature. After 2 months, a decrease in the monomer was observed, while aggregates and low molecular weight species were increasing. After month 2, the main peak started to significantly decrease too.

During the stability study, there were also some oscillations in subvisible particulate and relative binding values. The subvisible particulate ≥10 µm needs to be lower than 6000 particles *per* container. The values measured in this study, were too low compared to 6000. There was some variability in the measurements due to the sensitivity of the equipment. At all stability time points, the relative binding affinity was calculated by comparing the values obtained for the sample with respect to the reference. At each sampling point, biosimilar and the reference KD values were measured, and the relative value was calculated. The difference between the stability points was due to the variability in the measurements.

All this data proves to us that TUR01 can be kept at 5.0 ± 3.0˚C for 24 months and it does not get degraded at room temperature for 2 months. In case the cold chain is broken for 2 months, it can be still safe to use this drug product.

CONCLUSION

The results obtained to date for TUR01 DS indicate that the DS is stable, when stored under long-term storage conditions

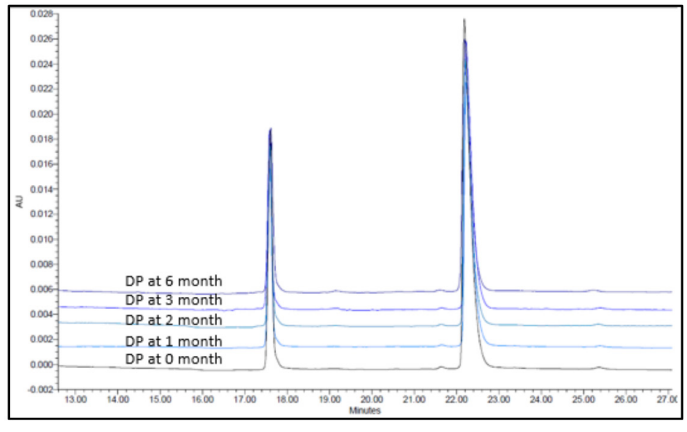

Figure 5. Reduced capillary electrophoresis-sodium dodecyl sulfate analysis results of drug products at months 0, 1, 2, 3, and 6 at 25.0 ± 2.0˚C

Table 4. Drug product stability results at 5.0 ± 3.0°C for 24 months

Test		Testing interval (months)								
		0 M	1 M	2 M	3 M	6 M	9 M	12 M	18 M	24 M
Protein content		49.9	49.8	49.6	50.1	49.9	49.7	51.5	49.7	50.5
Appearance		Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
Color		B8	B8	B8	B8	B8	B8	B8	B8	B8
Opalescence		8.3	9.6	10	8.9	8.3	10.1	10.7	10.2	9.5
Sub-visible particulates										
≥10 µm		147	92	83	137	228	245	49	235	13
≥25 µm		3	3	4	4	5	124	0	3	1
pH		5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3
Osmolality		299	300	316	313	317	318	313	311	316
Extractable volume		0.8	NT	NT	NT	0.8	NT	NT	NT	NT
SEC	Monomer	99.10%	98.90%	98.80%	98.70%	98.50%	98.30%	98.00%	98.00%	98.00%
	Aggregate	0.57%	0.70%	0.78%	0.84%	0.98%	1.09%	1.28%	1.28%	1.20%
	LMW	0.34%	0.43%	0.40%	0.47%	0.47%	0.60%	0.79%	0.70%	0.80%
Isoform profile icIEF		Comparable to ref.	Comparable to ref.	Comparable to ref.	Comparable to ref.	Comparable to ref.	Comparable to ref.	Comparable to ref.	Comparable to ref.	Comparable to ref.
pI range		AP: 8.66-8.77	AP: 8.66-8.76	AP: 8.68-8.79	AP: 8.67-8.77	AP: 8.66-8.77	AP: 8.67-8.76	AP: 8.66-8.77	AP: 8.67-8.78	AP: 8.68-8.80
		MP: 8.87	MP: 8.86	MP: 8.88	MP: 8.87	MP: 8.87	MP: 8.87	MP: 8.86	MP: 8.88	MP: 8.90
		BP: 8.95	BP: 8.94	BP: 8.97	BP: 8.95	BP: 8.94	BP: 8.95	BP: 8.94	BP: 8.98	BP: 8.97
% Peak area		AP: 22.28%	AP: 22.09%	AP: 21.01%	AP: 23.06%	AP: 22.90%	AP: 24.80%	AP: 18.56%	AP: 23.07%	AP: 24.62%
		MP: 75.15%	MP: 74.92%	MP: 76.28%	MP: 73.89%	MP: 74.29%	MP: 72.14%	MP: 76.66%	MP: 72.95%	MP: 71.71%
		BP: 2.57%	BP: 3.00%	BP: 2.72%	BP: 3.05%	BP: 2.82%	BP: 3.06%	BP: 4.78%	BP: 3.99%	BP: 3.69%
CE-SDS (red) (HC + LC)		99.41%	99.46%	99.47%	99.43%	99.48%	99.49%	99.48%	99.54%	99.58%
CE-SDS (Non-Red)	IgG	95.28%	95.84%	95.71%	95.23%	95.95%	95.73%	95.73%	94.82%	95.12%
	LC	0.62%	0.77%	0.68%	0.42%	0.60%	0.76%	0.90%	0.68%	0.69%
	HH	0.33%	0.30%	0.40%	0.46%	0.45%	0.32%	0.60%	0.39%	0.27%
	HHL	2.97%	2.49%	2.51%	2.79%	2.45%	2.57%	2.08%	2.87%	2.50%
	NG-IgG	0.79%	0.60%	0.70%	1.10%	0.55%	0.61%	0.69%	1.24%	1.42%
Biacore		110.50%	104.60%	108.10%	103.40%	116.10%	111.20%	96.20%	91.80%	82.80%
Endotoxins		0.0074	0.0011	0.0068	0.001	0.0048	0.001	0.0025	NT	0.026
Sterility		No growth	NT	NT	NT	No growth	NT	NT	NT	No growth

NT: Not tested, AP: Acidic peak, BP: Basic peak, MP: Main peak

Table 5. Drug product stability results at 25.0 ± 2.0°C/60% ± 5 RH

Test		Testing interval (months)				
		0 M	1 M	2 M	3 M	6 M
Protein content		49.9	49.8	49.9	49.2	50.7
Appearance		Pass	Pass	Pass	Pass	Pass
Color		B8	B8	B8	B8	B8
Opalescence		8.3	9.4	9.3	8.4	8.5
Sub-visible particulates						
≥10 µm		147	73	84	49	92
≥25 µm		3	1	1	0	0
pH		5.3	5.3	5.3	5.3	5.3
Osmolality		299	300	316	313	319
SEC	Monomer	99.10%	98.20%	98.00%	97.40%	96.20%
	Aggregates	0.57%	1.06%	1.09%	1.30%	1.53%
	LMW	0.34%	0.78%	0.90%	1.33%	2.25%
Extractable volume		0.8	NT	NT	NT	NT
Isoform profile icIEF		Comparable to reference pI	Comparable to reference pI	Comparable to reference pI	Comparable to reference pI	Comparable to reference pI
pI range		AP: 8.66-8.77	AP: 8.65-8.76	AP: 8.68-8.79	AP: 8.67-8.78	AP: 8.54-8.77
		MP: 8.87	MP: 8.85	MP: 8.89	MP: 8.87	MP: 8.87
		BP: 8.95	BP: 8.93	BP: 8.97	BP: 8.95	BP: 8.94
% Peak areas		AP: 22.28%	AP: 22.63%	AP: 26.19%	AP: 29.33%	AP: 38.78%
		MP: 75.15%	MP: 74.40%	MP: 69.21%	MP: 65.53%	MP: 55.49%
		BP: 2.57%	BP: 2.98%	BP: 4.61%	BP: 5.14%	BP: 5.74%
CE-SDS (red) (HC+LC)		99.41%	99.43%	99.39%	99.25%	98.90%
CE-SDS (Non-Red)	IgG	95.28%	95.54%	94.83%	93.89%	93.17%
	LC	0.62%	0.62%	1.00%	0.57%	0.87%
	HC	ND	ND	ND	ND	0.68%
	HH	0.33%	0.45%	0.64%	0.91%	1.54%
	HHL	2.97%	2.57%	2.51%	3.16%	2.60%
	NG-IgG	0.79%	0.81%	1.03%	1.47%	1.15%
Biacore		110.50%	109.70%	114.90%	107.90%	118.00%
Endotoxins		0.0074	0.0075	NT	0.001	0.0045
Sterility		No growth	NT	NT	NT	No growth

NT: Not tested, AP: Acidic peak, BP: Basic peak, MP: Main peak

≤-65°C for at least 18 months. TUR01 DS in Nalgene PETG bottles stability under short-term storage conditions at 5 ± 3°C is stable also at least 18 months. In this study, it is proved that the TUR01 DP is stable at 5 ± 3°C for 24 months and at 25 ± 2°C/60.5% RH for 2 months.

ACKNOWLEDGMENTS

The author thanks Dr. Ahmet Emin Atik, Yiğit Erdemgil, Zeynep Keleş, and Dr. Ozge Can for their support. Special thanks are given to Chairman of the Board and General Manager, Tunç Turgut from Turgut Pharmaceuticals.

Ethics

Ethics Committee Approval: Not applicable.

Informed Consent: Not applicable.

Peer-review: Externally peer-reviewed.

Financial Disclosure: The author declared that this study received no financial support.

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