



Extended Stability of Reconstituted and Diluted SB3 (Trastuzumab Biosimilar) Assessed by Physicochemical and Biological Properties

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Received: March 15, 2019 / Published online: May 17, 2019
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ABSTRACT

Introduction: Stability information for the trastuzumab biosimilar SB3 is limited to 48 h at 2–8 °C for the reconstituted solution and 24 h at up to 30 °C for diluted solutions. Extended physicochemical stability and biological activity were assessed to evaluate the advanced preparation of reconstituted and diluted SB3.

Methods: Under controlled and aseptic conditions, the stability of reconstituted and diluted SB3 was evaluated using several assessments and according to the UK's National Health Service guidance. Reconstituted SB3 was stored at 25 ± 2 °C with $60 \pm 5\%$ relative humidity for 3 days, and subsequently diluted SB3 (0.32–4 mg/mL) was stored in an infusion bag in the absence of light at 25 ± 2 °C with $60 \pm 5\%$ relative humidity for 28 days and 5 ± 3 °C for 28 days, respectively. Physicochemical stability (appearance, pH, protein concentration, size exclusion high-performance

liquid chromatography, non-reducing capillary electrophoresis–sodium dodecyl sulfate, imaged capillary isoelectric focusing), biological activity (competitive inhibition binding assay to human epidermal growth factor receptor 2 by fluorescence resonance energy transfer, anti-proliferation assay), and properties with a potential safety impact (subvisible particulates, submicronic aggregation by dynamic light scattering) were determined.

Results: No physicochemical instability signs or biological activity changes were observed for either reconstituted or diluted SB3 up to 28 days; all stability acceptance criteria were met. No major change was noted in the proportion of molecular weight variants (high molecular weight impurity, total purity) or relative percentages of acidic, main, and basic charge isoforms of the protein. No increases in particulates or aggregates in terms of a potential safety impact were noted.

Conclusion: The physicochemical stability and biological activity of reconstituted and diluted SB3 are maintained for extended time periods beyond those denoted in the product labeling, which allows for advanced SB3 preparation and may reduce drug wastage and preparation time.

Funding: Samsung Bioepis Co., Ltd.

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Keywords: Biosimilar; Extended stability; HER2; Herceptin; In-use; Ontruzant; SB3; Stability; Storage; Trastuzumab

INTRODUCTION

Trastuzumab (Herceptin[®]) is a recombinant humanized immunoglobulin G1 monoclonal antibody against the human epidermal growth factor receptor 2 (HER2) [1]. SB3 (Ontruzant[®]) is the first trastuzumab biosimilar approved by the European Union and received approval in November 2017 [2]. Pharmacokinetic equivalence between SB3 and its reference product has been demonstrated in healthy adults, and the efficacy and safety of SB3 have been shown to be comparable to those of the reference product in a randomized, double-blind, phase III trial of patients with HER2-positive early breast cancer [3–5].

According to the Summary of Product Characteristics (SmPC) for both products (SB3 and reference product), aseptic conditions during handling are paramount, as both products are only for single use and no preservative is present [1, 2]. Under aseptic conditions, SB3 and its reference product are physically and chemically stable for 48 h when reconstituted with sterile water for injection and stored at 2 °C to 8 °C; any remaining reconstituted solution should be discarded. Diluted SB3 solutions for intravenous infusion are physically and chemically stable for 24 h at temperatures up to 30 °C in polyvinylchloride (PVC), polyethylene, or polypropylene bags containing 0.9% normal saline [2]. Diluted reference product is physically and chemically stable for up to 7 days at 2–8 °C, and subsequently for 24 h at temperatures up to 30 °C in PVC, polyethylene, or polypropylene bags containing 0.9% normal saline [1].

Extended stability data have been published for Herceptin[®] supporting advanced preparation and dose-banding [6]. As stability data for the monoclonal antibody SB3 are limited, additional stability data are desired. Stability data are relevant to monoclonal antibodies because their therapeutic effects depend upon their structural integrity [5]. This study evaluated the extended physicochemical stability and biological activity of reconstituted and diluted SB3 for storage periods beyond those approved from the authorities and was conducted to show that advance preparation is feasible to avoid unnecessary delays in the

management of the patient's treatment without any impact on the quality or biological activity of the product. Under controlled and aseptic conditions, the stability of reconstituted SB3 stored at 25 ± 2 °C with $60 \pm 5\%$ relative humidity (RH) for 3 days and the stability of diluted SB3 stored in the absence of light at 5 ± 3 °C and 25 ± 2 °C with $60 \pm 5\%$ RH for 28 days were studied, respectively.

Although knowledge of physical and chemical stability of SB3 is important, assessment of biological activity is also important because it provides data on the potential efficacy of SB3. Trastuzumab binds to HER2 and inhibits the proliferation of HER2-overexpressing cells [7]. Based on the mechanism of action of trastuzumab, tests to measure HER2 binding and to quantify the anti-proliferation effect were used to assess biological activity. The biological assessments are a component of shelf-life assessment of a biosimilar [8]. Thus, this study was designed to provide biological assessment data related to the mechanism of action under the study conditions. In addition, assessment of parameters that might impact safety in the form of subvisible particle levels, degradation, and aggregation was included. Therefore, this study was designed to provide data regarding the physicochemical stability, biological activity, and any potential safety implication of both reconstituted and diluted SB3 when stored longer than periods stated in the approved labeling. The study was based on the UK's National Health Service (NHS) protocol [8].

METHODS

This article does not contain any studies with human participants or animals performed by any of the authors.

Sample Preparation

Reconstitution and Storage of SB3

Reconstituted drug solutions were prepared, stored, and analyzed as per Fig. 1. Three batches of SB3 (P1703019, P1703020, and P1703022; Ontruzant[®]; Samsung Bioepis NL B.V., Delft, the Netherlands) were used.

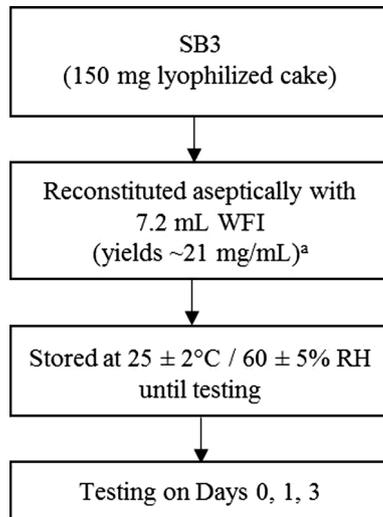


Fig. 1 Preparation, storage, and testing of reconstituted SB3. ^aIn accordance with the SmPC [2]; WFI manufactured by Daihan Pharmaceutical Co., Ltd. SmPC summary of product characteristics, RH relative humidity, WFI water for injection

Dilution and Storage of SB3

Dilutions of SB3 were prepared, stored, and analyzed as per Fig. 2. Three different batches of SB3 were reconstituted with 7.2 mL of water for

injection: batch 1 (P1703019; expiry date Jan 2022), batch 2 (P1703020; expiry date Jan 2022), and batch 3 (P1703022; expiry date Jan 2022). Polyethylene infusion bags (500 mL, Ecoflac[®], B. Braun) containing 500 mL of 0.9% sodium chloride were used. The actual volume of diluent in five of the 500-mL bags was measured, and the average volume was calculated. The average overfilled volume and an additional 250 mL were withdrawn from six 500-mL bags to yield a remaining 250 mL in each bag. These six bags were used to create the 80 mg/250 mL (batch 1), 140 mg/250 mL (batch 2), and 1000 mg/250 mL (batch 3) diluted solutions of SB3. As the reconstituted solution is 21 mg/mL, 3.8 mL was needed for 80 mg, 6.7 mL was needed for 140 mg, and 47.6 mL was needed for 1000 mg. The needed volume was removed from the 250-mL bag before adding the same volume of the drug solution for a total volume of 250 mL. The infusion bag was gently inverted 10 times to mix. The final concentrations were approximately 0.32 mg/mL (80 mg/250 mL), 0.56 mg/mL (140 mg/250 mL), and 4 mg/mL (1000 mg/250 mL).

^aDay 28 sample from both bags was further stored at $5 \pm 3^\circ\text{C}$ for 33 days.
NS normal saline (0.9% sodium chloride), RH relative humidity

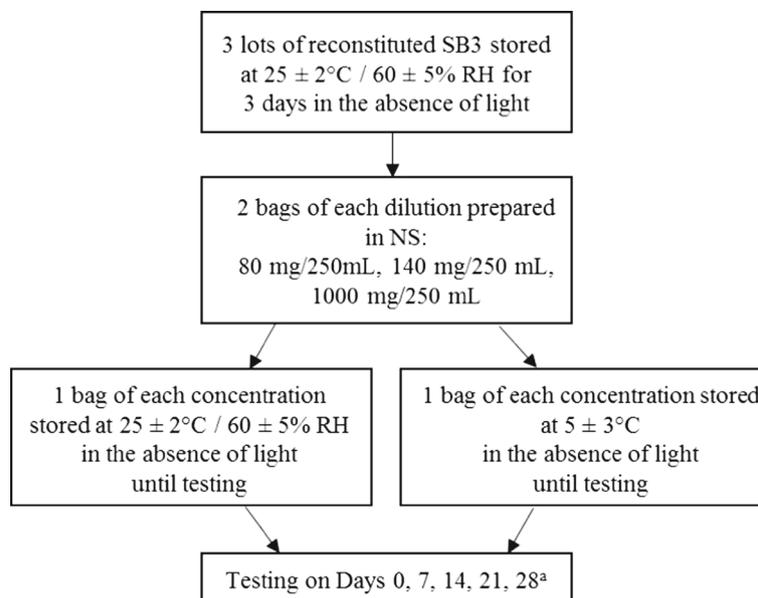


Fig. 2 Preparation, storage, and testing of diluted SB3

Sample Testing

The following validated methods were used to test samples: general tests, which included appearance (color, clarity, visible particulates) and pH; protein concentration; purity and impurity tests, which included size exclusion high-performance liquid chromatography (SE-HPLC), capillary electrophoresis–sodium dodecyl sulfate (CE-SDS), and imaged capillary isoelectric focusing (icIEF); biological activity tests, which included an anti-proliferation assay and an assessment of the relative binding activity of SB3 to the HER2 ligand by use of the competitive inhibition binding assay to HER2 by fluorescence resonance energy transfer (FRET); and tests for subvisible particle levels, degradation, and aggregation for potential safety implications (Table 1). The stability-indicating nature of physicochemical and biological assays used was demonstrated through forced degradation studies: SB3 product was stressed by temperature (40 ± 2 °C with $75 \pm 5\%$ RH) and stored for up to 6 months, and reconstituted SB3 underwent oxidation (0.1% hydrogen peroxide treatment and incubation at 5 ± 3 °C for 6 h), controlled acidic pH change (hydrochloric acid treatment and incubation at 25 ± 2 °C with $60 \pm 5\%$ RH for 7 days), and controlled basic pH change (sodium hydroxide treatment and incubation at 25 ± 2 °C with $60 \pm 5\%$ RH for 7 days).

General Tests and Protein Concentration

A manual visual inspection hood MIH DX (Bosch Packaging Technology) was used for the appearance tests of color, clarity, and visible particulates. The following standards were followed: Ph. Eur. 2.2.2 [9] for color, Ph. Eur. 2.2.1 [9] for clarity, and Ph. Eur. 2.9.20 [9] for visible particulates. The Ph. Eur. 2.2.3 [9] standard and Seven Excellence pH Meter and In Lab Micro Probe-ISM (Mettler Toledo) were used for pH measurement. An ultraviolet–visible spectrophotometer (Shimadzu Scientific instruments) was used for protein concentration determination.

Purity and Impurity Tests

For SE-HPLC, samples were injected onto a TSK gel G3000SW_{XL} column ($5 \mu\text{m}/7.8 \text{ mm} \times 300 \text{ mm}$; Tosoh) with a flow rate of 0.5 mL/min and a mobile phase that consisted of 100 mM sodium phosphate and 200 mM sodium chloride, pH 6.8. A high-performance capillary electrophoresis system (PA 800 plus Pharmaceutical Analysis System; Beckman Coulter, Inc.) was used for CE-SDS analyses, which were conducted in non-reducing conditions. Each sample was electrokinetically introduced onto a capillary ($50 \mu\text{m}/30.2 \text{ cm}$; Beckman Coulter, Inc.). UV radiation (wavelength, 220 nm) was passed through the capillary window and aperture ($100 \times 200 \mu\text{m}$; Beckman Coulter, Inc.) and was detected by the PA800 Plus. For icIEF, sample mixture with the carrier ampholytes was loaded onto the icIEF instrument using a capillary cartridge at 4 °C.

Biological Activity Tests

Time-resolved FRET was used to measure HER2 binding by samples. Fixed concentrations and volumes of europium (Eu)-labeled trastuzumab and cyanine 5-HER2 (Cy5-HER2) were added to the assay plate and incubated at ambient temperature with moderate agitation. Unlabeled SB3 competed against Eu-labeled trastuzumab for binding to Cy5-HER2-Fragment crystallizable (Cy5-HER2-Fc); the binding of unlabeled SB3 to Cy5-Her2-Fc resulted in inhibition of fluorescence, whereas a fluorescence signal was generated from Eu-labeled trastuzumab binding to Cy5-HER2-Fc. The plate was read by a microplate reader using Envision™, and relative binding activity was calculated by parallel line analysis software. The CellTiter-Blue® Cell Viability Assay (Promega) was used to quantify the anti-proliferation effect on BT474 cells, which were derived from human breast carcinoma tissue and overexpress HER2 receptor. The metabolic capacity of cells was measured with the indicator dye resazurin; only viable cells reduced resazurin into resorufin, which fluoresced. Cell proliferation that is inhibited by SB3 will not yield a fluorescent signal.

Table 1 Summary of tests

Test	Method	Objective
Appearance (color)	Visual inspection using yellow standard (Y1–Y7)	Evaluation of color
Appearance (clarity)	Visual inspection using turbidity standard (3 NTU–30 NTU)	Evaluation of clarity level
Appearance (visible particulates)	Visual inspection	Determination of visible particle number
pH	pH measurement	Evaluation of pH
Protein concentration	A_{280} measurement by UV–Vis spectrophotometer	Determination of protein concentration
SE-HPLC	UV detection (wavelength, 280 nm)	Determination of the percentage of high molecular weight impurities
CE-SDS (non-reducing)	UV radiation (wavelength, 220 nm)	Determination of total purity and the presence of low molecular weight impurities
icIEF	Capillary imaging at 4 °C	Determination of the change in charge variance including acidic, main, and basic variants
Competitive inhibition binding assay to HER2	Fluorescence resonance energy transfer	Determination of the relative binding to the reference standard
Anti-proliferation assay	CellTiter-Blue® Cell Viability Assay	Determination of the relative potency to the reference standard
Subvisible particulates	Light obscuration	Determination of particulate matter in subvisible particles (quantification of particles $\geq 10 \mu\text{m}$, and $\geq 25 \mu\text{m}$ in size)
Subvisible particulates	Microflow Imaging	Determination of particulate matter in subvisible particles (quantification of particles $\geq 2 \mu\text{m}$, $\geq 5 \mu\text{m}$, $\geq 10 \mu\text{m}$, and $\geq 25 \mu\text{m}$ in size)
Submicronic aggregation	Dynamic light scattering	Evaluation of the comparability of submicron particle size (1–1000 nm): hydrodynamic diameter and polydispersity index

CE-SDS capillary electrophoresis–sodium dodecyl sulfate, *HER2* human epidermal growth factor receptor 2, *icIEF* imaged capillary isoelectric focusing, *NTU* nephelometric turbidity units, *SE-HPLC* size exclusion high-performance liquid chromatography, *UV* ultraviolet, *UV–Vis* ultraviolet visible

Potential Safety Implication Tests

Reconstituted samples were used for subvisible particulate assessment via light obscuration with a HIAC 9703+ and HRLD 400 instrument

(Beckman Coulter, Inc.) because a relatively large sample volume was required. In contrast, microflow imaging (MFI) was used for subvisible particulate assessment in diluted samples, and

submicronic aggregation in diluted samples was determined by dynamic light scattering (DLS).

RESULTS

Results are similar among batches and concentrations, as applicable for each test.

General Tests and Protein Concentration

The general testing and protein concentration results for the reconstituted and diluted solutions of SB3 over time at the controlled conditions are summarized in Table 2; results met the acceptance criteria and were not changed over time.

Purity and Impurity Tests

Over the study period, no major change was noted in the proportion of high molecular weight (HMW) impurity or total purity for reconstituted and diluted solutions, and all data for SB3 met the acceptance criteria (Figs. 3a, b, 4a, b, 5a, b). No meaningful changes in the peak profile of SB3 solutions were noted by SE-HPLC, CE-SDS (non-reducing), or icIEF for reconstituted samples over 3 days or diluted samples at both controlled storage conditions over 28 days (Figs. 6, 7, and 8; data are not shown for

reconstituted solutions or diluted solutions at 5 ± 3 °C or for all concentrations for CE-SDS or icIEF). Concerning impurities of 1.0% or more, only the two heavy one light chain (2H1L) impurity was present in all samples for all storage conditions; the percentage of 2H1L ranged between 1.1% and 2.1%. In addition, the proportion of HMW impurity was not changed for the diluted samples that were removed at day 28 from both storage conditions and further stored in a microtube at 5 ± 3 °C for 33 more days (data are not shown for the extended storage data beyond the study period). The relative percentages for the acidic, main, and basic isoforms of the protein were stable over the 28-day storage period for all samples (Figs. 3c, 4c, 5c).

Biological Activity Tests

No substantial changes were noted in the percentage relative potency or binding activity over time for reconstituted and dilution solutions (Fig. 9a–f); the acceptance criteria were met for all measurements.

Potential Safety Implication Tests

All counts of subvisible particulates ≥ 10 μm and ≥ 25 μm by HIAC for reconstituted solutions met the acceptance criteria. Over the 3-day storage period, the number of

Table 2 General test results and protein concentrations of reconstituted and diluted SB3 stored under controlled conditions

Test	Result	
	Reconstituted SB3 at 25 °C for 3 days ^a	Diluted SB3 at 5 °C and 25 °C for 28 days ^b
Appearance	No change in color, clarity, or particulate matter (no fiber, flake, or particles present)	Slight variance in clarity between < 3 and < 6 NTU; no change in color or particulate matter (no fiber, flake, or particles present)
pH	6.1–6.2	6.1–6.5
Protein concentration	139–146 mg/vial	0.3–3.9 mg/mL

NTU nephelometric turbidity units, RH relative humidity

^a Reconstituted and stored at 25 ± 2 °C with $60 \pm 5\%$ RH over 3 days

^b Diluted SB3 stored at 5 ± 3 °C or 25 ± 2 °C with $60 \pm 5\%$ RH in the absence of light over 28 days

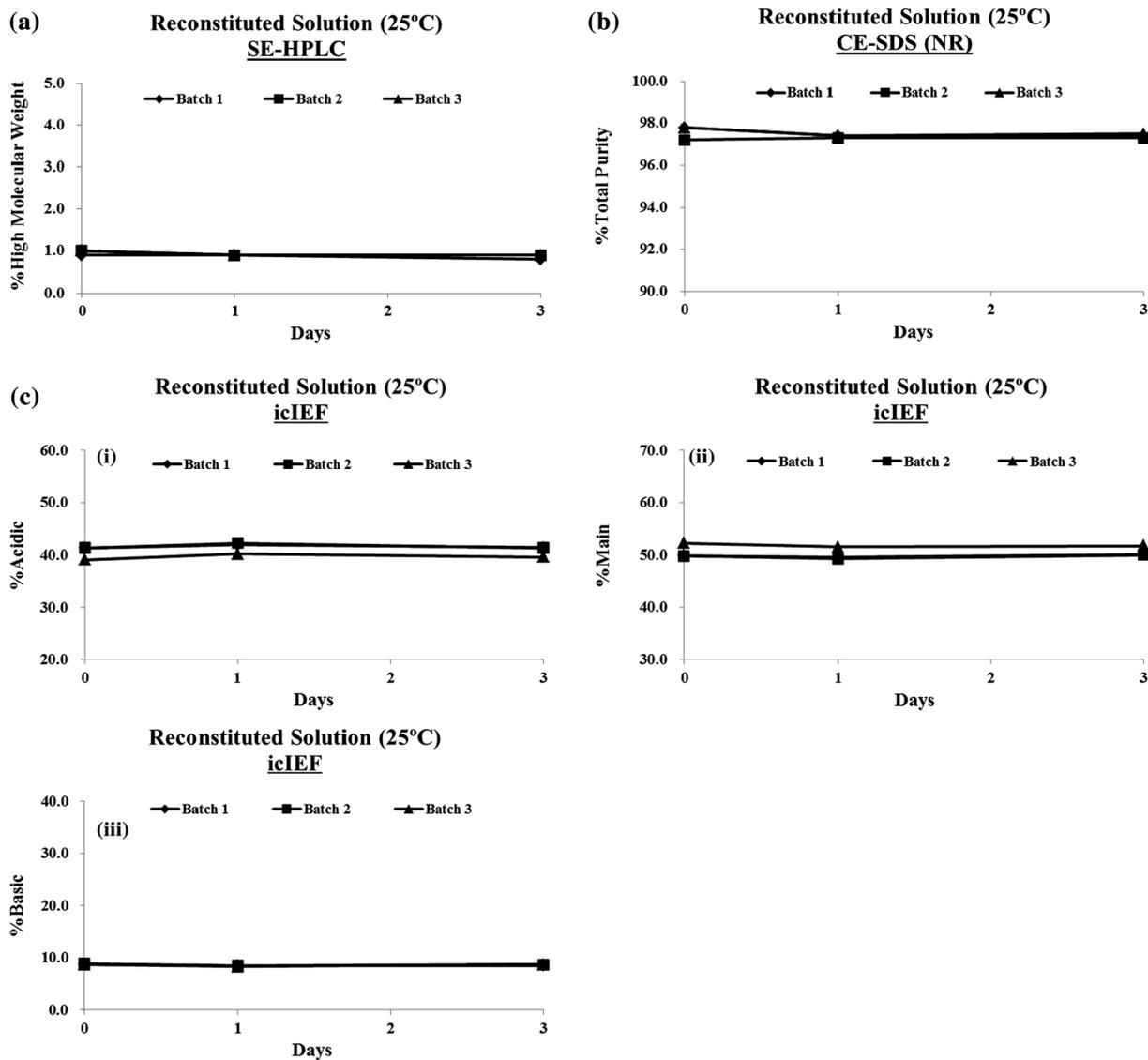


Fig. 3 Trends for purity and impurity analyses of reconstituted SB3 at 25 ± 2 °C with $60 \pm 5\%$ RH in the absence of light over 3 days. **a** Percentage of HMW as measured by SE-HPLC. **b** Percentage total purity as measured by CE-SDS (NR). **c** Percentage (i) acidic (ii) main, and (iii) basic isoforms as measured by icIEF. CE-

SDS capillary electrophoresis–sodium dodecyl sulfate, HMW high molecular weight, icIEF imaged capillary isoelectric focusing, NR non-reducing, RH relative humidity, SE-HPLC size exclusion high-performance liquid chromatography

particles ≥ 10 μm per vial ranged from 50 to 2054, and the number of particles ≥ 25 μm per vial ranged from 0 to 31.

For subvisible particulate assessments by MFI, no appreciable changes in the average number of subvisible particles sized ≥ 2 μm , ≥ 5 μm , ≥ 10 μm , or ≥ 25 μm were noted over

the 28 days for all diluted concentrations and for both storage conditions (data not shown).

For submicronic aggregation by DLS, no increase in either the hydrodynamic diameter (Fig. 10a) or the polydispersity index (Fig. 10b) was noted over the 28-day storage period for all diluted concentrations and for both storage conditions. Further, populations of high

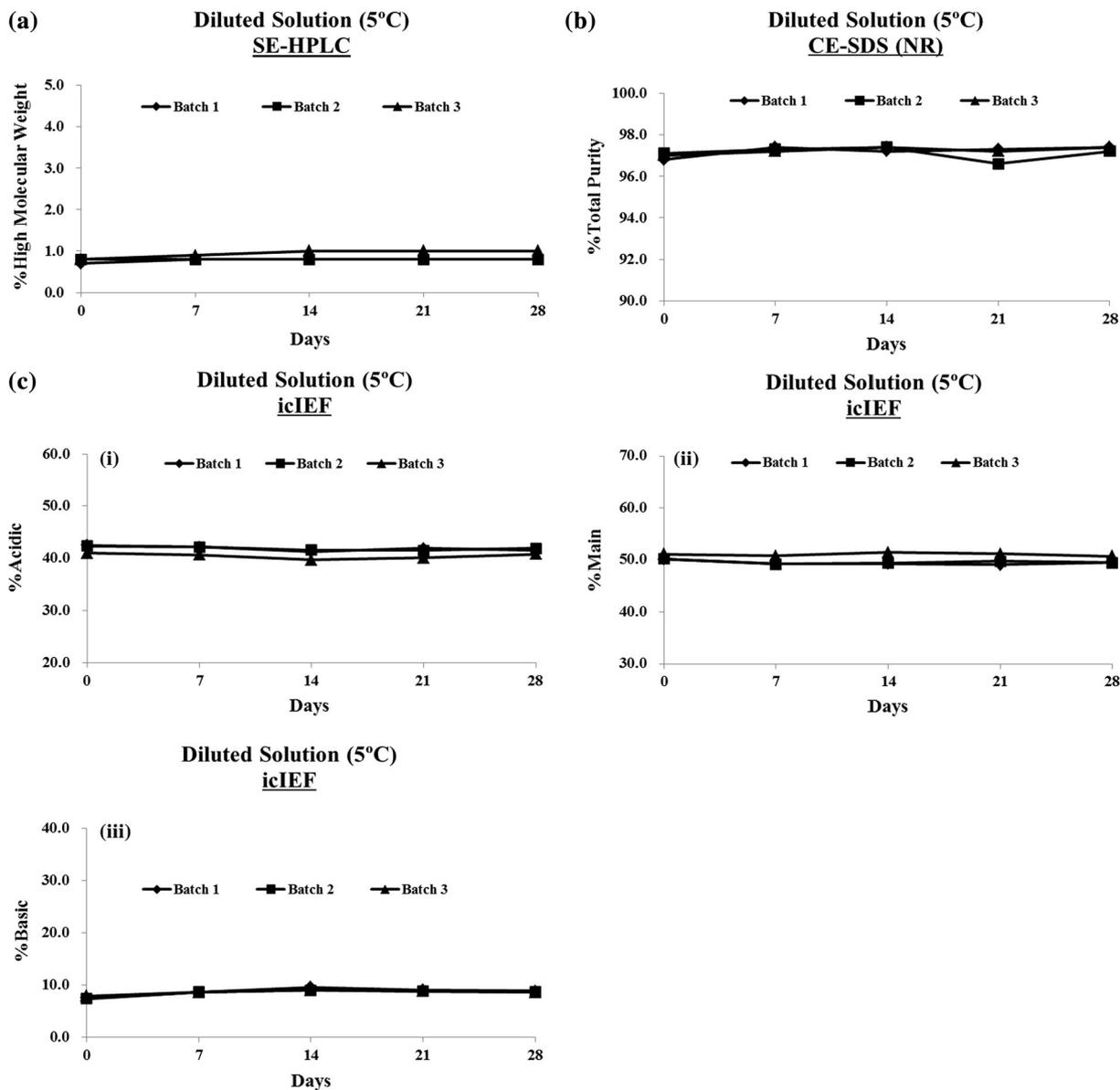


Fig. 4 Trends for purity and impurity analyses of diluted SB3 at 5 ± 3 °C in the absence of light over 28 days. **a** Percentage of HMW as measured by SE-HPLC. **b** Percentage total purity as measured by CE-SDS (NR). **c** Percentage (i) acidic, (ii) main, and (iii) basic isoforms as

measured by icIEF. *CE-SDS* capillary electrophoresis-sodium dodecyl sulfate, *HMW* high molecular weight, *icIEF* imaged capillary isoelectric focusing, *NR* non-reducing, *SE-HPLC* size exclusion high-performance liquid chromatography

hydrodynamic diameter were not present, particularly between 100 nm and 1000 nm.

Stability-Indicating Nature of Tests

For forced degradation studies (data not shown), the following changes were noted after exposure

of SB3 product to heat stress and subsequent reconstitution: the proportion of HMW impurities increased, and both relative percentage for main isoform and relative potency by anti-proliferation assay decreased. Under the oxidative stress condition, reconstituted SB3 showed an increase in the percentage oxidation of the

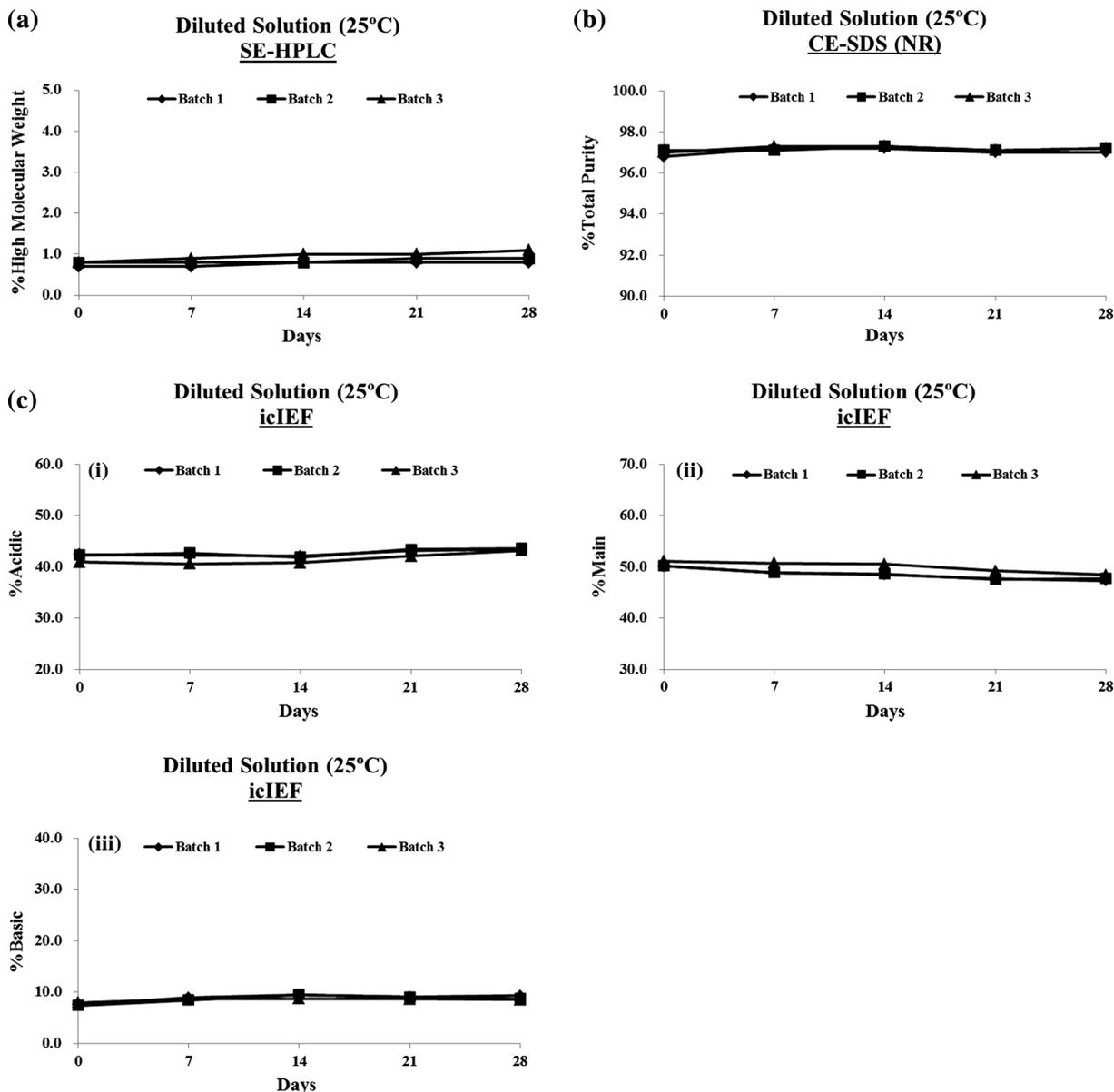


Fig. 5 Trends for purity and impurity analyses of diluted SB3 at 25 ± 2 °C with $60 \pm 5\%$ RH in the absence of light over 28 days. **a** Percentage of HMW as measured by SE-HPLC. **b** Percentage total purity as measured by CE-SDS (NR). **c** Percentage (i) acidic, (ii) main, and (iii) basic

methionine 255 residue with no major changes in physicochemical and biological properties. With exposure to high pH, an increase in the proportion of HMW impurities and relative percentage for the acidic isoform was observed. With exposure to low pH, slight variance in the proportion of HMW impurities was observed.

isoforms as measured by icIEF. CE-SDS capillary electrophoresis-sodium dodecyl sulfate, HMW high molecular weight, icIEF imaged capillary isoelectric focusing, NR non-reducing, RH relative humidity, SE-HPLC size exclusion high-performance liquid chromatography

DISCUSSION

Our study employed a design that is congruent with NHS standards for a robust evaluation of monoclonal antibody stability for a period of extended storage [8]. For reconstituted solutions, the study elements of storage condition,

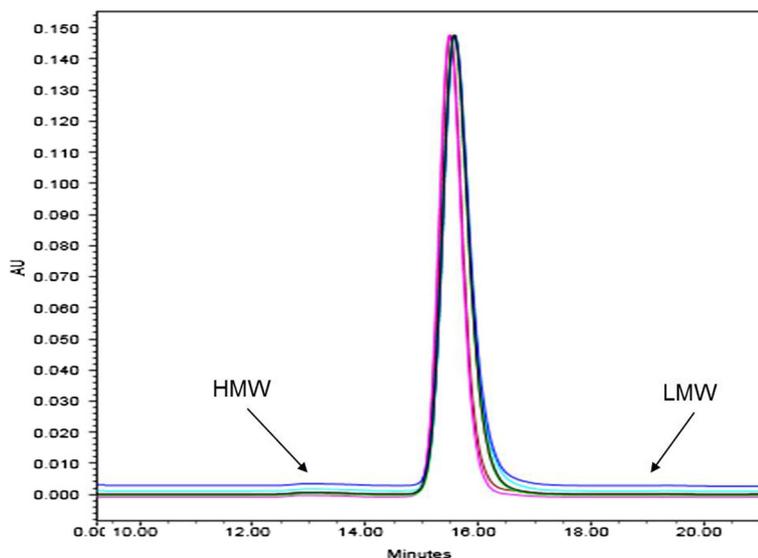


Fig. 6 Corresponding SE-HPLC peak profiles at day 0 and day 28 for all diluted SB3 concentrations at $25 \pm 2^\circ\text{C}$ with $60 \pm 5\%$ RH in the absence of light. Black line SB3 batch 1 (80 mg/250 mL) on day 0, dark blue line SB3 batch 1 (80 mg/250 mL) on day 28, green line SB3 batch 2 (140 mg/250 mL) on day 0, light blue

line SB3 batch 2 (140 mg/250 mL) on day 28, pink line SB3 batch 3 (1000 mg/250 mL) on day 0, brown line SB3 batch 3 (1000 mg/250 mL) on day 28. AU absorbance units, HMW high molecular weight, LMW low molecular weight, RH relative humidity, SE-HPLC size exclusion high performance liquid chromatography

storage period, and sampling strategy met the guidance standards with slight variance, as noted. The storage condition of $5 \pm 3^\circ\text{C}$ in the absence of light was not evaluated for reconstituted SB3, as reconstituted SB3 has demonstrated stability up to 48 h between 2°C and 8°C [2]. As only a 3-day storage period was evaluated for the reconstituted drug, the sampling strategy did not contain the minimum four sampling points in addition to the baseline data, as recommended by the NHS standards for studies less than 6 months [8]. For the diluted solutions tested up to 28 days, the study elements of diluent (0.9% sodium chloride), infusion container (non-PVC), concentrations, storage conditions, storage period, and sampling strategy met the NHS standards [8]. Our study also included evaluation of samples at one time point beyond the proposed storage period (28 days) [8]. At 33 days further storage after removal at day 28, the proportion of HMW impurity, which is one of the sensitive parameters indicating stability, was not changed. As for the study element of sample numbers, even though there were no replicate bags prepared for each storage condition, three independent

batches of starting material that covered a range of concentrations were used according to the guidance standards. Sample homogeneity was demonstrated by consistency of protein concentrations at every time point over the 28-day period, and validated test methods were used to evaluate all the diluted batches. Therefore, single preparation is considered to be adequate without the need for multiple repeated sampling. In addition, the stability-indicating nature of analytical techniques used in this study was demonstrated with forced degradation studies (e.g., exposure to heat, oxidative stress, and controlled change in pH).

None of the results indicated instability of reconstituted or diluted SB3 under the studied storage conditions. Several orthogonal and complementary methods were used to assess physicochemical stability, as a combination of techniques is required to give robust information. For example, non-reducing CE-SDS was used because of its ability to completely resolve or separate incomplete antibody fragments and low molecular weight variants as compared with SE-HPLC. The icIEF results of no change in charge isoforms indicated an absence of change

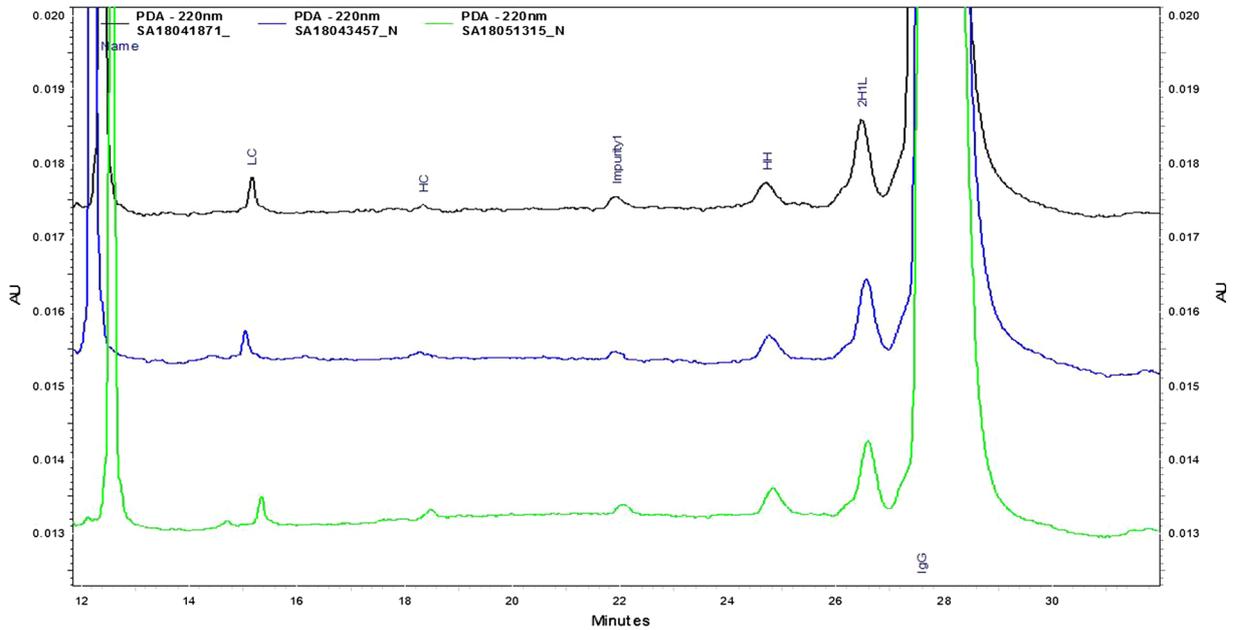


Fig. 7 Corresponding non-reducing CE-SDS electropherogram at day 0, day 14, and day 28 for 1000 mg/250 mL diluted SB3 at 25 ± 2 °C with $60 \pm 5\%$ RH in the absence of light. Black line SB3 batch 3 (1000 mg/250 mL) on day 0, blue line SB3 batch 3 (1000 mg/250 mL) on day 14, green line SB3 batch 3 (1000 mg/250 mL) on day 28. *2HIL* 2 heavy 1 light chain, *AU* absorbance unit, *HC* heavy chain, *HH* 2 heavy chain, *LC* light chain, *RH* relative humidity

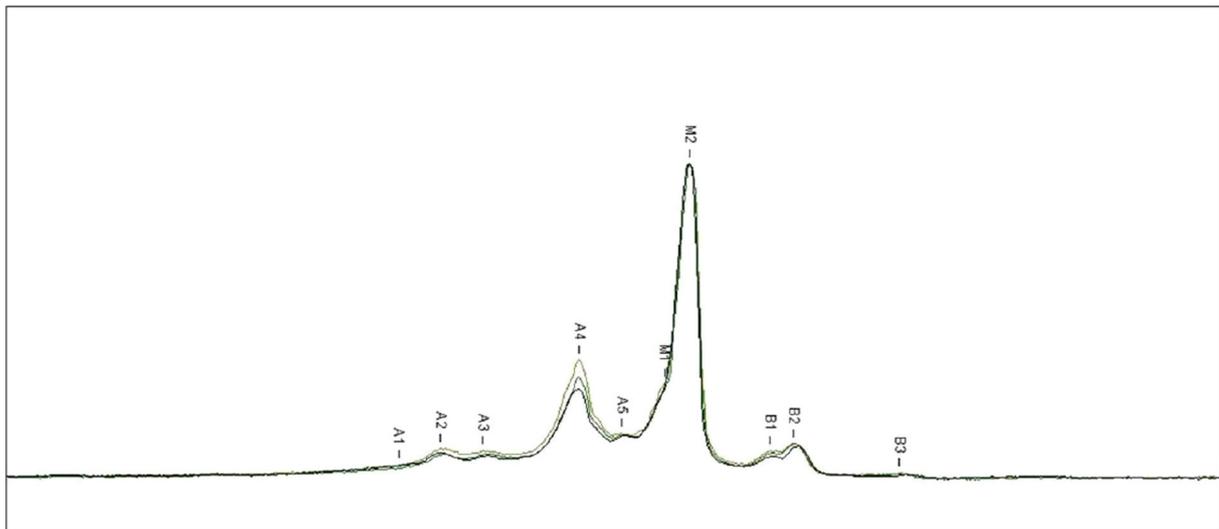


Fig. 8 Corresponding icIEF electropherogram at day 0, day 14, and day 28 for 1000 mg/250 mL diluted SB3 at 25 ± 2 °C with $60 \pm 5\%$ RH in the absence of light. Black line SB3 batch 3 (1000 mg/250 mL) on day 0, green line SB3 batch 3 (1000 mg/250 mL) on day 14, yellow line SB3 batch 3 (1000 mg/250 mL) on day 28. *A* acid, *B* basic, *M* main, *RH* relative humidity

to the initial structure. No meaningful changes in peak profiles, molecular weight variants, or additional impurities were noted for any of the

diluted SB3 solutions under both storage conditions for 28 days. The pH of the reconstituted SB3 solutions for 3-day storage at 25 °C varied

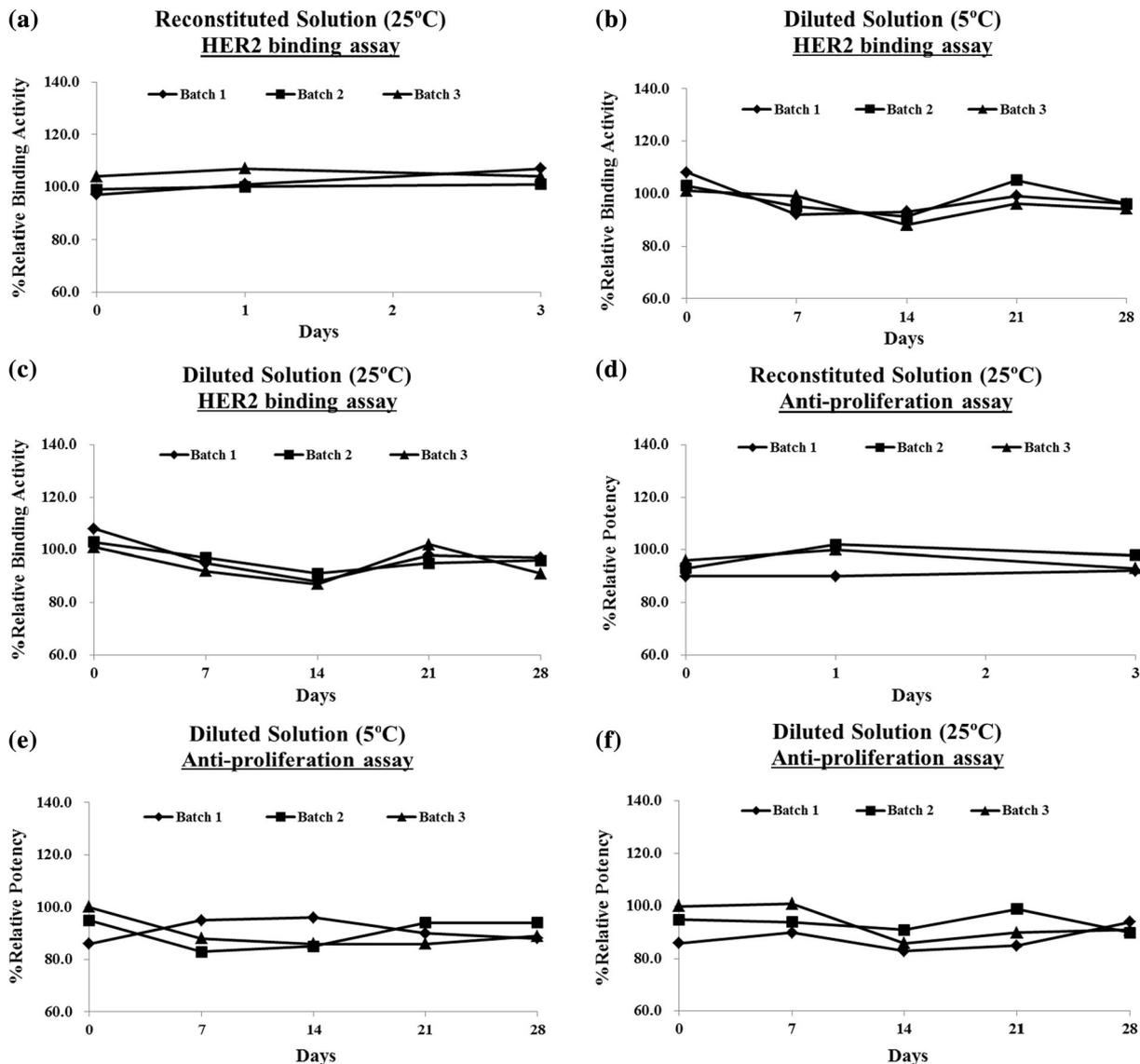


Fig. 9 Trends in biological activity analysis results. **a** Percentage relative binding activity as measured by HER2 binding assay for reconstituted solutions at 25 ± 2 °C with 60 ± 5% RH in the absence of light over 3 days. **b** Percentage relative binding activity as measured by HER2 binding assay for diluted solutions at 5 ± 3 °C in the absence of light over 28 days. **c** Percentage relative binding activity as measured by HER2 binding assay for diluted solutions at 25 ± 2 °C with 60 ± 5% RH in the absence of light over 28 days. **d** Percentage relative potency

as measured by anti-proliferation assay for reconstituted solutions at 25 ± 2 °C with 60 ± 5% RH in the absence of light over 3 days. **e** Percentage relative potency as measured by anti-proliferation assay for diluted solutions at 5 ± 3 °C in the absence of light over 28 days. **f** Percentage relative potency as measured by anti-proliferation assay for diluted solutions at 25 ± 2 °C with 60 ± 5% RH in the absence of light over 28 days. *HER2* human epidermal growth factor receptor 2, *RH* relative humidity

only by 0.1 units, and the pH values of the diluted solutions for 28-day storage at either 5 °C or 25 °C were fairly consistent and varied by a maximum of 0.4 units; minimal variability

in pH, defined as a change less than 0.5 pH units, is indicative of stability [8]. Additionally, in a laboratory setting, the biological function of SB3 appeared to be retained after storage of

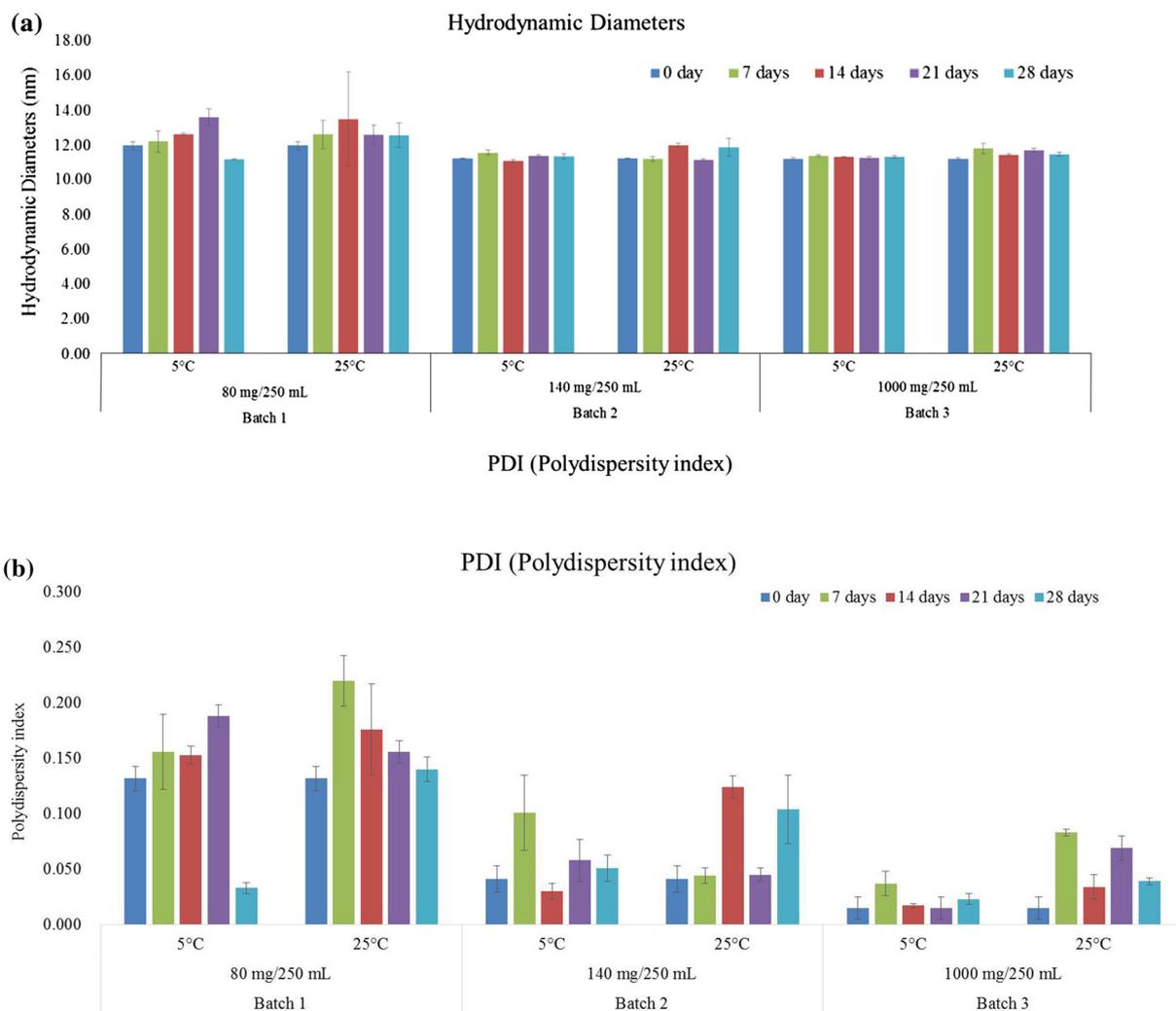


Fig. 10 Trends in submicronic aggregation assessed by dynamic light scattering for reconstituted solutions over 28 days. **a** Hydrodynamic diameters and **b** polydispersity

index for diluted solutions at $5 \pm 3^\circ\text{C}$ and $25 \pm 2^\circ\text{C}$ with $60 \pm 5\%$ RH in the absence of light. RH relative humidity

the reconstituted and diluted SB3 under the test conditions. The data, thus, provides an indirect indication that SB3 under the extended storage conditions studied would be efficacious. Lastly, no potential safety implication of reconstituted SB3 was demonstrated by particulate matter quantitation. The number of particles $\geq 10 \mu\text{m}$ per vial ranged from 50 to 2054, and the number of particles $\geq 25 \mu\text{m}$ per vial ranged from 0 to 31; these values are well within safety standards in USP <788> and Ph. Eur. 2.9.19, which are defined as an average of ≤ 6000 of particles $\geq 10 \mu\text{m}$ and ≤ 600 of particles $\geq 25 \mu\text{m}$ per container [9, 10]. For diluted SB3, no potential

safety implication was demonstrated by the MFI and DLS results. Microflow imaging is sensitive to $1 \mu\text{m}$ and, thus, may be a better option for biopharmaceuticals compared to light obscuration methods [8]. Dynamic light scattering is a method for providing size and polydispersity information for monomers, oligomers, and submicron-sized particles (1–1000 nm) and is used to detect HMW aggregates. Dynamic light scattering is, thus, a complementary test to MFI. Proteins such as trastuzumab may associate to form low-order oligomers. Further, denatured proteins tend to form aggregates. Populations of high hydrodynamic diameter were not present,

which suggests that a nucleation leading to aggregation is unlikely to occur. The MFI, DLS, and SE-HPLC results were consistent: storage of SB3 for 28 days at either temperature had no effect on its hydrodynamic diameter and did not trigger the formation of populations of submicron aggregates.

Potential limitations of the diluted stability data pertain to the methods and supplies utilized in this study, especially when compared to the standard preparation instructions in the SmPC. According to the NHS, product stability can be affected by the container including the type and size of the container as well as the amount of air headspace, and extrapolation of stability data is inappropriate when the handling of the product is not an exact match [8]. As a result of the aforementioned factors, stability studies should be performed in the containers typically used in practice. Additionally, it should be noted that polyolefin bags (e.g., polyethylene or polypropylene) may interact differently with monoclonal antibodies, so caution should be used when extrapolating data. According to the SmPC, the needed amount of the reconstituted solution is to be added to an infusion bag containing 250 mL of 0.9% sodium chloride [2]. In the current study, a 500-mL semi-rigid bag was selected, then 250 mL plus an approximate amount of overfill was removed. Additionally, the volume of the reconstituted SB3 to be added to the bag was removed as 0.9% sodium chloride volume to yield a total volume of 250 mL, thus differing from SmPC preparation instructions. Although a bag containing approximately 250 mL was used in both cases, the size of the infusion bag and, therefore, headspace was larger in this study than if a 250-mL bag had initially been selected. Protein unfolding and protein aggregation may occur at interfaces between the drug solution and the plastic container and/or between the drug solution and the air headspace; thus, a higher headspace and surface area to volume ratio is the worst-case scenario [8]. The DLS results validated no increase in either the hydrodynamic diameter or the polydispersity index over the 28-day storage period for all concentrations of SB3 under both storage conditions (5 °C and 25 °C) and, thus, suggest no protein unfolding or aggregation.

Although preparation of reconstituted and diluted SB3 solutions was done aseptically, the data are limited in terms of a bioburden assessment of the samples prepared in a real-world environment. Microbiological tests were not conducted on any of the samples. Because of the possibility of microbial contamination, SB3 is not intended to be stored after reconstitution and dilution unless these are performed under controlled and validated aseptic conditions, and any decisions to use storage times and conditions beyond those stipulated in the SmPC are the user's responsibility [2].

CONCLUSIONS

Because of the positive evidence from the numerous techniques used, data from this study support the physicochemical stability and maintained biological activity of reconstituted SB3 stored at 25 ± 2 °C with 60 ± 5 % RH for 3 days and of diluted SB3 stored in the absence of light for 28 days at either 25 ± 2 °C with 60 ± 5 % RH or 5 ± 3 °C. The dilutions of 0.32 mg/mL, 0.56 mg/mL, and 4 mg/mL represent a broad range of concentrations that may be prepared in clinical practice. These data may help reduce the need to discard unused SB3 doses and allow for advance preparation without impact on the quality or biological activity of SB3. Advance SB3 preparation may ease the pharmacy workload, as well as reduce any drug wastage.

ACKNOWLEDGEMENTS

The authors express their thanks to Sung Jae Kim, Suhyeon Eo, Daehee Kim, Eojin Jung, Jin Ah Han, Miju Cho, Hyunjung Ryu, Dayoung Kim, In Hye Sung (Quality evaluation team, Samsung Bioepis) for their helpful assistance on performing test.

Funding. Sponsorship for this study, article processing charges, and the open access fee were funded by Samsung Bioepis Co., Ltd. All authors had full access to all of the data in this study

and take complete responsibility for the integrity of the data and accuracy of the data analysis.

Medical Writing Assistance. Medical writing assistance was provided by Laurel Riemann, PharmD, BCPS and Carolyn Ragsdale, PharmD, BCPPS of Med Communications, Inc. (Memphis, TN), and was funded by Samsung Bioepis Co., Ltd.

Authorship. Authors (Jihoon Yun and Jihyun Kim) contributed equally to this article. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

Disclosures. Jihoon Yun is an employee of Samsung Bioepis Co., Ltd. Jihyun Kim is an employee of Samsung Bioepis Co., Ltd. Jihyun Chung is an employee of Samsung Bioepis Co., Ltd. Su-Jeong Hwang is an employee of Samsung Bioepis Co., Ltd. Su Jin Park is an employee of Samsung Bioepis Co., Ltd.

Compliance with Ethics Guidelines. This article does not contain any studies with human participants or animals performed by any of the authors.

Data Availability. The data sets generated during and/or analyzed during the current study are private property and are not publicly available but can be obtained from the corresponding author upon reasonable request.

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