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Short-term study on in-use stability of opened bevacizumab biosimilar PF-06439535 vials

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ABSTRACT

Objectives Aggregation is one of the key critical points limiting the stability of monoclonal antibodies in solution. The present study aimed to investigate the in-use stability of a residual monoclonal antibody solution after withdrawal of most of the filling volume of PF-06439535 (bevacizumab biosimilar), addressing the physical and chemical stability with respect to aggregation and fragmentation.

Methods The stability of residual PF-06439535 solution (25 mg/mL) after withdrawal of 80% (12.8 mL) filling volume with a 20G needle was monitored over a light-protected storage period of 8 days at 2–8°C and 25°C with measurement time points at D0 (start of storage), D2, D4, and D8 (2, 4, and 8 days of storage after start, respectively). Unopened vials stored under the same conditions served as control. For this purpose, the analytical results from size exclusion chromatography, dynamic light scattering, and micro-flow imaging obtained after the individual measurement time points up to 8 days were compared with those obtained at D0 and with those obtained for unopened vials stored under the same conditions.

Results No aggregation or ongoing fragmentation due to partial withdrawal of filling volume could be observed in the residual PF-06439535 solution. Moreover, no changes in the particle size distribution at D8 compared with the D0 values were identified upon storage at either 2–8°C or 25°C (both opened and unopened vials). The total concentration of particles ≥10 µm of all samples was <100 particles/mL. In addition, no variations in the pH values or in the visual appearance were detected over the whole study period in all samples at all storage conditions.

Conclusions Consequently, residual PF-06439535 solution (25 mg/mL) in opened vials may be regarded as stable when stored light-protected over a period of 8 days in the refrigerator (2–8°C) or at 25°C.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ For Avastin, the first bevacizumab monoclonal antibody registered in the EU, degradation of bevacizumab was <13% in pierced and sampled vials when stored at 4°C for 6 months.
⇒ PF-06439535 is a bevacizumab biosimilar; there is a great demand in clinical practice for stability data of the remaining bevacizumab concentrate solutions in sampled large volume vials of PF-06439535.

WHAT THIS STUDY ADDS

⇒ No changes in the physical and chemical stability of residual PF-06439535 concentrate (25 mg/mL) could be observed after partial volume (12.8 mL of 16 mL vial, 80% of the filling volume) was withdrawn and stored protected from light in a refrigerator at 2–8°C or in an incubator at 25°C for 8 days.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ There is a strong interest in clinical practice in the stability of residual PF-06439535 concentrate in opened vials. Based on the generated extended stability data obtained in the present study, the stability of PF-06439535 should be verified in more extensive studies.

INTRODUCTION

Monoclonal antibodies (mAbs) are among the most important classes of therapeutic proteins, in particular for oncology and rheumatoid and autoimmune diseases.[1] One of the most common mAbs used to treat cancer is the anti-vascular endothelial growth factor (anti-VEGF) antibody bevacizumab because of its anti-angiogenic activity.[2–5] Bevacizumab is additionally often used off-label as cost-effective anti-VEGF therapy in ophthalmology.[6]

The first bevacizumab mAb registered in the EU was Avastin in January 2005.[7] Avastin has been approved to treat a range of cancers in adult patients.[7 8] After loss of exclusivity of the originator bevacizumab, several biosimilars were launched to improve patient access to bevacizumab by increasing treatment options and reducing treatment costs. To be approved, biosimilars must demonstrate similarity to reference products in analytical, non-clinical, and clinical pharmacology as well as clinical efficacy/safety studies; and they must have no clinically meaningful differences in quality, safety, and efficacy compared with their reference product.[9 10] PF-06439535 (Zirabev; Pfizer) is a bevacizumab biosimilar. Similarity was established through a rigorous comparison of PF-06439535 and the reference product.[11–14] PF-06439535 was approved by the European Medicines Agency in February 2019[15] and launched in Germany in June 2020.[16] PF-06439535 is commercially supplied in 4 mL and 16 mL vials containing 100 mg and 400 mg bevacizumab, respectively.[16] These are concentrates for the preparation of diluted infusion solutions. Since the clinical doses are dependent on the indication and patient's body weight,[16] the whole volume is often not drawn and residual volumes are left in the vial (especially the 16-mL vials). In the summary of product characteristics it is stated that bevacizumab

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vials are intended for single-use only and any residual volumes have to be discarded immediately.[16] Nevertheless, there is a great demand in clinical practice to obtain stability data on the residual volume of bevacizumab concentrate solutions in opened large volume vials of PF-06439535.

Many reports exist for the originator on bevacizumab stability and sterility when repackaged in syringes for intravitreal application.[17–20] The stability of residual bevacizumab concentrate volumes in fractionated vials has also been investigated,[18] revealing <10% degradation and preservation of the anti-VGEF activity, as well as sterility for up to 6 months, when the remaining content of multiple-dose vials of the originator product (Avastin) was stored at 4°C and aseptic conditions were followed during sampling. Another study reported a 9.6% and 12.7% degradation of bevacizumab in pierced and sampled vials of the originator product compared with unpierced unopened vials at 3 and 6 months, respectively.[17]

Due to the complexity of mAbs in terms of their structure and proprietary nature of their manufacturing process, some levels of heterogeneity usually exist not only between batches of the originator biologic product but also between biosimilars and the originator. Therefore, the stability data already collected for residual bevacizumab concentrates in fractionated vials of the originator product cannot be extrapolated to PF-06439535. Based on that background, we looked at the physical and chemical stability of bevacizumab PF-06439535 solution (25 mg/mL) with regard to fragmentation and aggregation over a period of 8 days in vials from which 80% (12.8 mL) of the initial volume (16 mL) was removed. These vials were stored protected from light at 4°C and 25°C, respectively, and subjected to stability analysis by applying size exclusion chromatography (SEC), dynamic light scattering (DLS), and micro-flow imaging (MFI), along with visual inspection of the appearance and measurement of pH.

METHODS

Chemicals, reagents, and equipment

Commercially available Zirabev 16 mL concentrate vials for infusion (PF-06439535) containing 400 mg bevacizumab-bvzr (Lot DK 1277) with expiry date 03/2023 were provided by Pfizer Pharma GmbH (Berlin, Germany). PF-06439535 is formulated at 25 mg/mL and excipients include succinic acid, sucrose, edetate disodium dihydrate (EDTA), polysorbate 80, sodium hydroxide to titrate solution to pH 5.5, and water for injection.[16 21] The protein standard mix consisting of ribonuclease A type I-A from bovine pancreas (1 mg/mL, 13.7 kDa), albumin chicken egg grade VI (1 mg/mL, 44.3 kDa), and thyroglobulin bovine (0.5 mg/mL, 670 kDa) used for calibration was bought from Sigma Aldrich (Darmstadt, Germany). Thermo Scientific COUNT-CAL count standard 10 µm, Thermo Scientific Nanosphere Particle size standard 20 nm, and Thermo Scientific Nanosphere Particle size standard 50 nm were purchased from Fisher Scientific (Schwerte, Germany).

Potassium dihydrogen phosphate, sodium chloride, and sodium hydroxide were purchased from Merck (Darmstadt, Germany). Purified water (as defined by the United States Pharmacopeia) was purified by using a Milli-Q purification system (Merck Millipore Corporation, Bedford, MA, USA).

SEC was carried out on an Agilent 1200 liquid chromatography system equipped with a binary pump SL of the Agilent 1200 series, an autosampler (Agilent 1200 series G1367C with coupled thermostat G1330B), and an ultraviolet detector (Agilent 1200 series G1315C). MFI analysis was conducted on an MFI-5200 particle analyser system (ProteinSimple, Santa Clara, CA, USA) equipped with a silane-coated high-resolution 100-µm flow cell. DLS was measured on a Zetasizer Nano ZS system (Malvern Instruments, Worcestershire, UK) using single-use 220–1600 nm UVette plastic cuvettes from Eppendorf (Hamburg, Germany).

Study design

A total of 84 PF-06439535 16 mL vials were received and stored for approximately 1 week at 2–8°C until study initiation.

At the start of the study, approximately 80% of the filling volume (12.8 mL) was withdrawn from the vials using a 20G needle connected to a 10-mL syringe. The pierced vials were not covered by parafilm but were stored protected from light upright at 2–8°C in a temperature-controlled refrigerator. An equivalent set of samples was kept in a temperature-controlled incubator at 25±2°C with 60±5% relative humidity. Analysis was carried out at the starting point (D0), and at the second (D2), fourth (D4), and eighth (D8) day of storage. Unopened (ie, not punctured) control vials were stored in the same refrigerator and incubator to be tested at D8.

At each time point the respective number of vials were removed from the storage location and analysed on the same day. The vials stored in the refrigerator were equilibrated to room temperature before analysis. Any necessary dilutions were carried out immediately before analysis. The analytical investigations are shown in table 1.

Analyses

Visual inspection

The samples were visually inspected for the presence of visible particles under a white light in front of a matte black panel and a non-glare white panel.

pH

pH measurements were made using a pH metre (SevenMulti pH/LF S47-KS with InLab Routine Pro electrode; Mettler Toledo,

Table 1 Overview of the analytical investigations, number of vials tested at each time point, and the applied test concentrations

		Number of samples measured at each time point and storage condition			
Analytical test	Test concentration	D0	D2 2–8°C/25°C	D4 2–8°C/25°C	D8 2–8°C/25°C
Visual inspection	25 mg/mL	3	3+3	3+3	3+3 + 3*+3*
pH	25 mg/mL	3	3+3	3+3	3+3 + 3*+3*
SEC†	0.5 mg/mL and 5.0 mg/mL	3	3+3	3+3	3+3 + 3*+3*
MFI	25 mg/mL	3	3+3	3+3	3+3 + 3*+3*
DLS	10 mg/mL	3	3+3	3+3	3+3 + 3*+3*

*Indicates additional unopened control vials that have been tested after storage in the same refrigerator and incubator at D8.
†Test concentration of 0.5 mg/mL was used for molecular weight analysis and the test concentration of 5.0 mg/mL was applied for side peak analysis.
D0, day 0 (start); D2, day 2; D4, day 4; D8, day 8; DLS, dynamic light scattering; MFI, micro-flow imaging; SEC, size exclusion chromatography.

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Gießen, Germany) calibrated under European Pharmacopoeia conditions. For each storage period, the pH measurements were performed in triplicate for both storage conditions.

SEC

Any formation of aggregates (high molecular weight impurities) or fragments (low molecular weight impurities) was detected by SEC using a Thermo Scientific MabPac SEC1 300 Å, 4.0×300 mm, 5 µm column, and Thermo Scientific MabPac SEC1 300 Å, 4.0×50 mm, 5 µm precolumn (Fisher Scientific, Schwerte, Germany). The samples were diluted with mobile phase to a concentration of 0.5 mg/mL for the determination of molecular weight and 5.0 mg/mL for the determination of side peaks via peak area percent. Three solutions were prepared from three vials, respectively, and each solution was measured twice. The mobile phase consisted of 100 mM potassium dihydrogen phosphate, containing 200 mM sodium chloride adjusted to pH 6.8 with sodium hydroxide. Chromatographic separation was carried out isocratically at a flow rate of 0.3 mL/min, applying an injection volume of 1 µL and adjusting the column temperature to 30°C and the autosampler temperature to 15°C. The isocratic elution profile obtained over a run time of 30 min was monitored using ultraviolet detection at 214 nm. The SEC column was calibrated by measuring the elution volume of molecular weight markers commercially available in a protein standard mix solution, the composition of which is mentioned above. Log molecular weight values of the standards were plotted against the elution volume to determine the equivalent molecular weight of the sample.

The method was validated by verifying linearity, repeatability, and accuracy. For verifying linearity, five different dilutions of the bevacizumab PF-06439535 sample (0.5, 1, 2, 4, and 5 mg/mL) were analysed in duplicate, respectively, yielding $R^2=0.98832$. The repeatability was verified by six-fold measurement of the PF-06439535 sample at both concentrations of 0.5 mg/mL and 5 mg/mL, yielding variation coefficients corresponding to 0.17% and 0.09%, respectively. For verifying accuracy, the molecular weight of five different dilutions of the PF-06439535 sample (0.5, 1, 2, 4, and 5 mg/mL) were analysed in duplicate, yielding molecular weights not deviating by more than 10% from the declared molecular weight. Based on the signal to noise ratio, the limit of quantitation was determined to correspond to 0.025%. A detailed overview of the individual values is presented in the online supplemental tables S4–S6 and in the online supplemental figure S1.

DLS

For DLS analysis, the samples were diluted with purified water to a final protein concentration of 10 mg/mL; 250 µL of the diluted sample were pipetted into the cuvette and each sample was equilibrated to 20°C for 120 s before measurement.

For stability testing, three vials at each time point and storage condition were analysed in triplicate by applying the automatic measurement mode at 20°C at an angle of 90°. Instrument parameters, such as attenuator settings, were optimised by the software to obtain the recommended count rates.

Malvern Zetasizer Software version 7.03 was used to fit the autocorrelation function by using the default settings for protein solutions and water (refractive index=1.333; viscosity=0.890 mPa*s) to calculate the Z-average diameter, polydispersity index (PdI), and particle size distribution by intensity. The average of the triplicate analyses was reported as analytical results.

At each day of measurement, a system suitability test was performed with two sizing standards (Nanosphere series 3000, sizing standards 20 nm and 50 nm).

MFI

Samples were analysed undiluted in MFI. The background illumination was performed using purified water. Samples of 1.0 mL were loaded and a pre-run volume of 0.25 mL was followed by a sample run of 0.58 mL at a flow rate of 0.17 mL/min. MFI View System Software version 2-R2-6.1.20.1915 was used to perform the measurements, and MFI View Analysis Suite (MVAS) version 1.3.0.1007 was used to analyse the samples for the presence of subvisible particles in a size range between 2–100 µm.

For the discrimination of silicone oil droplet-like particles (SP) and protein particles (non-silicone oil particles (NSP)) in the size range starting from 5 µm, the 'find similar' operation in the MVAS software was used. SP were selected manually as a basis for the automatic search function by the software. The particles were then categorised into three groups: the total number of particles, SP only, and NSP only, including the respective images.

Before sample measurement, a particle count control measurement was performed using a polystyrene counting standard of a defined particle size and particle concentration.

Statistical analysis

Student's t-test at the common significance level $\alpha=0.05$ was applied to compare the individual values obtained by SEC, DLS, and MFI for particles ≥2 µm for the opened vials at the different time points at different storage conditions, and for the unopened vials at different storage conditions with the D0 values, respectively.

RESULTS

Visual inspection

The visual assessment of the vials stored in the refrigerator or at 25°C revealed clear, colourless solutions, free of visible particles.

pH measurements

No significant changes in pH values were observed. The average pH values of opened vials were 5.49 at D0 and D8 when stored at 2–8°C and between 5.49 (D0) and 5.47 (D8) when stored at 25°C, being comparable to the D8 pH values of the unopened vials when stored at 2–8°C (5.48) and at 25°C (5.47).

SEC

Using the MabPac SEC1 300 Å column, the molecular mass of PF-06439535 could be determined accurately indicating the proper implementation of the method following the calibration with known molecular weight standards.

As can be depicted from the SEC chromatograms presented in figure 1A and B, the chromatographic profiles obtained for PF-06439535 after 8 days of storage at 2–8°C and 25°C were comparable to those at D0. Moreover, there was no difference between samples from opened or unopened vials.

Also, no additional side peaks were detected when comparing the SEC chromatograms of PF-06439535 diluted to a concentration of 5 mg/mL after storage for 8 days at 2–8°C and 25°C, respectively, to D0. The visible side peaks 1, 2, and 3 were already present at the start point. The same applies for the unopened vials (see figure 1C). Only peak 3a could not be seen at D0. It is assumed that it probably co-eluted with peak 3 at the start point and eluted at a later retention time in the following time points. For peak area evaluation, peaks 3 and 3a were calculated as sum.

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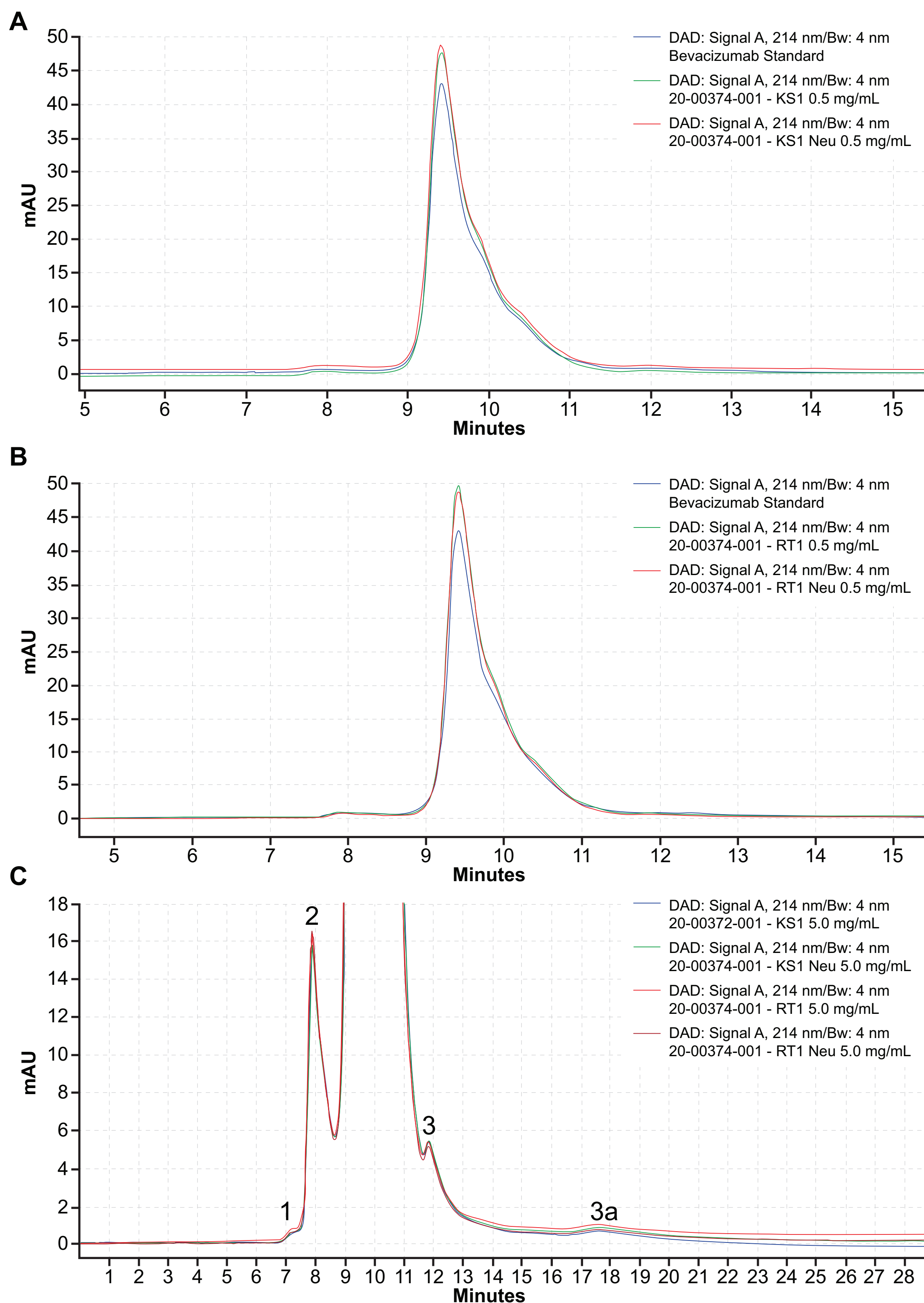

Figure 1 SEC chromatogram of PF-06439535. (A) SEC chromatogram of PF-06439535 at concentration of 0.5 mg/mL obtained at D0 in comparison with those obtained at D8 when stored at 2–8°C for the opened and unopened vials (blue=PF-06439535 at D0, green=PF-06439535 at D8, and red=PF-06439535 at D8 (unopened vial)). (B) SEC chromatogram of PF-06439535 at concentration of 0.5 mg/mL obtained at D0 in comparison with those obtained at D8 when stored at 25°C for the opened and unopened vials (blue=PF-06439535 at D0, green=PF-06439535 at D8, and red=PF-06439535 at D8 (unopened vial)). (C) SEC profile evolution of PF-06439535 at concentration of 5 mg/mL obtained at D8 when stored at 2–8°C and 25°C for the opened and unopened vials (blue=PF-06439535 at D8 (opened, 2–8°C), green=PF-06439535 at D8 (unopened, 2–8°C), red=PF-06439535 at D8 (opened, 25°C), and dark-red=PF-06439535 at D8 (unopened, 25°C)). D0, day 0 (start); D8, day 8; DAD, diode-array detection; SEC, size exclusion chromatography.

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Table 2 Overview of the average individual area percentages (mean %) compared with the bevacizumab PF-06439535 peak*

	Side peak 1 (RT about 7.7 min)	Side peak 2 (RT about 8.1 min)	Side peak 3 (RT about 12.2 min)
Time point/storage conditions	Mean %	Mean %	Mean %
D0/2–8°C unopened	0.059	2.869	0.635
D2/2–8°C opened	0.061	3.031	1.449
D2/25°C opened	0.052	3.003	1.436
D4/2–8°C opened	0.050	2.887	1.380
D4/25°C opened	0.052	2.940	1.355
D8/2–8°C opened	0.045	2.906	1.435
D8/25°C opened	0.048	2.993	1.325
D8/2–8°C unopened	0.043	2.923	1.450
D8/25°C unopened	0.048	3.017	1.361

*The bevacizumab PF-06439535 peak was determined from triplicate measurements of 3 opened vials at each test condition (D0, D2, D4, D8 at 2–8°C and 25°C, respectively), and for 3 unopened vials for D8 at 2–8°C and 25°C, respectively. Mean % represents the average individual area percentage of the respective side peak compared with the PF-06439535 peak.
D0, day 0 (start); D2, day 2; D4, day 4; D8, day 8; RT, retention time.

At the start, side peak 1 was present in a size of about 0.06 area percent. During stability analysis, it slightly decreased to values below 0.04% in the unopened control vials as well as in the opened stability vials stored in the refrigerator or at room temperature. This side peak eluted earlier than PF-06439535, which means that this compound has a higher molecular weight.

Side peak 2 also eluted earlier than PF-06439535 in a quite constant size of 2.8–3.0% at all conditions and time points tested.

Side peak 3 (with 3a) eluted later than PF-06439535, indicating a lower apparent molecular weight. As peak 3a was also detected in the unopened control vials, it is assumed that the appearance of this peak may be attributed to a change in the binding performance of the SEC column rather than to an ongoing degradation effect after piercing the vials and withdrawing 80% of the filling volume. This assumption is further supported by the fact that the peak area was quite constant from D2 until D8 under all conditions tested. An overview of the average individual area percentages obtained for the individual side peaks is presented in table 2, and a detailed overview of the individual values is presented in the online supplemental material tables S1–3.

Statistical analysis revealed significant differences for all values listed in table 2 compared with the respective D0 values for all side peaks except for side peak 1 at D0/2–8°C. Nevertheless, in no case did the sum of the three side peaks exceed an area percentage of 4.541% compared with the PF-06439535 peak. This indicates that the specified range of ±10% for PF-06439535 is complied with under all testing conditions. Consequently, PF-06439535 is considered stable for the investigated storage time and condition, even if statistical differences are detected.

DLS

The size distributions by volume for storage at 2–8°C and 25°C (figure 2A and B) suggest that all analysed samples were characterised by one dominating main species (≥98% main peak) at about 11–12 nm, which is the expected size for an mAb.[22 23] No differences were observed for up to 8 days of storage at 2–8°C and 25°C. The additional unopened vials analysed at D8 exhibited a similar profile as compared with the opened vials.

A summary of Z-average diameters and PdI values calculated from triplicate DLS measurements for all tested samples at all stability time points is presented in table 3. Statistical analysis revealed no significant changes in the Z-average values when comparing the individual values for the opened vials at the different time points at different storage conditions, and for the unopened vials stored at 25°C for 8 days with the D0 values. Only the unopened vial stored at 2–8°C revealed a significant difference to the D0 value, which might be attributed to inherent different Z-average diameters in both unopened vials.

No substantial changes or trends in sample polydispersity or Z-average diameter were determined in the PF-06439535 samples stored at 2–8°C or 25°C for up to 8 days. At D8, the vials with a residual filling volume of approximately 20% showed similar results as compared with unopened control vials.

MFI

MFI measurements were performed to evaluate the particle concentrations in the larger subvisible size range of approximately 2–100 µm. In general, low levels of subvisible particles were found in the samples. No clear trend of changing particle concentrations upon storage at both temperatures or between opened and unopened vials was identified (table 4).

Statistical analysis was solely carried out on particles ≥2 µm as they best represent any possible trend. No significant changes in the total particle concentration could be detected when comparing the individual values for the opened vials at the different time points at different storage conditions, and for the unopened vials stored at different storage conditions with the D0 values. The differentiation between SP and NSP is feasible for particles >5 µm based on their characteristic appearance.[24] Overall, the majority of particles were NSP for all sampling time points.

DISCUSSION

Bevacizumab is labelled as stable for 3 years in the source vials when stored unopened at 2–8°C, protected from light and not frozen.[16] Furthermore, in-use stability data are provided in the summary of product characteristics of PF-06439535 (Zirabev) for the diluted concentrate in 0.9% saline solution, but not for the concentrate remaining in the opened vial.[16] In protein solutions like bevacizumab, concentrate aggregation can only occur with temperature changes, protein concentration changes, or mechanical stress.[25] In pharmaceutical practice, storage temperature and protein concentration are generally well controlled, representing no risk factors. However, the impact of mechanical stress from partial volume withdrawal on the quality of the remaining solution is not known. Since the collision of native and/or denatured proteins can be considered the major step in protein aggregation, it cannot be excluded that withdrawing a certain volume from the vial may affect the stability of the residual solution.[26]

The summary of product characteristics of PF-06439535 states that the product is for single-use only and any unused medicinal product should be disposed of since it contains no preservatives.[16] Nevertheless, there is a strong interest in clinical practice in the stability of the residual bevacizumab concentrate solutions in sampled large volume vials. Therefore, the present study aimed to evaluate the physical and chemical stability of fractionated PF-06439535 concentrate samples following the withdrawal of 80% (12.8 mL) of the prefilled volume stored under various storage conditions over a period of 8 days, applying different complementary stability-indicating analytical techniques.

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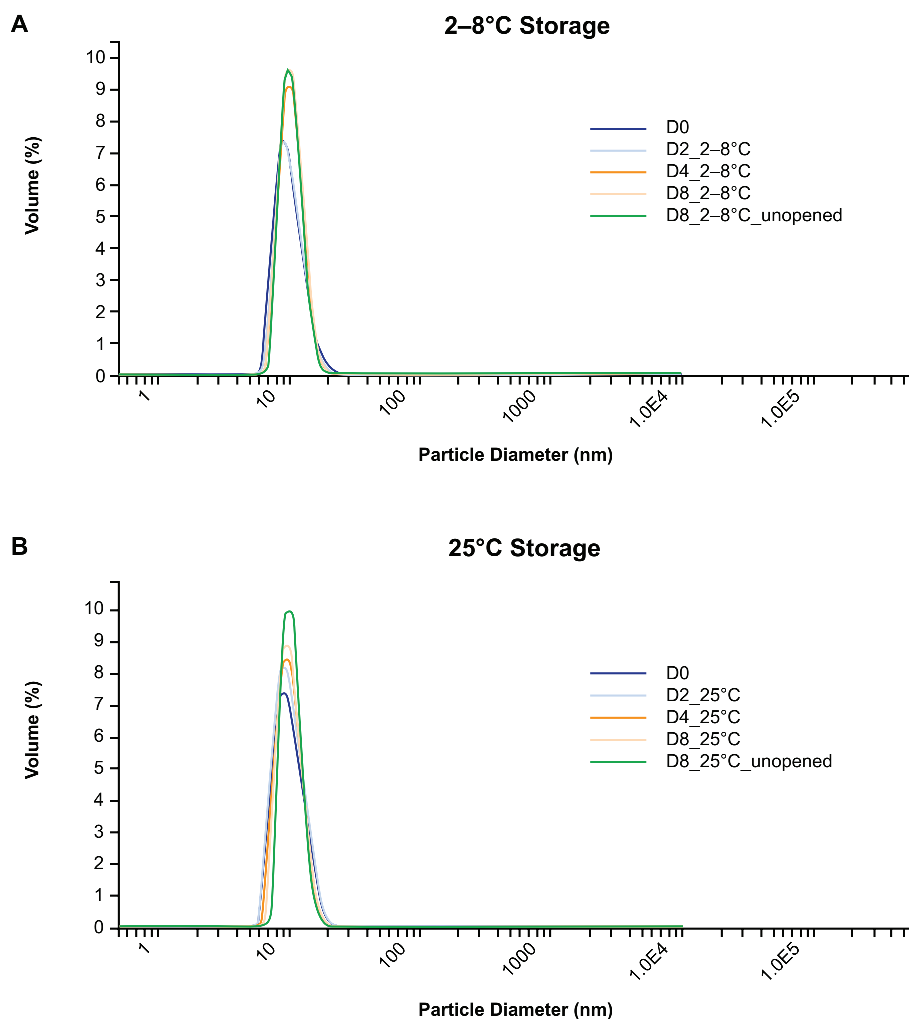

Figure 2 Size distribution by volume. (A) Size distribution by volume of the analysed stability samples of all time points stored at 2–8°C (mean of n=3). (B) Size distribution by volume of the analysed stability samples of all time points stored at 25°C (mean of n=3).

D0, day 0 (start); D2, day 2; D4, day 4; D8, day 8.

SEC-high-performance liquid chromatography (HPLC) was used to detect a possible formation of aggregates and fragments, as this technique can separate the mAb monomer from the polymeric form (dimers or more) and fragments. DLS allowed for estimation of the presence and size distribution of any formed protein aggregates in the nanometre size range. MFI was applied to quantify subvisible particles >1 µm and distinguish between different particle types such as protein aggregates, SP, and air bubbles in the size range ≥5 µm, thereby providing insight into the nature of the observed particles.

Table 3 Summary of Z-average diameter and PdI values

Time point	Storage condition	Z-average, nm	PdI	Main peak, %	Main peak, nm
D0	–	12.08±0.39	0.13±0.04	99.69±0.28	13.00±0.64
D2	2–8°C	11.52±0.26	0.08±0.03	99.40±1.04	12.32±0.45
	25°C	11.28±0.08	0.06±0.02	99.40±1.04	11.91±0.28
D4	2–8°C	11.55±0.02	0.05±0.01	100.00±0.00	12.18±0.10
	25°C	11.70±0.31	0.08±0.04	100.00±0.00	12.41±0.51
D8	2–8°C	11.41±0.07	0.05±0.02	100.00±0.00	11.94±0.19
	25°C	11.70±0.31	0.08±0.04	100.00±0.00	12.41±0.51
	2–8°C, unopened vial	11.36±0.10*	0.05±0.01	100.00±0.00	11.92±0.15
	25°C, unopened vial	11.54±0.19	0.06±0.03	100.00±0.00	12.11±0.35

PdI values were obtained as mean values from triplicate DLS measurements (main peak (%) deriving from size distribution by volume) for all tested samples and storage conditions.
*Indicates statistically significant difference compared with the respective Z-average value at D0.
D0, day 0 (start); D2, day 2; D4, day 4; D8, day 8; DLS, dynamic light scattering; PdI, polydispersity index.

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Table 4 Total particle concentrations determined at the individual time points in stability samples stored at 2–8°C and 25°C

Time point	Storage temperature, °C	Population	Total particle concentration/mL			
			≥2 µm	≥5 µm	≥10 µm	≥25 µm
D0	–	Total	3343±1490	198±141	15±22	1±2
		SP		4	1	0
		NSP		193	15	1
D2	2–8	Total	2812±572	158±26	12±7	1±1
		SP		5	1	0
		NSP		153	11	1
	25	Total	2943±380	226±86	23±11	2±2
		SP		14	1	0
		NSP		212	23	2
D4	2–8	Total	3079±188	157±12	16±7	1±1
		SP		26	3	0
		NSP		130	13	1
	25	Total	3422±704	225±85	29±13	5±4
		SP		16	1	0
		NSP		209	28	6
D8	2–8	Total	3143±1102	197±93	25±16	2±2
		SP		2	1	0
		NSP		196	24	2
	25	Total	3364±270	179±49	11±3	2±2
		SP		8	1	0
		NSP		171	11	2
D8 unopened	2–8	Total	2499±810	121±53	11±7	0±0
		SP		2	1	0
		NSP		119	11	0
	25	Total	2884±385	189±52	20±7	1±1
		SP		1	1	0
		NSP		188	19	1

Values are mean±SD of n=3 measurements, which have been independently calculated for SP, NSP, and total particle concentration. Therefore, the total particle concentration may slightly differ from the sum SP and NSP. The differentiation between SP and NSP is shown for sizes ≥5 µm.
D0, day 0 (start); D2, day 2; D4, day 4; D8, day 8; NSP, non-silicone oil particles; SP, silicone oil droplets.

A limitation of this study is that microbiological tests were not conducted in order to exclude any microbiological contamination of the residual solution after withdrawal of 80% of the volume. Another limitation is the concentration on size exclusion chromatography as the only method for evaluating the chemical stability of bevacizumab. Of course, ion exchange chromatography could have been used to monitor the stability of bevacizumab PF-06439535. Since, however, the aim of the study was to establish a preliminary idea of the effect of volume withdrawal on the stability of PF-06439535, and since it is clearly recommended in the summary of product characteristics[16] to dispose of any unused residual volume, we are of the opinion that SEC is sufficient for the purpose of the present study. Therefore, the study was designed so that stability assessment of the fractionated vial was deduced from the comparison of the analytical results obtained at D0 with those obtained at different time points and at the end of the 8 day storage period. In addition, unopened vials serving as control and stored under the same conditions as the fractionated vials for the same period of time were also comparatively evaluated.

Based on the results obtained in the present study, no aggregation or ongoing fragmentation due to partial withdrawal of filling volume could be observed in SEC-HPLC. Moreover, no changes in the DLS particle size distribution at D8 compared with the D0 values were identified upon storage at either 2–8°C or 25°C, neither in the pierced nor in the unopened vials. The total concentration of particles ≥10 µm of all samples was <100 particles/mL; the other size ranges showed comparable results. In addition, no significant variations in the pH values or in the visual appearance were detected over the whole study period in all samples at all storage conditions. Based on the above, no changes in the physical and chemical stability could be observed for PF-06439535 formulation (25 mg/mL) over a period of 8 days when stored protected from light at 2–8°C or 25°C after partial withdrawal of filling volume.

CONCLUSIONS

Based on the results of several complementary stability-indicating analytical techniques including SEC, DLS, and MFI, no changes in the physical and chemical stability of residual PF-06439535 (Zirabev) concentrate (25 mg/mL) could be observed after partial volume withdrawal of 80% of the filling volume and stored protected from light in the refrigerator at 2–8°C or at 25°C for 8 days.

Correction notice This paper has been amended since it was first published. Affiliation 3 has been corrected.

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Competing interests MA-T and MW are employees of the Central Laboratory of German Pharmacists. JW was an employee of the Central Laboratory of German Pharmacists when the study was carried out. AH is an employee of and has stock and/or other ownership interests in Coriolis Pharma Research GmbH, Germany. FG was an employee of Coriolis Pharma Research GmbH, Germany. AMS is an employee of and has stock and/or other ownership interests in Pfizer. JHS was an employee of Pfizer.

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REFERENCES

1 Awwad S, Angkawinitwong U. Overview of antibody drug delivery. *Pharmaceutics* 2018;10:83.
2 Garcia J, Hurwitz HI, Sandler AB, *et al*. Bevacizumab (Avastin®) in cancer treatment: A review of 15 years of clinical experience and future outlook. *Cancer Treat Rev* 2020;86:102017.
3 Thomas KA. Vascular endothelial growth factor, a potent and selective angiogenic agent. *J Biol Chem* 1996;271:603–6.
4 Mukherji SK. Bevacizumab (Avastin). *AJNR Am J Neuroradiol* 2010;31:235–6.
5 Dirix LY, Van Dam PA, Prove AM, *et al*. Bevacizumab in the treatment of patients with advanced breast cancer: where have we landed? *Ther Adv Med Oncol* 2010;2:331–42.
6 Peterson JS, Rockwell K, Scott IU, *et al*. Long-term physical stability, sterility, and anti-VEGF bioactivity of repackaged bevacizumab in 2-mL glass vials. *Retina* 2019;39:1802–9.
7 European Medicines Agency (EMA). Avastin summary of product characteristics [Internet], 2015. Available: https://www.ema.europa.eu/en/documents/product-information/avastin-epar-product-information_en.pdf [Accessed 14 Jul 2022].
8 Melosky B, Reardon DA, Nixon AB, *et al*. Bevacizumab biosimilars: scientific justification for extrapolation of indications. *Future Oncol* 2018;14:2507–20.
9 European Medicines Agency (EMA). Biosimiar medicines: overview [Internet], 2019. Available: https://www.ema.europa.eu/en/human-regulatory/overview/biosimilar-medicines-overview [Accessed 14 Jul 2022].
10 European Medicines Agency (EMA), European Commission. Biosimilars in the EU: information guide for healthcare professionals [Internet], 2019. Available: https://www.ema.europa.eu/en/documents/leaflet/biosimilars-eu-information-guide-healthcare-professionals_en.pdf [Accessed 14 Jul 2022].
11 Peraza MA, Rule KE, Shiue MHI, *et al*. Nonclinical assessments of the potential biosimilar PF-06439535 and bevacizumab. *Regul Toxicol Pharmacol* 2018;95:236–43.
12 Grunder B, Costigan L, Johnson K. Characterization and similarity assessment of bevacizumab and a proposed biosimilar, Poster W5349. In: *American Association of Pharmaceutical Scientists (AAPs) Annual Meeting and Exhibition*. San Diego, CA, 2014.
13 Knight B, Rassam D, Liao S, *et al*. A phase I pharmacokinetics study comparing PF-06439535 (a potential biosimilar) with bevacizumab in healthy male volunteers. *Cancer Chemother Pharmacol* 2016;77:839–46.
14 Reinmuth N, Bryl M, Bondarenko I, *et al*. PF-06439535 (a bevacizumab biosimilar) compared with reference bevacizumab (Avastin®), both plus paclitaxel and carboplatin, as first-line treatment for advanced non-squamous non-small-cell lung cancer: a randomized, double-blind study. *BioDrugs* 2019;33:555–70.
15 European Medicines Agency (EMA). Zirabev (bevacizumab) EPAR – medicine overview [Internet], 2020. Available: https://www.ema.europa.eu/en/medicines/human/EPAR/zirabev [Accessed 14 Jul 2022].
16 European Medicines Agency (EMA). Zirabev summary of product characteristics [Internet], 2021. Available: https://www.ema.europa.eu/en/documents/product-information/zirabev-epar-product-information_en.pdf [Accessed 14 Jul 2022].
17 Bakri SJ, Snyder MR, Pulido JS, *et al*. Six-month stability of bevacizumab (Avastin) binding to vascular endothelial growth factor after withdrawal into a syringe and refrigeration or freezing. *Retina* 2006;26:519–22.
18 Chen Y-H, Wu P-C, Shiea J, *et al*. Evaluation of the sterility, stability, and efficacy of bevacizumab stored in multiple-dose vials for 6 months. *J Ocul Pharmacol Ther* 2009;25:65–70.
19 Palmer JM, Amoaku WM, Kamali F. Quality of bevacizumab compounded for intravitreal administration. *Eye* 2013;27:1090–7.
20 Khalili H, Sharma G, Froome A, *et al*. Storage stability of bevacizumab in polycarbonate and polypropylene syringes. *Eye* 2015;29:820–7.
21 European Medicines Agency (EMA). Assessment report: Zirabev [Internet], 2018. Available: https://www.ema.europa.eu/en/documents/assessment-report/zirabev-epar-public-assessment-report_en.pdf [Accessed 14 Jul 2022].
22 Bermudez O, Forciniti D. Aggregation and denaturation of antibodies: a capillary electrophoresis, dynamic light scattering, and aqueous two-phase partitioning study. *J Chromatogr B Analyt Technol Biomed Life Sci* 2004;807:17–24.
23 Sukumar M, Doyle BL, Combs JL, *et al*. Opalescent appearance of an IgG1 antibody at high concentrations and its relationship to noncovalent association. *Pharm Res* 2004;21:1087–93.
24 Zölls S, Weinbuch D, Wiggenhorn M, *et al*. Flow imaging microscopy for protein particle analysis--a comparative evaluation of four different analytical instruments. *AAPS J* 2013;15:1200–11.
25 Wang W. Instability, stabilization, and formulation of liquid protein pharmaceuticals. *Int J Pharm* 1999;185:129–88.
26 Colombié S, Gaunand A, Lindet B. Lysozyme inactivation under mechanical stirring: effect of physical and molecular interfaces. *Enzyme Microb Technol* 2001;28:820–6.

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