Report

Physicochemical stability of Vegzelma 25 mg/mL concentrate for solution for infusion in original vials after first opening

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University Medical Centre of the Johannes Gutenberg University, Pharmacy,
Langenbeckstr. 1, 55131 Mainz, Germany

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	Signatures
Helen Linxweiler	H. Linxweiler 31.10.2023
Laura Knoll	L. Knoll 31.10.2023
Dr. Judith Thiesen	J. Th 31.10.2023
Prof. Dr. Irene Krämer	Krämer 31.10.23

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Helen Linxweiler, Laura Knoll, Dr. Judith Thiesen, Prof. Dr. Irene Krämer

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1. Objective

The aim of the study was to determine the physicochemical stability of Vegzelma 25 mg/mL concentrate for solution for infusion (a bevacizumab biosimilar) in the original vial after first opening. Vials containing the concentrated solutions were punctured and stored light protected at 25 °C or 2-8 °C for 28 days.

Samples were withdrawn immediately after puncturing the vials and after 1, 7, 14, 21, 28 days. Physicochemical stability was determined by ion-exchange and size-exclusion high performance liquid chromatography (IE-HPLC, SE-HPLC), dynamic light scattering (DLS), pH measurement, and visual inspection for particles and colour changes.

2. Specifications

Table 1: Specifications of the finished medicinal product Vegzelma 25 mg/mL concentrate for solution for infusion.

Test parameter	Specification	Method
Appearance and visible particles	Clear to slightly opalescent, colourless to pale brown liquid, without visible particles	Visual inspection Ph.Eur. 2.9.20
pH	pH unit change < 0.5[1]	Ph.Eur. 2.2.3
Bevacizumab concentration	≤ 5% loss of the initial bevacizumab concentration measured	SE-HPLC assay
Bevacizumab charge variants	Main peak loss ≤ 5%[1]	IE-HPLC assay
Particle size	Variation in size of < 3 nm and polydispersity index (PdI) < 0.2[2]	Dynamic light scattering Ph.Eur. 2.9.31

[1] NHS Pharmaceutical Quality Assurance Committee: Stability Part 2 Aseptic Preparations (Biopharmaceuticals), 2021
[2] Vieillard, V., Paul, M. Physicochemical stability study of a biosimilar of Bevacizumab in vials and after dilution in 0.9% NaCl in polyolefin intravenous bags. Pharm Technol Hosp Pharm 2023; 8(1): 20220007

3. Investigation period and investigation sites

Experiments, validation, and calibration were performed between August 28th, 2023 and October 25th, 2023 at the Department of hospital pharmacy, University Medical Center of the Johannes Gutenberg-University Mainz, Building 704, Langenbeck-straße 1, 55131 Mainz, Germany.

DLS measurements were carried out at the Institute for Pharmaceutical and Biomedical Science, Johannes Gutenberg-University, Staudinger Weg 5, 55128 Mainz, Germany.

4. Material

Vegzelma 25 mg/mL finished medicinal product

Stability tests were executed with 6 vials of the marketed medicinal product Vegzelma 25 mg/mL concentrate for solution for infusion, 400 mg/16 mL (batch 3KCC021, expiry date 31.01.2027). For method validation and calibration 3 Vegzelma 100 mg/4 mL vials (batch 2K3C027, expiry date 30.06.2024) were used.

Dilution solution

Water HPLC grade (PanReac AppliChem, batch 3H010486, expiry date 31.01.2027)

SE-HPLC

Mobile phase: 150 mM Phosphate buffered saline (PBS): 5 phosphate buffered saline tablets (Sigma Aldrich, batch 2701602) diluted in 1000 mL water HPLC grade, pH 7.2-7.6

Column: TSKgel G3000SWXL 7.8 x 300 mm, 5 µm, Tosoh Bioscience

IE-HPLC

Mobile Phase:

Buffer A solution: 20 mM morpholinoethanesulfonic acid (MES) / 60 mM NaCl, pH 6.0:
3.9 g MES (Tokyo Chemical Industry Japan, batch L2KXI-SH) and 3.51 g NaCl (Fagron, batch 08/22-21H26) were dissolved with water HPLC grade to 1000 mL and the pH adjusted to pH 6.0 with NaOH 1 M (Merck, Germany, batch HC27801937)

Buffer B solution: 20 mM MES / 180 mM NaCl pH 6.0:
3.9 g MES and 10.53 g NaCl were dissolved with water HPLC grade to 1000 mL and the pH was adjusted to pH 6.0 with NaOH 1 M

Guard column: Propac WCX-10G BioLC Guard 4 mm x 50 mm, Thermo Fisher Scientific

Column: Propac WCX-10 BioLC Analytical 4 mm x 250 mm, 10 µm Thermo Fisher Scientific, Reinach, Switzerland

DLS measurements

UV cuvettes mikro, 12.5 x 12.5 x 45 mm (Brand GmbH + Co. KG, Wertheim, DE)

5. Apparatus

SE-HPLC

Waters Alliance 2695 HPLC system connected to a Waters photodiode array detector 2990 (Waters, Eschborn, DE)

Waters Empower Pro, Empower 2 software, version 6.10.01.00 (Waters, Eschborn, DE)

IE-HPLC

Waters Acquity Arc HPLC system connected to a Waters photodiode array detector 2998 (Waters, Eschborn, DE)

Waters Empower Pro, Empower 3 software, version 3.7.0 (Waters, Eschborn, DE)

pH metre

SevenCompact S210 pH metre equipped with an InLab Micro Pro-ISM electrode (Mettler Toledo, Greifensee, CH), daily calibration with buffer solutions pH 2.00 (batch 1H028J), pH 4.01 (batch 1H315K), pH 7.00 (batch 1H218A), pH 9.21 (batch 1J110D), pH 11.00 (batch 1G340H) (Mettler Toledo, Greifensee, CH)

DLS system

Zetasizer Nano – ZS (Malvern Instruments Ltd., Malvern, UK)

Climatic chamber

Memmert ICH260L (Memmert, Schwabach, DE, serial number Y618.0124)

6. Methods

6.1. SE-HPLC

Table 2: Characteristics of the SE-HPLC assay

Column	TSKgel G3000SWXL 7.8 x 300 mm, 5 µm, Tosoh Bioscience
Flow rate	1.0 mL/min
Injection volume	15 µL
Sample temperature	5 °C ± 3 °C
Column temperature	35 °C ± 5 °C
Detection wavelength	280 nm
Mobile Phase	PBS buffer (150 mM)
Gradient profile	Isocratic
Run time	20 min

Each sample was injected by an autosampler in triplicate. Sample preparation is described in section 7.

6.1.1. Calibration and validation of the SE-HPLC assay

To demonstrate the precision and robustness of the analyses, the HPLC assay used was validated according to the ICH Guideline Q2 (R1).[3]

Accuracy and precision

Accuracy, intra-day and inter-day precision of the method were validated on 3 consecutive days by injecting 9 bevacizumab 2.5 mg/mL standard solutions in duplicate. Test solutions were freshly prepared on each day by puncturing a new vial of Vegzelma 100 mg/4 mL concentrated solution, withdrawing 100 µL aliquots and diluting with 900 µL water HPLC grade.

[3] ICH Guideline Q2 (R1) Validation of analytical procedures: Text and Methodology, EMA 2006

Linearity

For linearity testing Vegzelma 100 mg/4 mL concentrated solution was diluted with water HPLC grade to achieve seven calibration standards (1.25 mg/mL, 2.00 mg/mL, 2.25 mg/mL, 2.50 mg/mL, 2.75 mg/mL, 3.00 mg/mL, 3.75 mg/mL). Aliquots of the standards were injected in triplicate. The calibration curve was constructed by plotting the peak area versus the nominal concentration of bevacizumab.

6.2. IE-HPLC

Table 3: Characteristics of the IE-HPLC assay

Column	Propac WCX-10 BioLC Analytical 4 mm x 250 mm, 10 µm Thermo Fisher Scientific
Flow rate	0.8 mL/min
Injection volume	20 µL
Sampler temperature	5 °C ± 3 °C
Column temperature	35 °C ± 5 °C
Detection wavelength	280 nm
Mobile phase	Buffer A: 20 mM MES + 60 mM NaCl pH 6.0 Buffer B: 20 mM MES + 180 mM NaCl pH 6.0
Gradient profile	0 min → 30 min: starting with 100% buffer A, 0% buffer B linearly decreasing/increasing to 40% buffer A, 60% buffer B 31 min → 41 min: 0% buffer A, 100% buffer B 41 min → 51 min 100% buffer A, 0% buffer B
Run time	51 min

6.2.1. Validation of the IE-HPLC assay

Precision

The intra- and inter-day precision was determined on 3 consecutive days by injecting 9 bevacizumab solutions with a concentration of 2.5 mg/mL in duplicate. Test solutions were freshly prepared on each day by puncturing a new vial of Vegzelma 100

mg/4 mL concentrated solution, withdrawing 100 µL aliquots and diluting with 900 µL water HPLC grade.

6.3. DLS

The particle size of the solutions was determined using dynamic light scattering. At each measuring time point approximately 1 mL of undiluted test solution was transferred to a disposable cuvette and analyzed with the Zetasizer Nano ZS. Measuring conditions are listed in Table 4. Particle sizes were determined as average intensity-based particle diameter (Z-average) and polydispersity index (PdI).

Table 4: Characteristics of DLS measurement

Cuvettes	UV cuvette mikro, 12.5 x 12.5 x 45 mm
Refractive index sample	1.342
Refractive index dispersant	1.342
Viscosity	1.0178 mPa s
System temperature	25.0 ± 0.1 °C
Measurement duration	10 sec
No. of measurements per sample	3
Repetitions per sample measurement	11

6.4. pH

pH was measured in approximately 1 mL aliquots of test solutions transferred to HPLC vials prior to the measurement.

6.5. Visual inspection

All test solutions were visually examined for colour changes and particles with the unaided eye whenever samples were withdrawn.

7. Storage, preparation of test solutions and samples

7.1. Test solutions

Three vials each of bevacizumab containing Vegelzma 25 mg/mL were stored either at 25 °C ± 2 °C in the climatic chamber, light protected or at 2-8 °C ± 2 °C in the refrigerator, light protected for a maximum period of 28 days. At each sampling time point vials were (re-)punctured with 1 mL syringes (BD, 1 mL syringe Luer-Lok Tip, batch 21958109, expiry date 30.06.2027) connected with canulas (BD Microlane 3, batch 230101, expiry date 12/2027) and samples withdrawn.

7.2. Sampling

Samples were withdrawn from each test solution after initial puncturing of the vial (T0) and at day 1, 7, 14, 21, and 28 of storage.

7.3. Sample preparation for IE-HPLC, SE-HPLC

At each sampling time point, 100 µL aliquots of bevacizumab 25 mg/mL test solution were withdrawn and mixed with 900 µL water HPLC grade, resulting in 2.5 mg/mL bevacizumab samples.

7.4. Preparation of samples for DLS, pH measurement and visual inspection

Undiluted samples were used for DLS, measurement of pH and inspection for visible particles and colour changes at each sampling time point.

8. Results

8.1. SE-HPLC

8.1.1. Calibration and validation

The SE-HPLC chromatograms revealed a bevacizumab monomer peak with a retention time of about 8 minutes and a second peak with a retention time of about 6.6 minutes, which is considered to be a bevacizumab oligomer peak. The coefficient of correlation obtained by plotting the peak areas against the nominal bevacizumab concentrations was $R^2 = 0.999$, thereby proving linearity of the assay. The accuracy was 99.83% (± 0.2%). The intra-day precision tests revealed a mean bevacizumab concentration of 25.02 mg/mL ± 0.49% relative standard deviation (RSD). The inter-day precision tests revealed a mean bevacizumab concentration of 24.96 mg/mL ± 0.41% RSD. The results met the criteria based on ICH Q2 (R1) and proved reproducibility.

8.1.2. Vegzelma 25 mg/mL analysed by SE-HPLC

Bevacizumab concentrations remained within the specification (≥ 95% of the initial concentration measured) stored in the original vials either at 2-8 °C (see Table 5) or at 25 °C (see Table 6) over the 28 day-period. A slight decrease of the peak height of the bevacizumab monomer got obvious over time, but the decrease did not succeed the specification limit (see Figure 1, 2). At day 28 day, the concentration of intact bevacizumab monomer amounted to 98.6% (vials stored at 2-8 °C) or 97.8% (vials stored at 25 °C) of the initial concentration. The overlay chromatograms for Vegzelma 25 mg/mL stored at 2-8 °C and 25 °C show an unchanged peak area of the bevacizumab oligomer peak.

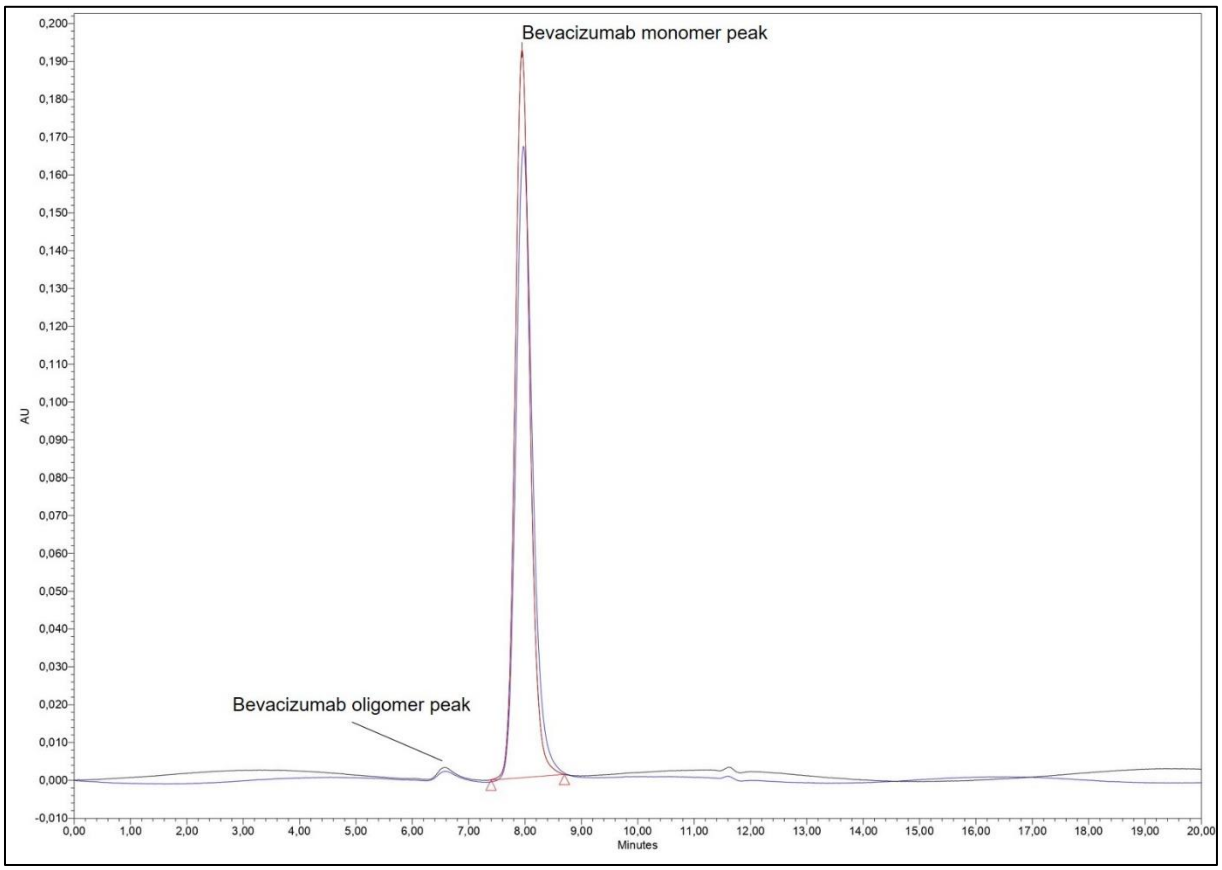

Figure 1: Overlay of SE-HPLC chromatograms of Vegzelma 25 mg/mL solutions on day 0 (red) and day 28 (blue) stored at 2-8 °C.

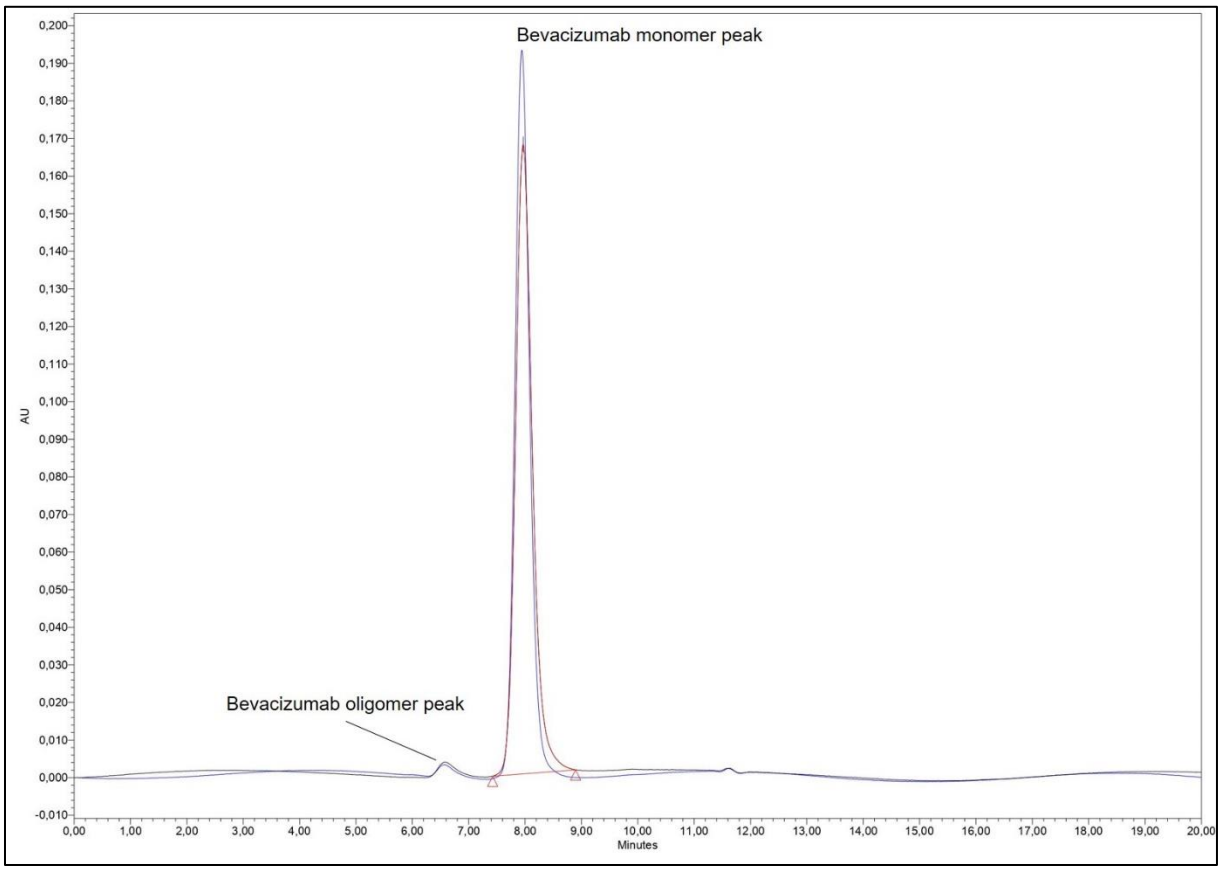

Figure 2: Overlay of SE-HPLC chromatograms of Vegzelma 25 mg/mL solutions on day 0 (blue) and day 28 (red) stored at 25 °C.

Table 5: Bevacizumab concentrations in punctured Vegzelma 25 mg/mL concentrate vials, stored at 2-8 °C protected from light for 28 days determined by SE-HPLC (n=9).

Vegzelma 25 mg/mL, 2-8 °C						
	T0	T1	T7	T14	T21	T28
Measured bevacizumab concentration [mg/mL]	24.98	24.91	24.95	24.98	24.9	24.62
RSD [%]	0.3	0.3	0.3	0.4	0.7	1.0
% remaining bevacizumab concentration (initial concentration is taken as 100%)	100	99.72	99.88	100.00	99.68	98.56

Table 6: Bevacizumab concentrations in puctured Vegzelma 25 mg/mL concentrate vials, stored at 25 °C protected from light for 28 days determined by SE-HPLC (n=9).

Vegzelma 25 mg/mL, 25 °C						
	T0	T1	T7	T14	T21	T28
Measured bevacizumab concentration [mg/mL]	25.03	24.77	24.79	24.74	24.73	24.48
RSD [%]	0.2	0.3	0.3	0.2	0.2	1.0
% remaining bevacizumab concentration (initial concentration is taken as 100%)	100	98.96	99.04	98.84	98.80	97.80

8.1. IE-HPLC

8.1.1. Validation

The assay resulted in five peaks, with peak 1 and 2 corresponding to the acidic charge variants and peak 4 and 5 corresponding to the basic charge variants of the main bevacizumab peak 3 (see Figure 3). The results of intra-day and inter-day precision tests for each peak are shown in Table 7 and 8.

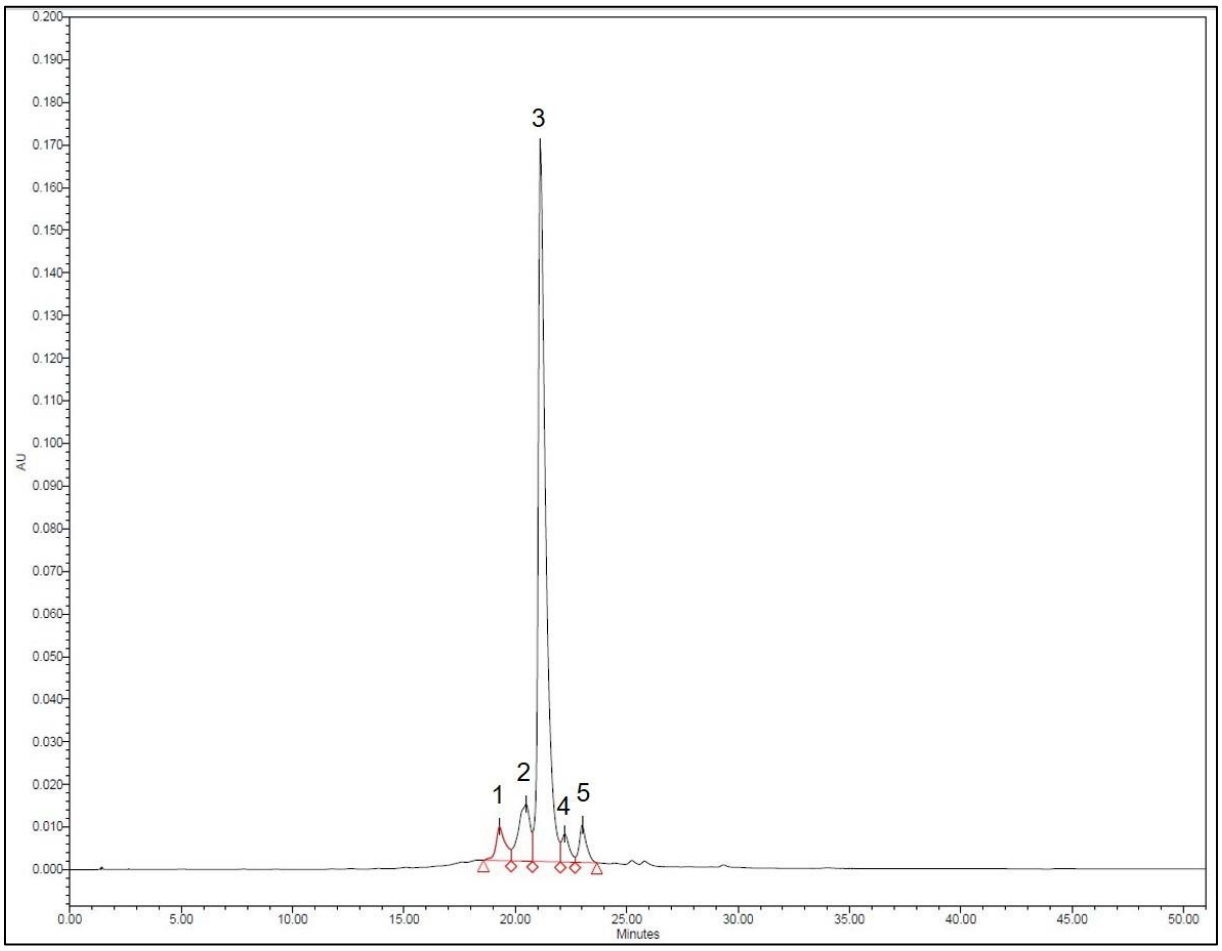

Figure 3: IE-HPLC chromatogram of a freshly prepared Vegzelma 25 mg/mL solution diluted to a bevacizumab concentration of 2.5 mg/mL. Peak 1 and 2 correspond to the acidic variants, peak 4 and 5 to the basic variants of the bevacizumab main peak 3.

Table 7: Intra-day precision of the IE-HPLC assay measured in 9 bevacizumab solutions injected in duplicate. Results expressed as % peak area of the total peak area (100%) ± RSD.

Peak area [%] ± RSD [%]				
Acidic Peak (1)	Acidic Peak (2)	Main Peak (3)	Basic Peak (4)	Basic Peak (5)
4.07 ± 0.71	9.42 ± 0.28	79.5 ± 0.12	3.16 ± 1.4	3.84 ± 0.56

Table 8: Inter-day precision of the IE-HPLC assay measured in 9 bevacizumab solutions injected in duplicate on 3 consecutive days. Results expressed as % peak area of the total peak area (100%) ± RSD.

Peak area [%] ± RSD [%]				
Acidic Peak (1)	Acidic Peak (2)	Main Peak (3)	Basic Peak (4)	Basic Peak (5)
4.05 ± 1.03	9.39 ± 0.43	79.49 ± 0.09	3.23 ± 2.03	3.84 ± 0.44

8.1.2. Vegelzma 25 mg/mL analyzed by IE-HPLC

Detailed quantitative results of the IE-HPLC analysis are shown in Tables 9 and 10. During the observation period, no changes in the peak pattern were detected in any of the test solutions. The peak areas of the acidic, basic, and main peaks remained almost unchanged over time.

Table 9: Stability of Vegzelma 25 mg/mL concentrate in punctured vials determined by IE-HPLC stored for 28 days at 2-8 °C, protected from light. Peak areas calculated as percentage rates of the total peak area expressed as mean ± RSD (n=9).

Vegzelma 25 mg/mL, 2-8 °C						
		T0	T7	T14	T21	T28
Area [%] (± RSD%) (n=9)	Acidic Peak (1)	3.44 (± 1.78)	3.94 (± 9.33)	3.68 (± 1.91)	3.52 (± 3.53)	3.66 (± 1.61)
	Acidic Peak (2)	8.76 (± 0.92)	8.54 (± 3.02)	8.66 (± 0.67)	8.62 (± 1.84)	8.20 (± 2.86)
	Main Peak (3)	80.41 (± 0.21)	79.35 (± 0.61)	79.97 (± 0.38)	79.73 (± 0.67)	80.15 (± 0.33)
	Basic Peak (4)	4.12 (± 7.03)	4.38 (± 4.96)	4.37 (± 5.85)	4.83 (± 8.92)	4.56 (± 7.18)
	Basic Peak (5)	3.27 (± 3.82)	3.80 (± 5.20)	3.31 (± 0.89)	3.30 (± 7.00)	3.43 (± 1.30)

Data of day 1 are not evaluable because of system failure.

Table 10: Stability of Vegzelma 25 mg/mL concentrate in punctured vials determined by IE-HPLC stored for 28 days at 25 °C, protected from light. Peak areas calculated as percentage rates of the total peak area expressed as mean ± RSD (n=9).

Vegzelma 25 mg/mL, 25 °C						
		T0	T7	T14	T21	T28
Area [%] (± RSD%) (n=9)	Acidic Peak (1)	3.60 (± 5.63)	4.29 (± 3.79)	4.96 (± 3.70)	5.01 (± 0.94)	5.60 (± 0.23)
	Acidic Peak (2)	9.32 (± 4.27)	8.87 (± 1.01)	9.72 (± 1.36)	9.55 (± 0.37)	9.63 (± 0.61)
	Main Peak (3)	79.76 (± 0.62)	79.08 (± 0.59)	77.96 (± 0.33)	77.23 (± 0.42)	77.07 (± 0.28)
	Basic Peak (4)	4.04 (± 6.00)	4.29 (± 5.57)	4.03 (± 2.40)	4.57 (± 5.27)	4.24 (± 5.28)
	Basic Peak (5)	3.27 (± 6.24)	3.46 (± 7.46)	3.33 (± 2.42)	3.63 (± 4.21)	3.46 (± 1.30)

Data of day 1 are not evaluable because of system failure.

8.2. DLS

The DLS particle analysis did not reveal any signs of aggregation within the test solutions over the study period of 28 days. The particle distribution was the same in all test solutions showing only one peak, i.e. one single size population. The variation of

hydrodynamic diameters of the test solutions remained within the acceptance limit of < 3 nm. The PdIs remained below 0.2, showing the monodispersity of the solutions.

Table 11: Hydrodynamic diameters (Z-Average) and polydispersity index (PdI) for Vegzelma 25 mg/mL concentrate stored at 2-8 °C and 25 °C protected from light for 28 days (n=3).

Vegzelma 25 mg/mL							
		T0	T1	T7	T14	T21	T28
Stored at 2-8 °C	Z-Average [nm]	14.02	13.47	14.04	14.24	13.84	14.74
	PdI	0.051	0.049	0.055	0.043	0.067	0.080
Stored at 25 °C	Z-Average [nm]	13.60	13.43	13.81	13.78	13.61	13.88
	PdI	0.056	0.050	0.063	0.043	0.056	0.053

8.3. pH

The average pH values of the test solutions are shown in Table 12. The pH values remained constant in each test solution over the study period of 28 days.

Table 12: pH values of Vegzelma 25 mg/mL concentrate stored at 2-8 °C and 25 °C protected from light for 28 days (n=3).

Vegzelma 25 mg/mL						
	T0	T1	T7	T14	T21	T28
Stored at 2-8 °C	6.13	6.07	6.13	6.13	6.13	6.13
Stored at 25 °C	6.14	6.09	6.14	6.13	6.15	6.12

8.4. Visual inspection

The visual inspection of the test vials did not reveal any visible particles or colour changes within the observation period. The solutions remained clear and colourless.

9. Conclusion

Vegzelma 25 mg/mL concentrate for solution for infusion is physicochemically stable for at least 28 days stored light protected at 2-8 °C or 25 °C in original vials after first opening. Due to microbiological reasons, Vegzelma vials should be stored at 2-8 °C.