

Supplementary Materials

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

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Table S1 Overview of the area percent determined for the side peaks observed in PF-06439535 via SEC after storage at 2–8°C at different timepoints

Timepoint at 2–8°C	Sample #	Side peak 1 (RT about 7.7 min)		Side peak 2 (RT about 8.1 min)		Side peak 3 (RT about 12.2 min)	
		Area %	Mean %	Area %	Mean %	Area %	Mean %
D0	Vial 1	0.057	0.056	2.791	2.829	0.538	0.630
	Vial 1	0.060		2.836		0.669	
	Vial 1	0.051		2.858		0.682	
	Vial 2	0.062	0.060	2.860	2.872	0.691	0.642
	Vial 2	0.058		2.876		0.548	
	Vial 2	0.061		2.880		0.688	
	Vial 3	0.060	0.060	2.908	2.906	0.683	0.632
	Vial 3	0.058		2.896		0.537	
Vial 3	0.062	2.914		0.676			
D2	Vial 1	0.068	0.063	3.014	3.023	1.495	1.413
	Vial 1	0.059		3.031		1.386	
	Vial 1	0.062		3.025		1.357	
	Vial 2	0.056	0.058	3.034	3.032	1.539	1.482
	Vial 2	0.060		3.032		1.406	
	Vial 2	0.058		3.031		1.500	
	Vial 3	0.063	0.062	3.034	3.039	1.474	1.452
	Vial 3	0.061		3.053		1.538	
Vial 3	0.062	3.030		1.345			
D4	Vial 1	0.045	0.050	2.856	2.872	1.400	1.396
	Vial 1	0.056		2.869		1.421	
	Vial 1	0.050		2.891		1.367	
	Vial 2	0.047	0.050	2.883	2.887	1.383	1.374
	Vial 2	0.051		2.879		1.358	
	Vial 2	0.051		2.898		1.381	
	Vial 3	0.047	0.049	2.896	2.903	1.360	1.371
	Vial 3	0.052		2.907		1.392	
Vial 3	0.047	2.905		1.362			
D8	Vial 1	0.057	0.049	2.877	2.890	1.391	1.466
	Vial 1	0.045		2.895		1.466	
	Vial 1	0.044		2.897		1.542	
	Vial 2	0.047	0.043	2.893	2.908	1.405	1.403
	Vial 2	0.039		2.916		1.444	
	Vial 2	0.042		2.915		1.361	
	Vial 3	0.041	0.044	2.927	2.919	1.425	1.435
	Vial 3	0.043		2.901		1.404	
Vial 3	0.048	2.930		1.475			

Area % represents the individual area percentage of the respective side peak compared to the bevacizumab PF-06439535 peak. Mean % represents the average individual area percentage of the respective side peak compared to the PF-06439535 peak based on triplicate measurement.

D0, day 0 (start); D2, day 2; D4, day 4; D8, day 8; RT, retention time; SEC, size exclusion chromatography.

Table S2 Overview of the area percent determined for the side peaks observed in PF-06439535 via SEC after storage at 25°C at different timepoints

Timepoint at 25°C	Sample #	Side peak 1 (RT about 7.7 min)		Side peak 2 (RT about 8.1 min)		Side peak 3 (RT about 12.2 min)	
		Area %	Mean %	Area %	Mean %	Area %	Mean %
D0	See table S1						
D2	Vial 1	0.049	0.053	2.937	2.984	1.446	1.432
	Vial 1	0.051		3.004		1.417	
	Vial 1	0.058		3.010		1.433	
	Vial 2	0.056	0.050	3.016	3.015	1.419	1.431
		0.050		3.013		1.398	
		0.044		3.015		1.476	
	Vial 3	0.053	0.053	3.009	3.011	1.406	1.444
		0.054		3.012		1.521	
		0.051		3.013		1.406	
D4	Vial 1	0.047	0.052	2.923	2.922	1.315	1.364
	Vial 1	0.056		2.919		1.378	
	Vial 1	0.053		2.924		1.400	
	Vial 2	0.055	0.052	2.941	2.948	1.351	1.346
		0.054		2.947		1.352	
		0.047		2.957		1.335	
	Vial 3	0.049	0.051	2.950	2.950	1.359	1.354
		0.052		2.952		1.369	
		0.052		2.947		1.334	
D8	Vial 1	0.047	0.044	2.988	2.984	1.306	1.284
	Vial 1	0.039		2.988		1.203	
	Vial 1	0.045		2.975		1.344	
	Vial 2	0.048	0.053	2.980	2.986	1.356	1.360
		0.056		2.975		1.373	
		0.054		3.004		1.350	
	Vial 3	0.049	0.048	3.004	3.008	1.323	1.332
		0.047		3.007		1.328	
		0.049		3.014		1.346	

Area % represents the individual area percentage of the respective side peak compared to the bevacizumab PF-06439535 peak. Mean % represents the average individual area percentage of the respective side peak compared to the PF-06439535 peak based on triplicate measurement.

D0, day 0 (start); D2, day 2; D4, day 4; D8, day 8; RT, retention time; SEC, size exclusion chromatography.

Table S3 Overview of the area percent determined for the side peaks observed in PF-06439535 via SEC when stored unopen at 2–8°C and 25°C

Timepoint	Sample #	Side peak 1 (RT about 7.7 min)		Side peak 2 (RT about 8.1 min)		Side peak 3 (RT about 12.2 min)	
		Area %	Mean %	Area %	Mean %	Area %	Mean %
D8 2–8°C	Vial 1	0.043	0.043	2.907	2.916	1.453	1.439
	Vial 1	0.044		2.905		1.425	
	Vial 1	0.043		2.935		1.439	
	Vial 2	0.039	0.041	2.930	2.939	1.435	1.448
	Vial 2	0.042		2.953		1.352	
	Vial 2	0.042		2.935		1.558	
	Vial 3	0.042	0.045	2.903	2.915	1.440	1.463
	Vial 3	0.053		2.911		1.439	
	Vial 3	0.041		2.930		1.510	
D8 25°C	Vial 1	0.046	0.046	3.003	3.034	1.326	1.342
	Vial 1	0.043		3.059		1.343	
	Vial 1	0.050		3.040		1.357	
	Vial 2	0.048	0.050	3.019	3.005	1.307	1.363
	Vial 2	0.054		3.011		1.317	
	Vial 2	0.048		2.984		1.466	
	Vial 3	0.050	0.048	3.006	3.013	1.329	1.377
	Vial 3	0.049		3.021		1.415	
	Vial 3	0.044		3.013		1.388	

Area % represents the individual area percentage of the respective side peak compared to the bevacizumab PF-06439535 peak. Mean % represents the average individual area percentage of the respective side peak compared to the PF-06439535 peak based on triplicate measurement.

D8, day 8; RT, retention time; SEC, size exclusion chromatography.

Table S4 Overview on PF-06439535 sample dilutions tested for linearity of the SEC method and the corresponding peak areas*

Concentration of PF-06439535 sample solution (mg/mL)	Peak area
0.5	21,173,353
	21,532,498
	21,352,926
1	42,239,922
	42,634,521
	42,437,222
2	82,330,125
	82,867,169
	82,598,647
4	140,603,960
	141,365,353
	140,984,657
5	160,849,579
	161,323,314
	161,086,447

*The corresponding calibration line is presented in figure S1.

SEC, size exclusion chromatography.

Table S5 Overview on the results obtained for precision of SEC

PF-06439535 concentration	Peak area	
	0.5 mg/mL	5.0 mg/mL
Sample		
1	22,558,035	184,282,430
2	22,653,565	184,445,365
3	22,604,336	184,165,762
4	22,569,942	184,424,478
5	22,559,188	184,638,158
6	22,558,396	184,391,471
Mean	22,583,910	184,391,277
Standard deviation	38,459.856	159,801.607
Variation coefficient (%)	0.17	0.09

SEC, size exclusion chromatography.

Table S6 Overview on the results obtained for accuracy of the SEC method*

	Molecular weight				
PF-06439535 concentration	0.5 mg/mL	1.0 mg/mL	2.0 mg/mL	4.0 mg/mL	5.0 mg/mL
Sample					
1	149,000	147,477	147,477	145,969	150,537
2	147,477	145,969	147,477	149,000	150,537
Mean	148,239	146,723	147,477	147,485	150,537

*The molecular weight determined from different bevacizumab PF-06439535 sample dilutions was compared with the declared molecular weight of bevacizumab corresponding to 149 kDa.

SEC, size exclusion chromatography.

Figure S1 Calibration line corresponding to table S4