SUPPORTING INFORMATION

Sustained Release of Vascular Endothelial Growth Factor (VEGF) from Poly(ε-caprolactone-PEG-ε-caprolactone)-*b*-poly(L-lactide) Multi-block Copolymer Microspheres

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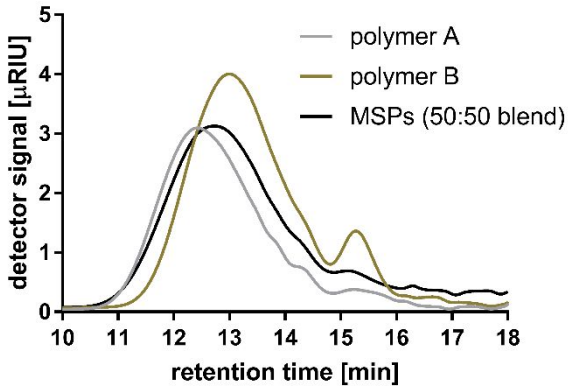


Figure S1: GPC chromatograms of multi-block copolymers A and B ("polymer A" and "polymer B"), and of (placebo) microspheres prepared with a 50:50 blend of polymer A and B (MSPs: microspheres).

Table S1: Molecular weights of multi-block copolymers A and B and placebo microspheres.

	M_n [kDa]	M_w [kDa]	M_p [kDa]	PDI [M_w / M_n]
polymer A	21.7	53.7	52.4	2.59
polymer B	13.7	31.6	30.5	2.31
placebo microspheres (50:50 blend)	17.2	45.6	39.1	2.64

Polymer A: multi-block copolymer A, Polymer B: multi-block copolymer B. Placebo microspheres (50:50 blend): Microspheres prepared with a 50:50 blend of polymer A and B. Molecular weights are based on GPC measurements (as shown in figure S1).

Table S2: Size characteristics of VEGF-loaded microspheres (0.2 wt% target loading) prepared with various polymer A and B blends.

blend ratio polymer A : polymer B	average size [μm] ; CV [%]
100:0	45 ; 14
90:10	n.d.*
80:20	46 ; 28
70:30	51 ; 12
60:40	42 ; 16
50:50	48 ; 21
0:100	43 ; 28

*not determined.

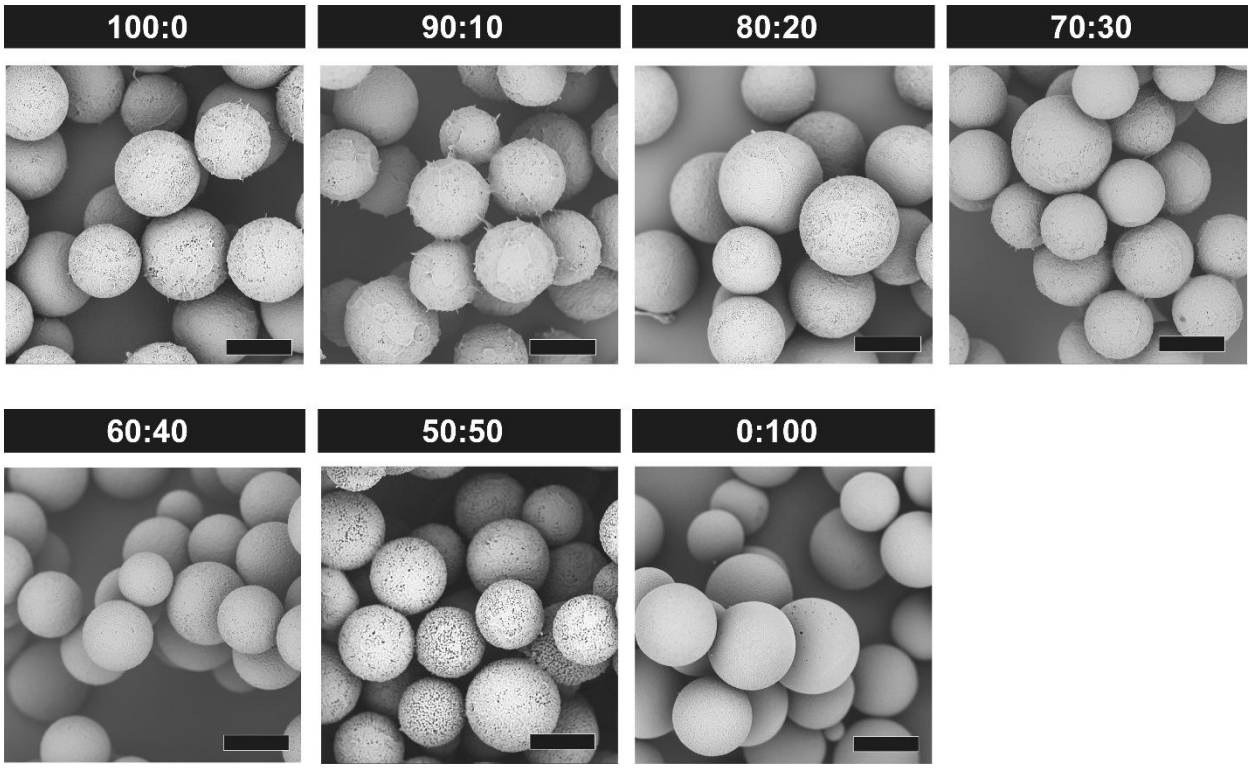


Figure S2: SEM images of VEGF-loaded microspheres (0.2 wt% target loading). The blend ratio of polymer A and B used for each batch is indicated on the bar above each image. Black scale bar represents 30 µm.

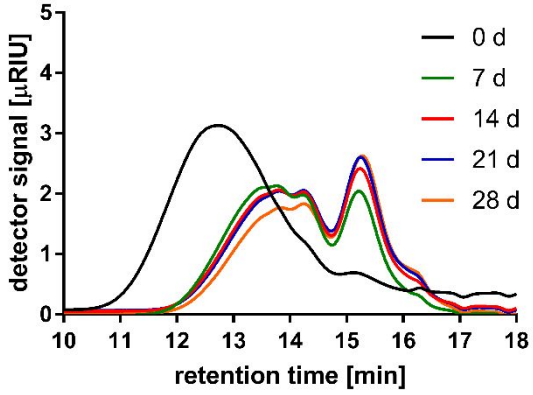


Figure S3: GPC chromatograms of (placebo) microsphere degradation samples. The time of incubation in IVR buffer (PBS pH 7.4, supplemented with 0.025% Tween 20 and 0.02% NaN_3) at 37 ºC is indicated in the legend (d: days). The GPC chromatrogram of freshly prepared microspheres is shown in black ("0 d").

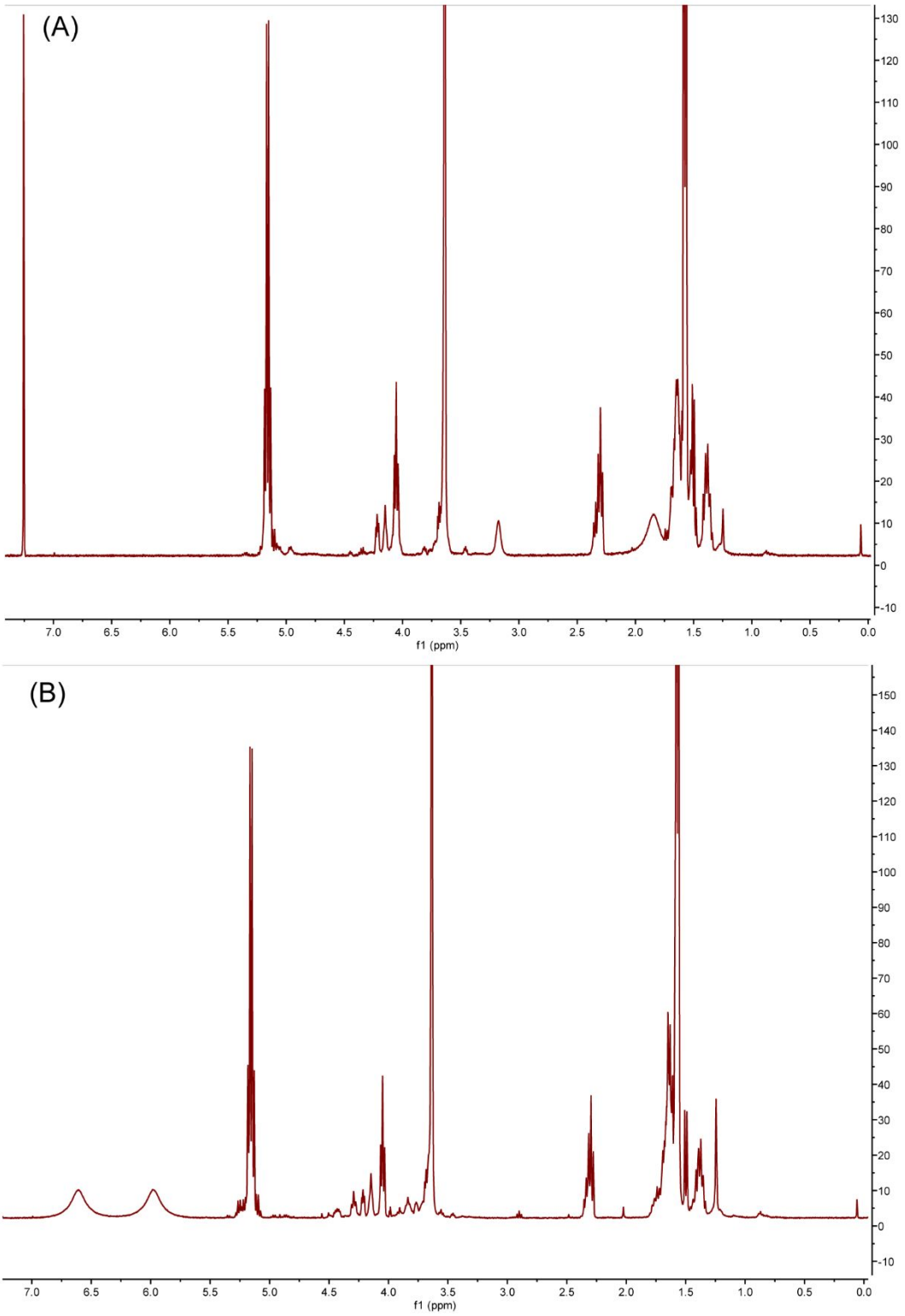
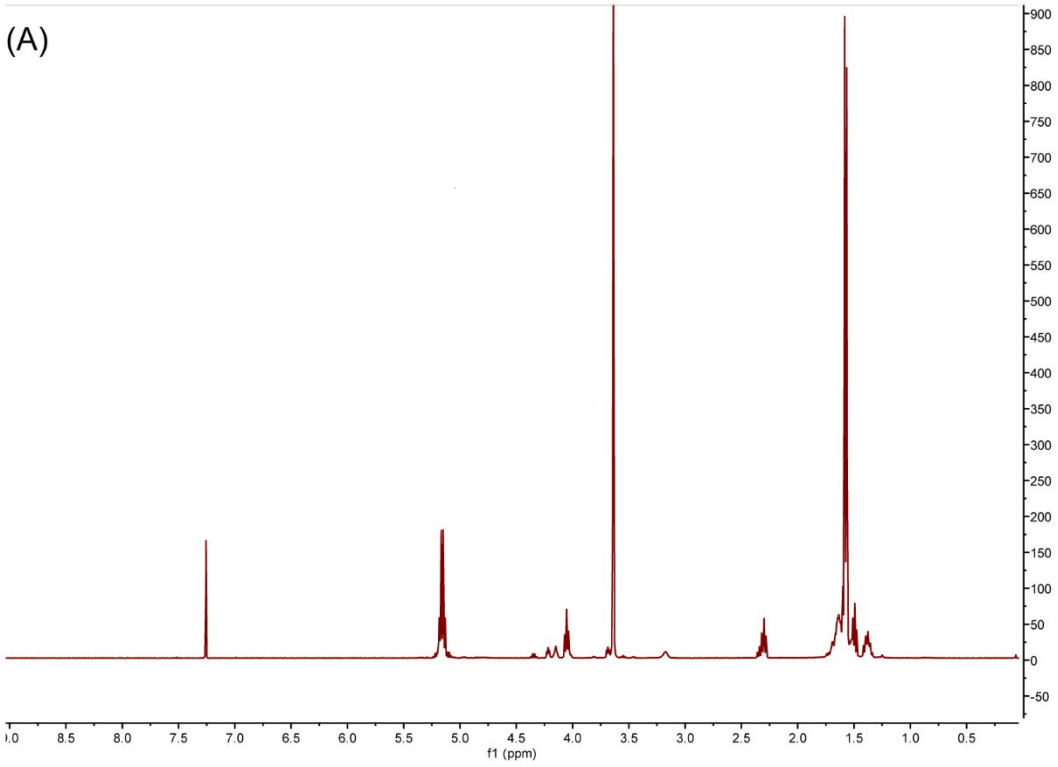


Figure S4: ^{1}H-NMR spectra of freeze-dried placebo microspheres directly after preparation (A). Figure B: After addition of shift reagent TAIC a small additional peak at ~ δ 4.5 is visible.


(A)
f1 (ppm)

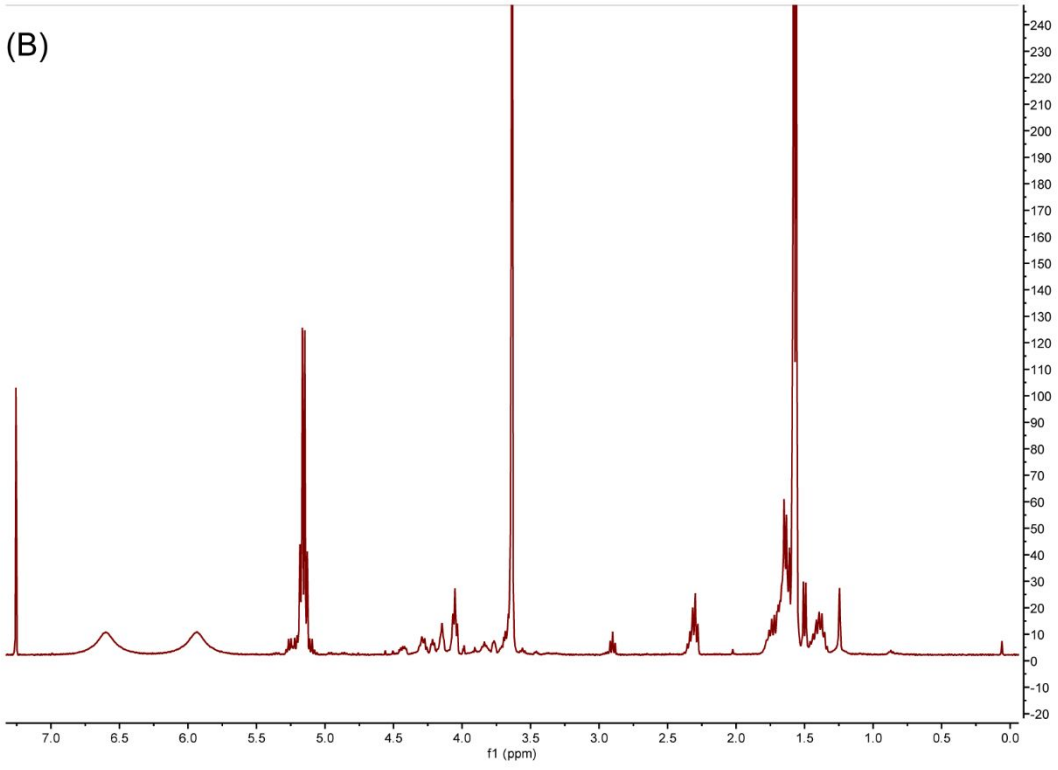

(B)
f1 (ppm)

Figure S5: ^{1}H-NMR spectra of freeze-dried placebo microspheres after incubation in IVR buffer (PBS pH 7.4, 0.025% Tween 20, 0.02% NaN_3) at 37 ºC for 28 days (figure A). Figure B: After addition of shift reagent TAIC a small additional peak at ~ δ 4.5 is visible.

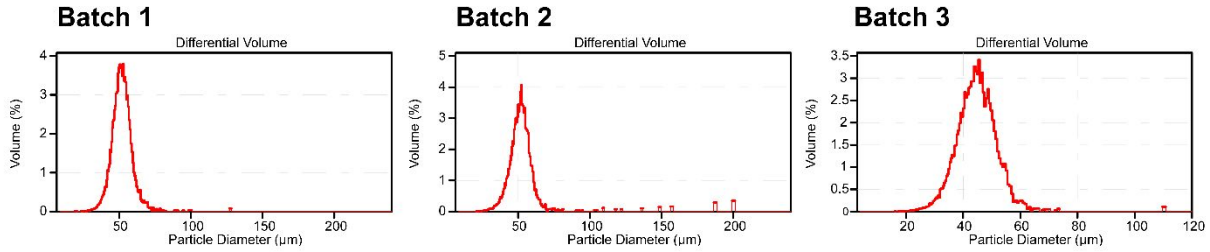


Figure S6: Size distribution plots, measured by Coulter Counter, of 3 microsphere batches prepared with 50:50 blend ratio of polymer A and B, 1 wt% target VEGF loading.

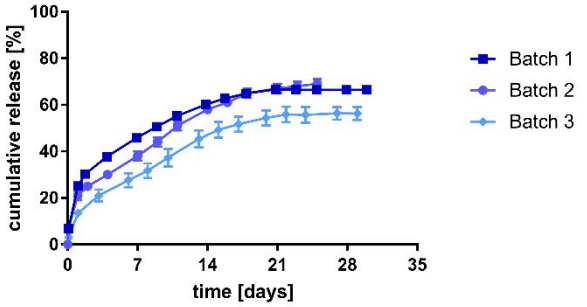


Figure S7: Cumulative release of VEGF from three microsphere batches (50:50 blend ratio polymer A and B) prepared with a target VEGF loading of 1wt%. The release was performed at 37 ºC in IVR buffer (PBS pH 7.4, supplemented with 0.025% Tween 20 and 0.02% NaN_3). Released VEGF was quantified by SE-UPLC.

Table S3: VEGF quantification by SE-UPLC and ELISA.

theoretical VEGF concentration [µg/ml]	measured VEGF concentration [µg/ml]	
	SE-UPLC	ELISA
6	6 ± 0	4 ± 0
16	60 ± 1	44 ± 2
160	158 ± 0	92 ± 3

Theoretical concentrations [µg/ml] are based on the amount of lyophilized VEGF per vial, as received by the supplier. The content of a vial (1 mg lyophilized VEGF) was reconstituted in 1 ml IVR buffer, resulting in a 1 mg/ml VEGF solution. This solution was further diluted to the concentrations stated in this table.

Table S4: Quantification of released and freshly spiked VEGF by SE-UPLC and ELISA.

IVR sample	VEGF spike [µg/ml]	measured VEGF concentration [µg/ml]	
		SE-UPLC	ELISA
Day 1	0	18 ± 0	5 ± 1
	50	70 ± 1	45 ± 4
Day 11	0	8 ± 0	3 ± 0
	50	57 ± 0	32 ± 1
Day 18	0	5 ± 0	2 ± 0
	50	55 ± 1	38 ± 9
-	50	52 ± 1	31 ± 1

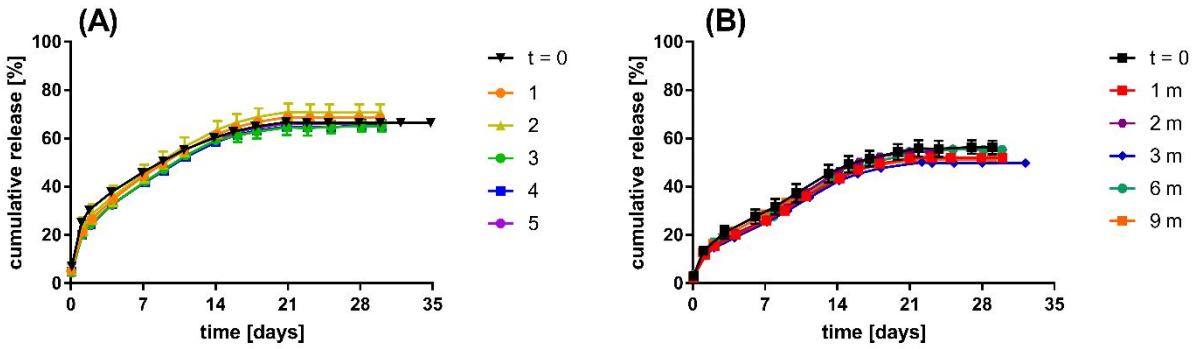


Figure S8: Cumulative release of VEGF [%] from freeze-dried microspheres. A: after repeated freezing and storage -20 ºC, B: after long-term storage at -20 ºC for 1, 2, 3, 6 months compared to directly after freeze-drying (t=0). The release was performed at 37 ºC in PBS pH 7.4, supplemented with 0.025% Tween 20 and 0.02% NaN_3. Released VEGF was quantified by SE-UPLC.

Table S5: Similarity factor f_2 of VEGF release curves from microspheres after 1 to 5 times of freezing to and storage at -20 ºC compared to VEGF release curve from microspheres after preparation and freeze-drying.

freezing cycles	f_2
1	81

2	74
3	74
4	74
5	75

Table S6: Similarity factor f_2 of VEGF release curves from microspheres after various months of storage at -20 ºC and VEGF release curves from microspheres after preparation and freeze-drying (batch 3).

storage time	f_2
1 month	63
2 months	68
3 months	59
6 months	73
9 months	65

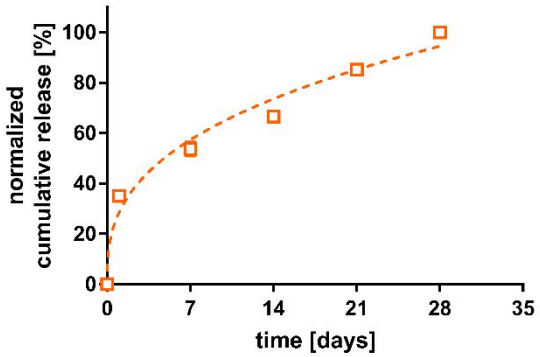


Figure S9: Normalized cumulative release profiles of VEGF from microspheres. Release was performed at 37 ºC in bioactivity IVR buffer (PBS pH 7.4, supplemented with 0.5% BSA, 30 µg/ml and 15 ng/ml amphotericin). Korsmeyer-Peppas model fitting (orange dotted line): correlation coefficient (R^2): 0.98, Diffusional exponent (n): 0.36 ± 0.05, 95% confidence interval: 0.22 – 0.55.

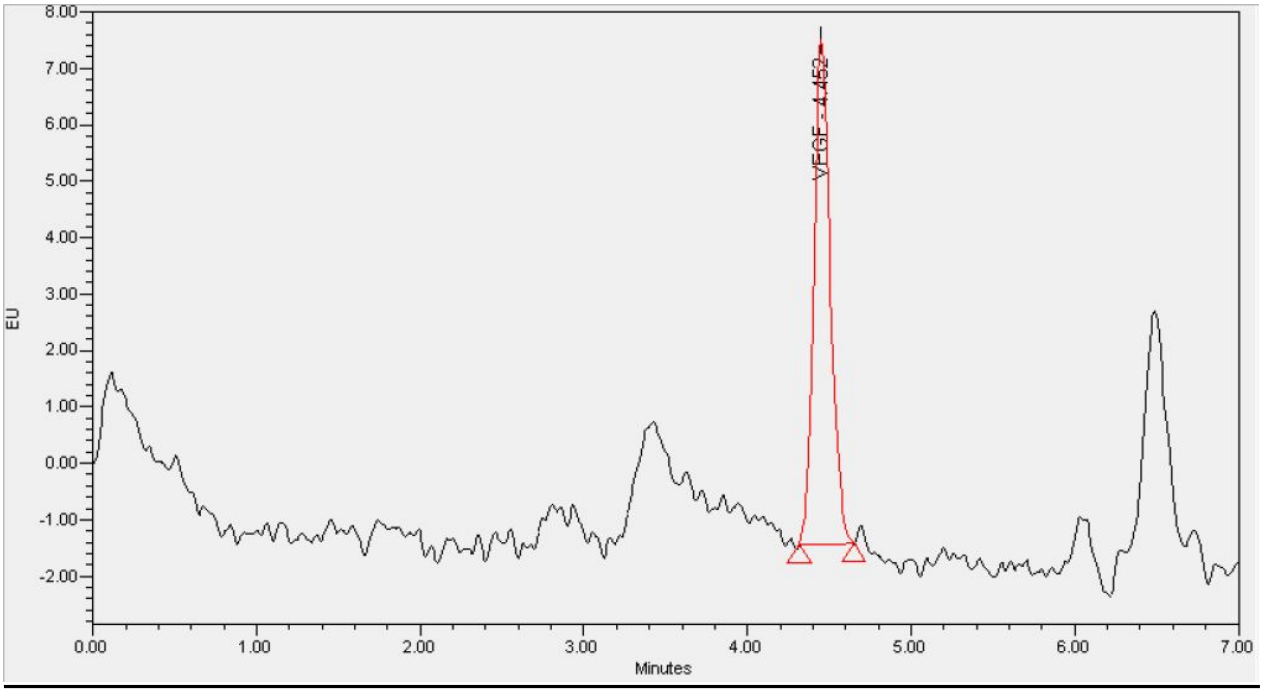


Figure S10: Representative SE-UPLC chromatogram for VEGF quantification. Peaks at 3.5 minutes, 6 minutes and 6.5 minutes have not been identified.