#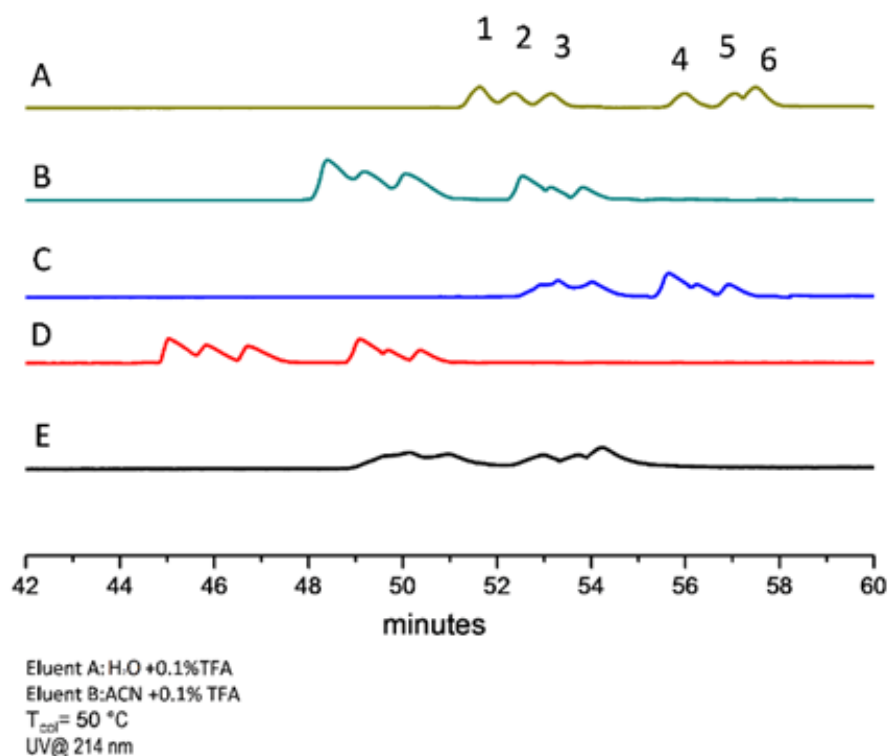 Boosting basic-peptide separation through dynamic electrostatic-repulsion reversed-phase (d-ERRP) liquid chromatography

Giulia Mazzoccanti*, Simone Manetto, Michele Bassan, Alberto Foschini, Andrea Orlandin, Antonio Ricci*, Walter Cabri, Omar H. Ismail, Martina Catani, Alberto Cavazzini, Francesco Gasparrini*

ELECTRONIC SUPPLEMENTARY INFORMATION (ESI)

Figure S1. Chromatograms recordered by using mobile phases **1** described above. **A)** Column A, flow rate: 0.4 mL/min, gradient elution 23% B (0 min), 23% B (1 min), 28% B (61 min), 50% B (65min), 50% B (70 min) and 23% B (71min). **B)** Column B, flow rate: 0.4 mL/min, gradient elution 23% B (0 min), 23% B (1 min), 28% B (61 min), 50% B (65min), 50% B (70 min) and 23% B (71min). **C)** Column C, flow rate: 0.2 mL/min, gradient elution 24% B (0 min), 24% B (1 min), 29% B (61 min), 50% B (65min), 50% B (70 min) and 24% B (71min). **D)** Column D,flow rate: 0.4 mL/min, gradient elution 25% B (0 min), 25% B (1 min), 29% B (61 min), 50% B (65min), 50% B (70 min) and 25% B (71min). **E)** Column E, flow rate: 1.0 mL/min, gradient elution 26% B (0 min), 26% B (1 min), 31% B (61 min), 50% B (65min), 50% B (70 min) and 26% B (71min).
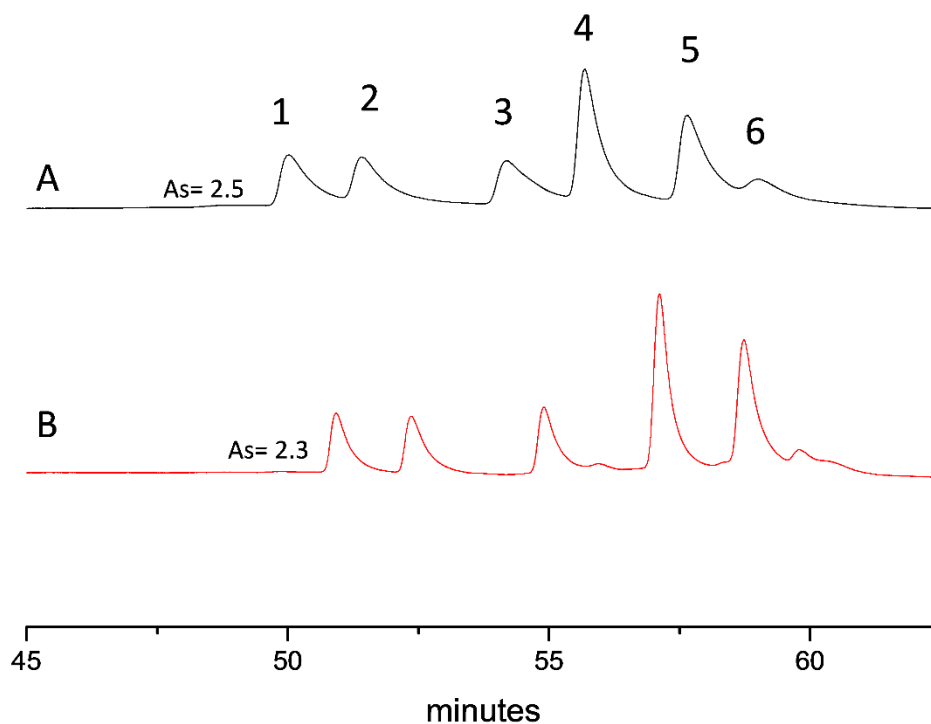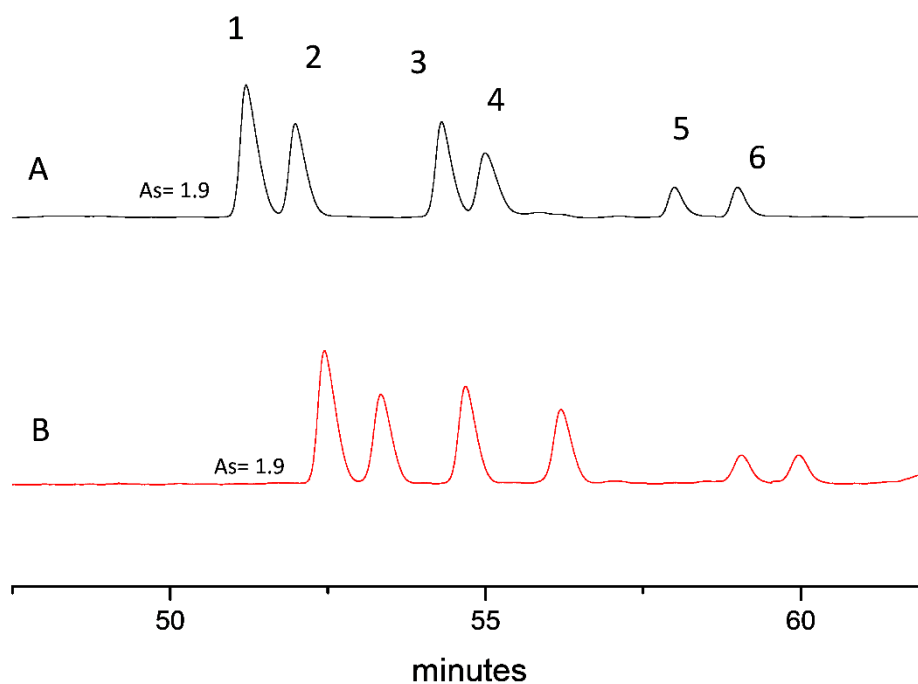
Eluent A: $H_2O+ H_3PO_4$ 15 mM+TBAOH 10 mM (^wpH = 2.2);
Eluent B: ACN + H_3PO_4 15 mM+TBAOH 10 mM (^{App}pH = 3.8)
T_{col}= 50 °C
UPLC Waters
UV@ 214 nm

Figure S2. Chromatograms recordered by using mobile phases **2** described in the main text (2.3 Chromatographic conditions). **A)** ACQUITY UPLC® BEH C18 (150 mm × 2.1 mm L. × I.D.) 1.7 μm 300Å, flow rate: 0.2 mL/min, gradient elution 18% B (0 min), 18% B (1 min), 23% B (61 min), 50% B (65min), 50% B (70 min) and 18% B (71min), **B)** Halo peptide C18 (150 mm × 3.0 mm L. × I.D.) 2.0 μm 160Å flow rate: 0.4 mL/min, gradient elution 18% B (0 min), 18% B (1 min), 23% B (61 min), 50% B (65min), 50% B (70 min) and 1% B (71min); columns and conditions were used.

Table S1. Chromatographic data for separation of the six reference standards in Figure S3 ESI.

|	Column	Rs 1-2	Rs 2-3	Rs 3-4	Rs 4-5	Rs 5-6	μ_0 (mm/s)	$t_G = 60$ minutes
A	ACQUITY UPLC® BEH C18 (150 mm x 2.1 mm L. x I.D.) 1.7 μm 300Å	1.40	2.54	1.60	2.41	-	1.40	from 18% B to 23% B
B	Halo peptide C18 (150 mm x 3.0 mm L. x I.D.) 2.0 μm 160Å	2.43	4.28	4.19	3.00	1.99	1.67	from 18% B to 23% B

Eluent A: H$_2$O+ TFA 13.1 mM+TBAOH 10 mM (^{w}pH = 2.5)
Eluent B: ACN + TFA 13.1 mM+TBAOH 10 mM (^{App}pH = 4.2)
T$_{col}$= 50 °C
UPLC Waters
UV@ 214 nm

Figure S3. Chromatograms recordered by using mobile phases **3** described above in the main text (2.3 Chromatographic conditions). **A)** ACQUITY UPLC® BEH C18 (150 mm × 2.1 mm L. × I.D.) 1.7 µm 300Å, flow rate: 0.2 mL/min, gradient elution 18% B (0 min), 18% B (1 min), 23% B (61 min), 50% B (65min), 50% B (70 min) and 18% B (71min), **B)** Halo peptide C18 (150 mm × 3.0 mm L. × I.D.) 2.0 µm 160Å flow rate: 0.4 mL/min, gradient elution 18% B (0 min), 18% B (1 min), 23% B (61 min), 50% B (65min), 50% B (70 min) and 18% B (71min); columns and conditions were used.

Table S2. Chromatographic data for separation of the six reference standards in Figure S4 ESI.

|	Column	Rs 1-2	Rs 2-3	Rs 3-4	Rs 4-5	Rs 5-6	μ_0 (mm/s)	$t_G = 60$ minutes
A	ACQUITY UPLC® BEH C18 (150 mm x 2.1 mm L. x I.D.) 1.7 µm 300Å	1.20	4.28	2.26	2.76	2.17	1.40	from 18% B to 23% B
B	Halo peptide C18 (150 mm x 3.0 mm L. x I.D.) 2.0 µm 160Å	1.99	3.24	3.86	4.67	2.20	1.67	from 18% B to 23% B
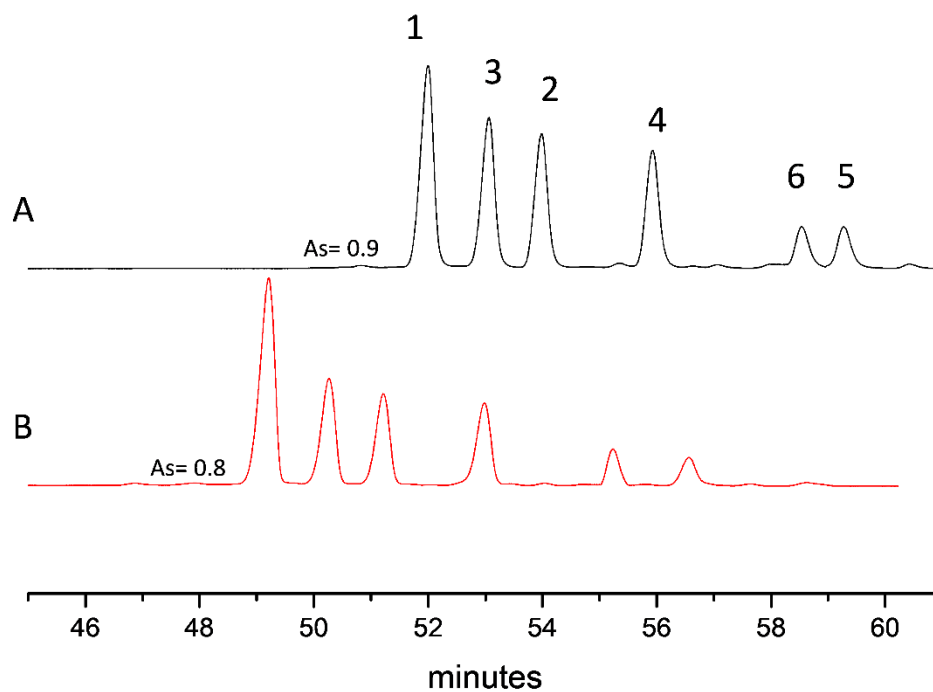
Eluent A: H_2O+ TBAHSO$_4$ 10 mM (^{w}pH = 2.0)
Eluent B: ACN + TBAHSO$_4$ 10 mM (^{App}pH =2.7)
T_{col}= 50 °C
UPLC Waters
UV@ 214 nm

Figure S4. Chromatograms recordered by using mobile phases **4** described above in the main text (2.3 Chromatographic conditions). **A)** ACQUITY UPLC® BEH C18 (150 mm × 2.1 mm L. × I.D.) 1.7 μm 300Å, flow rate: 0.2 mL/min, gradient elution 23% B (0 min), 23% B (1 min), 28% B (61 min), 50% B (65min), 50% B (70 min) and 23% B (71min), **B)** Halo peptide C18 (150 mm × 3.0 mm L. × I.D.) 2.0 μm 160Å flow rate: 0.4 mL/min, gradient elution 23% B (0 min), 23% B (1 min), 28% B (61 min), 50% B (65min), 50% B (70 min) and 23% B (71min); columns and conditions were used.
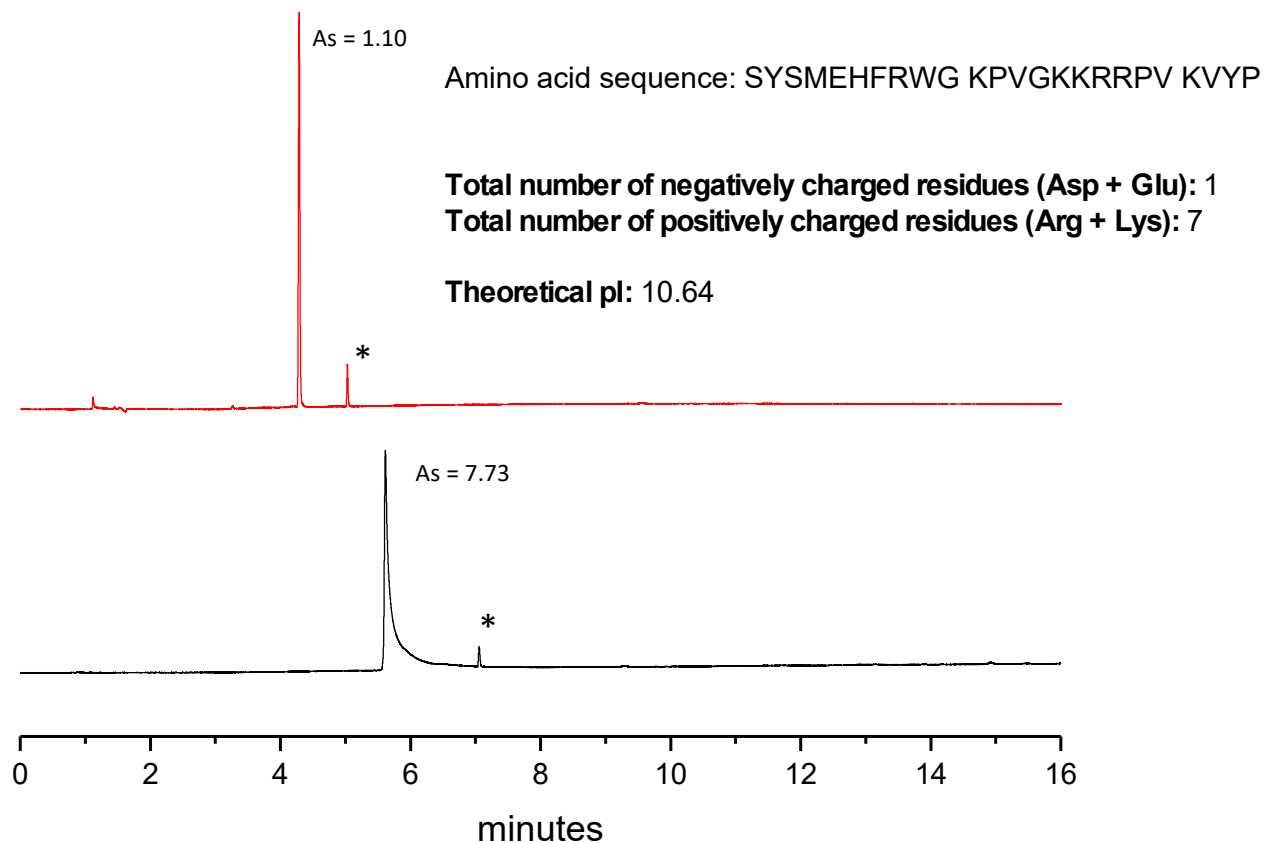
Table S3. Chromatographic data for separation of the six reference standards in Figure S5 ESI.

|	Column	Rs 1-3	Rs 3-2	Rs 2-4	Rs 4-6	Rs 6-5	μ_0 (mm/s)	t_G= 60 minutes
A	ACQUITY UPLC® BEH C18 (150 mm x 2.1 mm L. x I.D.) 1.7 μm 300Å	2.56	2.12	4.74	5.56	1.73	1.40	from 23% B to 28% B
B	Halo peptide C18 (150 mm x 3.0 mm L. x I.D.) 2.0 μm 160Å	2.24	2.09	3.87	2.93	1.93	1.67	from 23% B to 28% B
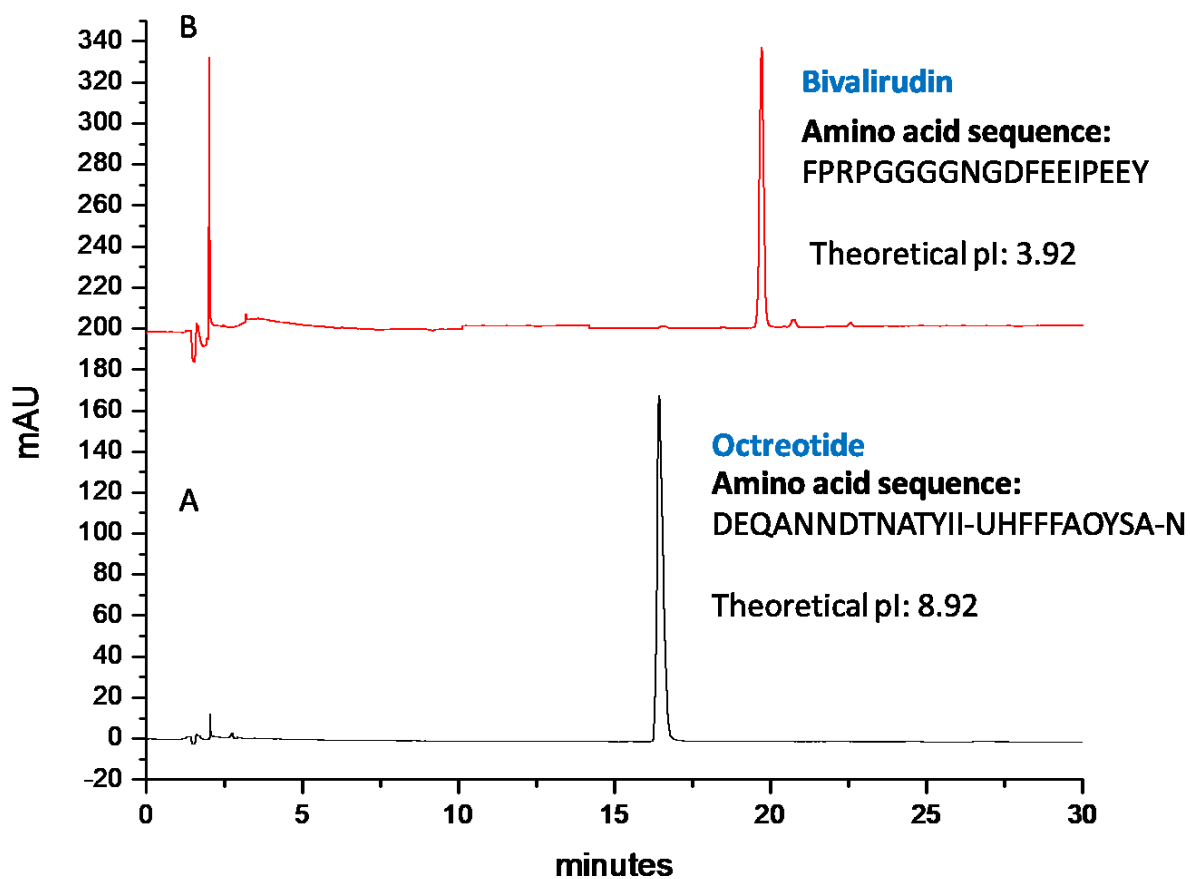
Figure S5. Analysis of A) octreotide and B) bivalirudin. The mobile phases 2 and column B were employed. Both analyses were carried out by using a gradient time of 30 minutes, gradient elution: A)13% B (0 min), 13% B (1 min), 19% B (31 min), 19% B (35min), 13% B (36 min) and 13% B (37min): B) 5% B (0 min), 5% B (1 min), 8% B (31 min), 8% B (35min), 5% B (36 min) and 5% B (37 min). Flow rate 0.4 mL/min, UV detection 214 nm.

As = 1.10

Amino acid sequence: SYSMEHFRWG KPVGKKRRPV KVYP

Total number of negatively charged residues (Asp + Glu): 1
Total number of positively charged residues (Arg + Lys): 7

Theoretical pl: 10.64

*

As = 7.73

*

minutes

Figure S6. Analysis of a tetracosactide sample. The black trace refers to analysis in *"static"* ERRP mode [mobile phases 1 and column A (CSH column)]. The red trace refers to analysis in d-ERRP mode [mobile phases 5 and column E]. Both analyses were carried out by using a gradient time of 15 minutes (gradient elution: 10% B (0 min), 10% B (1 min), 60% B (16 min), 60% B (20min), 10% B (21 min) and 10% B (31min)) and a flow rate of 0.4 mL/min. The asterisk denotes an unknown impurity.
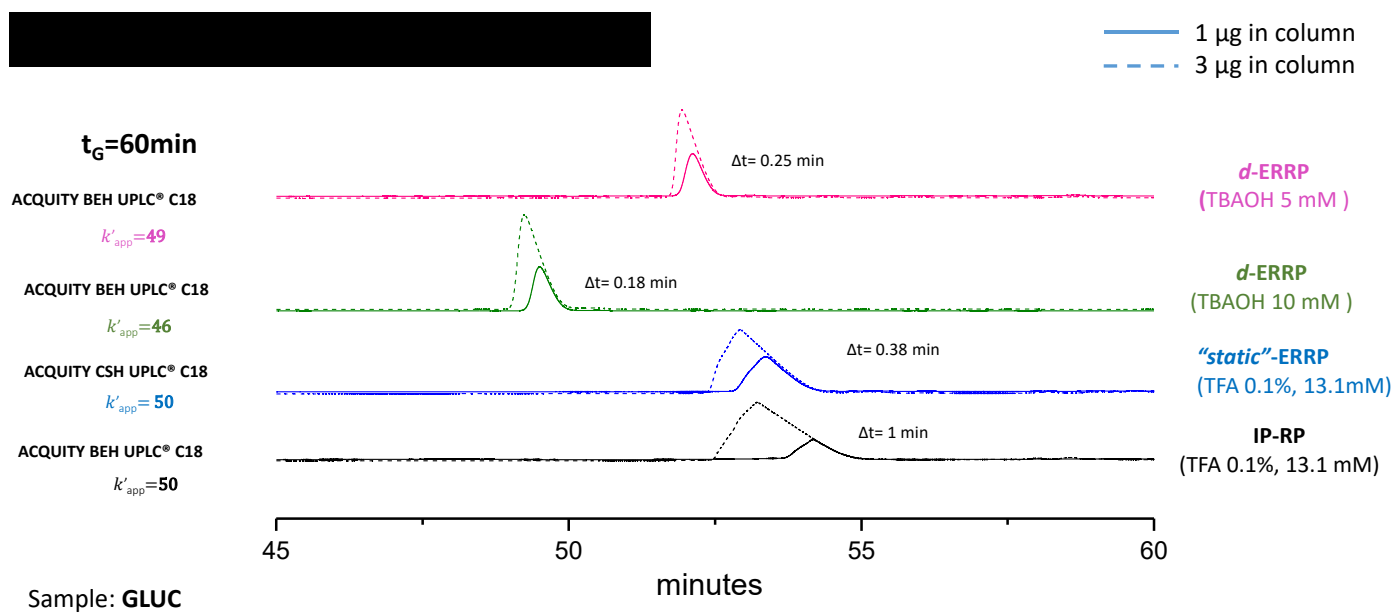
Figure S7. Overlaid chromatograms for 1.0–3.0 µg GLUC. Columns packed with BEH and CSH particles (1.7 µm, 130 Å) with same geometry: 100 mm × 3.0 mm L. × I.D.
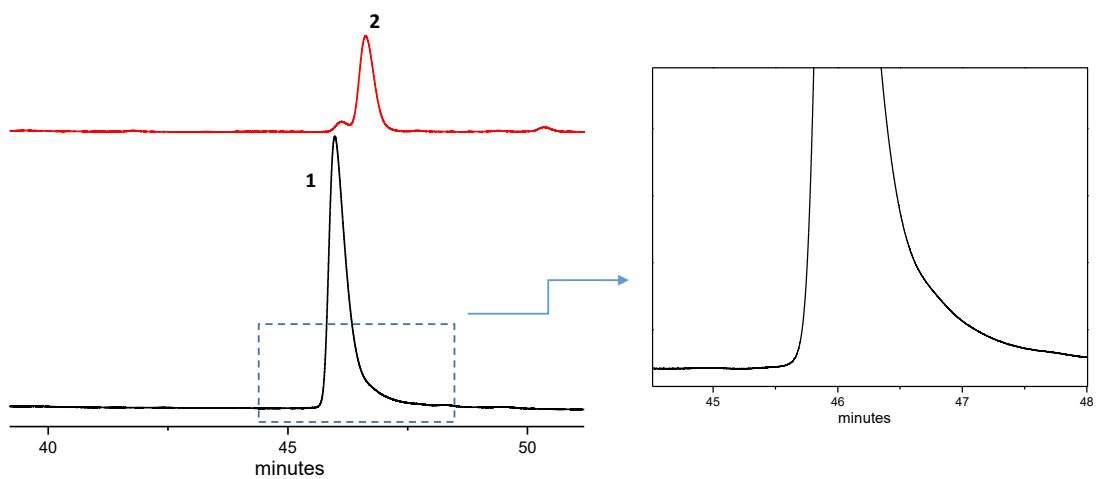
Column: Halo peptide C18 (150 mm x 3.0 mm L. x I.D.) 2.0 μm 160Å

Eluent A: 90% TEA-TFA/10% ACN
Eluent B: 40% TEA-TFA/60% ACN
Flow rate: 0.4 mL/min; Detector UPLC Waters
UV@ 214 nm T_{col}=50°C t_G= 60 min

Figure S8. Analysis of **1** and **2** samples. Mobile phases: eluent A) 90:10 Buffer TFA+ TEA / ACN, eluent B) 60:40 Buffer TFA+ TEA/ACN , and Halo peptide C18 (150 mm × 3.0 mm L. × I.D.) 2.0 μm 160Å column (flow rate: 0.4 mL/min) were used. Analyses were carried out by using a gradient time of 60 minutes (gradient elution: 25% B (0 min), 25% B (1 min), 65% B (61 min), 100% B (65min), 100% B (70 min) and 25% B (71min)).