Supporting Information

Harnessing polarity and viscosity to identify green binary solvent mixtures as viable alternatives to DMF in solid-phase peptide synthesis

Vincent Martin,[a] Sandip Jadhav,[b] Peter H. G. Egelund,[a] Raphael Liffert,[b] Henrik Johansson Castro,[a] Tobias Krüger,[b] Kim F. Haselmann,[c] Sebastian Thordal Le Quement,[a] Fernando Albericio,[d,e] Frank Dettner,[b] Carolin Lechner,[b] Ralph Schönleber,[b] Daniel Sejer Pedersen[a]

[a] Novo Nordisk A/S, CMC API Development, Smørmosevej 17-19, DK-2880 Bagsværd, Denmark

[b] Bachem AG, Hauptstrasse 144, 4416 Bubendorf, Switzerland

[c] Novo Nordisk A/S, GRT MS Characterisation, Novo Nordisk Park 1, DK-2760 Måløv, Denmark

[d] School of Chemistry and Physics, University of KwaZulu-Natal, University Road, Westville, Durban, South Africa

[e] CIBER-BBN, Networking Centre on Bioengineering, Biomaterials and Nanomedicine, and Department of Organic Chemistry, University of Barcelona, Barcelona, Spain

Table of Contents

1. General information

All reagents and solvents were obtained from standard suppliers of raw materials for peptide synthesis and were used as received. All reactions were carried out under ambient temperature.

1.1 HPLC, UPLC, and LC-MS instruments and methods

Analytical HPLC was performed on Thermo Scientific™ Vanquish™ UHPLC system using AQUITY UPLC BEH 130 C18 (1.7 µm, 2.1 × 150 mm) column, with a flow rate of 0.4 mL/min and UV detection at 220 nm. The MS analysis was performed on UHPLC coupled with Bruker maXis II™ spectrometer with ultra-high resolution QTOF technology equipped with electron-transfer dissociation (ETD) capabilities. Buffer A: 0.05% TFA in MeCN/H_2O (1:99, v/v/v) and buffer B: 0.05% TFA in MeCN (v/v). **Method A**: 0-100% B in 30 min.

Analytical HPLC was performed on Thermo Scientific™ Scientific Ultimate UHPLC system using Kinetex UPLC column C18 100Å 1.7 µm, 150 × 2.1 mm, with a flow rate of 0.5 mL/min and UV detection at 210 nm. The MS analysis was performed on MSQ Plus Mass spectrometer, operating in positive mode ESI. Buffer A: 0.1% HCOOH in MeCN/H_2O (10:90, v/v/v), buffer B: 0.1% HCOOH in MeCN/H_2O (90:10, v/v/v). **Method B**: 0-100% B in 5 min **Method C**: 0-60% B in 12 min followed by 60-100% B in 2 min. **Method D**: 0-20% B over 2 min, 20-50% over 10 min and 50-100% B over 2 min.

Analytical HPLC was performed on Waters Acquity UPLC H-Class using Waters Column Acquity BEH UPLC C18 1.7 µm, 2.1 × 50 mm with a flowrate of 0.5 mL/min and UV detection at 214 nm and 280 nm. Buffer A: 0.1% TFA in MeCN/H_2O (10:90, v/v/v). Buffer B: 0.1% TFA in MeCN/H_2O (90:10, v/v/v). **Method E:** 0-60% B in 12 min followed by 60-100% B in 2 min.

Analytical HPLC was performed on a Waters Acquity UPLC i-Class system using Waters Column Acquity CSH C18 1.7 µm, 1.0 × 150 mm with a flowrate of 0.1 mL/min and UV detection at 215 nm. The MS analysis was performed on Waters Synapt G2S high definition mass spectroscopy equipped with an electrospray interference operating in positive mode. Buffer A: 0.1% formic acid in H_2O (v/v/v). Buffer B: 0.1% formic acid in MeCN (v/v/v). **Method F**: 20-40% B in 20 min.

2. Experimental methods and results

2.1 Solubility of Fmoc-amino acids, coupling reagents and *N,N'*-diisopropylurea

For the solubility assessment of Fmoc-amino acids, coupling reagents and *N,N'*-diisopropylurea (DIU), 2 mmol of compound were weighed in 20 mL high performance glass vials for liquid scintillation counting

(Perkin Elmer®). The designated solvent system (5 mL) was then added to the solid to reach a concentration of 0.4 M, and the vial was agitated for 30 min before visual inspection. A clear solution was noted as soluble (S) while cloudy solution or solution containing insoluble material were diluted further by adding 3 mL of solvent to the scintillation vial to reach a concentration of 0.25 M. The mixture was again agitated for 30 min before visual inspection. A clear solution was noted as soluble at 0.25 M (S (0.25 M)) while cloudy solutions or solutions containing insoluble material were noted as insoluble (I).

2.2 Assessment of solvent stability

H-Gly-Ala-Phe-Phe-Ala-Ramage resin (200 mg, loading = 0.45 mmol/g) was suspended in the indicated solvent (1.5 mL) and incubated at 40 °C. In separate experiments, HOBt monohydrate (26.8 mg, 0.17 mmol, 1.9 equiv.) and DIPEA (33.7 µL, 0.19 mmol, 2.1 equiv.) were added separately to the suspension and the mixtures were incubated at 40 °C. After seven days the suspension was filtered and the resin was washed with DMF and IPA (3 × 10 mL, alternating), IPA (3 × 10 mL), and lastly diisopropyl ether (DIPE; 1 × 10 mL). The peptide was cleaved from the resin with TFA/H_2O (95/5, v/v), precipitated with cold DIPE and dried under vacuum to obtain a solid powder. In the experiments with GVL (neat and with DIPEA), gummy solids were obtained. The isolated peptides were subjected to HPLC analysis (Method A) without further purification.

2.3 Resin swelling

2.3.1 Starting resin swelling

Each resin (1.0 g) was dispersed into the indicated solvent (10 mL) in a graduated cylinder (10 ± 0.2 mL) at room temperature. The resin was shortly stirred with a spatula and then allowed to sit at room temperature for one hour, after which the final resin volume was noted (rounded to the nearest 0.5 mL).

Supplementary Table S1. Additional resin swelling experiments.

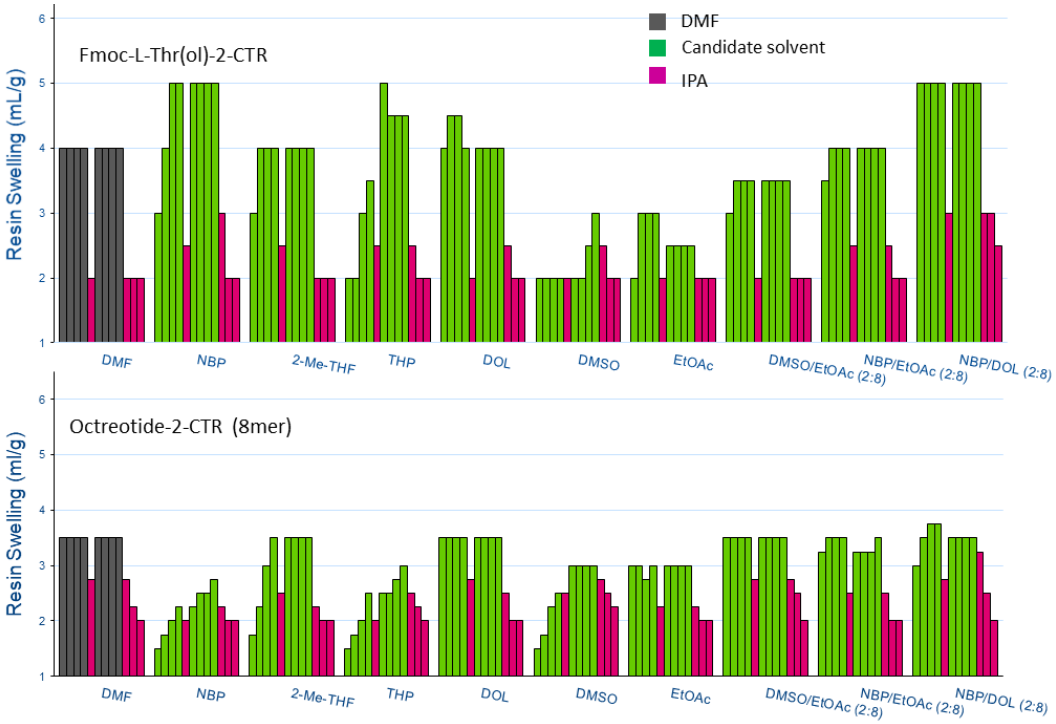
Solvent/resin	Trityl-OH ChemMatrix® [a]	Ramage ChemMatrix® [b]	H-Rink amide ChemMatrix® [c]
DMF	8.5	8.5	9.0
NBP	7.5	10.0	10.0
DMSO	6.5	8.0	8.0
DOL	>10	>10	>10
NFM	8.0	9.0	9.0
2-Me-THF	4.5	4.0	4.5
THP	7.0	8.0	7.0
DMSO/EtOAc (2:8)	7.0	6.5	7.5
NBP/EtOAc (2:8)	8.5	9.0	9.0
NFM/EtOAc (2:8)	8.5	9.0	9.5

Resin loading (mmol/g) and particle mesh size: [a] 0.31 (35-100 mesh); [b] 0.47 (35-100 mesh); [c] 0.48 (35-100 mesh).
Green: 4-7 mL/g (sufficient swelling); Blue > 7 mL/g (excessive swelling)

2.3.2 Peptidyl resin swelling

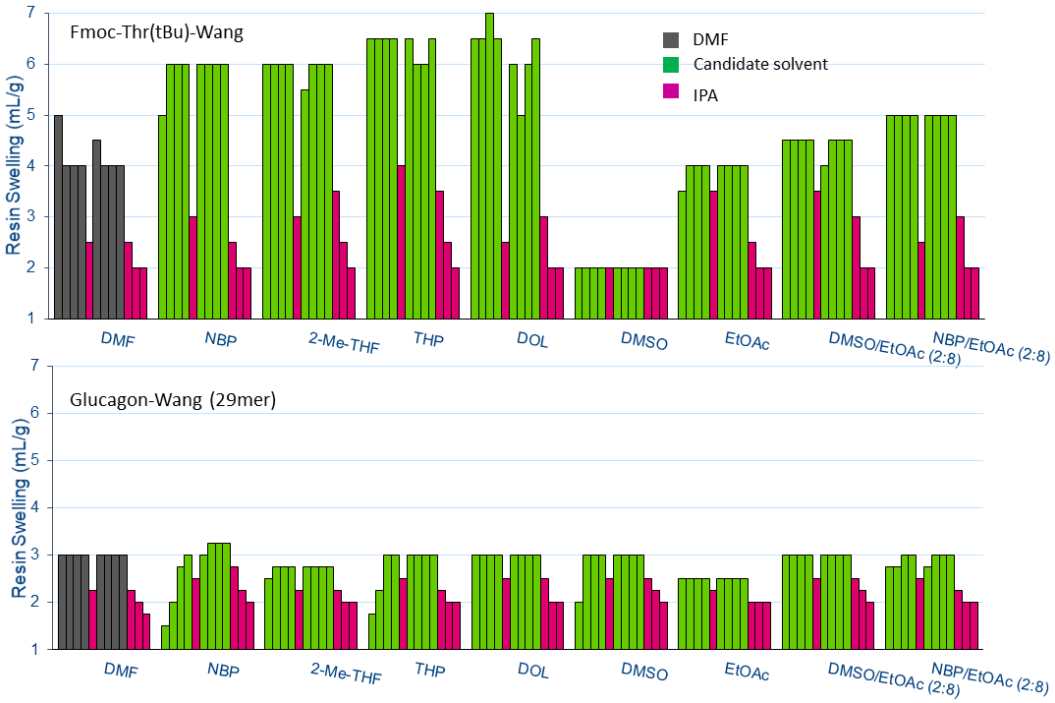
Dry peptidyl resin (1.0 g) was added to a graduated syringe (10 mL), fitted with a porous frit (volume of 0.4 mL). The resin was subjected to series of swelling and shrinkage process in candidate solvent and IPA respectively (10 mL each). The resin swelling was performed in the candidate solvent (neat or binary mixture) by shaking the syringe on an orbital shaker. After each swelling/shrinkage step, the solvent was drained via gentle aspiration using a vacuum manifold and swelling volume was noted on the graduated syringe. The resin was swelled in the candidate solvent for 2 × 10 minutes followed by 2 × 30 minutes. At this stage the resin was washed with IPA for 1 × 2 minutes to remove candidate solvent from resin beads which resulted into resin shrinkage. The shrinked resin was subjected for re-swelling in a candidate solvent for 2 × 2 minutes, followed by 1 × 10 minutes and finally for 1 × 30 minutes. In the end, the resin was washed with IPA for 3 × 2 minutes. The swelling capacity of the solvent was determined using the following equation (1), and the results presented in Supplementary Figure S1A-D.

Swelling of resin (mL/g) = Measured volume (mL) – Frit volume (mL) / Weight of resin (g) (1)



Supplementary Figure S1A. Swelling/shrinkage cycles of protected Octreotide-2-CTR (8-mer, loading = 0.37 mmol/g) and its corresponding starting resin Fmoc-L-Thr(ol)-2-CTR (loading = 0.84 mmol/g). The experiments in neat NBP showed slower draining.

Supplementary Figure S1B. Swelling/shrinkage cycles of protected Bivalirudin-2-CTR (20-mer, loading = 0.25 mmol/g) and its corresponding starting resin H-Leu-2-CTR (loading = 1.1 mmol/g). The experiments in neat NBP showed slower draining.



Supplementary Figure S1C. Swelling/shrinkage cycles of protected Glucagon-Wang resin (29-mer, loading = 0.14 mmol/g) and its corresponding starting resin Fmoc-Thr(tBu)-Wang resin (loading = 0.64 mmol/g). The experiments in neat NBP showed slower draining.

Supplementary Figure S1D. Swelling/shrinkage cycles of protected Aprotinin-Wang resin (58mer, loading = 0.073 mmol/g) and its corresponding starting resin Fmoc-Ala-Wang resin (loading = 0.43 mmol/g). The experiments in neat NBP showed slower draining.

2.4 Solvent viscosity

The solvent viscosities were determined using a DV2T™ viscometer (Brookfield, model LVDV2T) equipped with RheocalcT software. The cylinder-shaped spindle (ULA, material: 316 s/s) was used at the rotation of 60 rpm with shear rate (1/s) of 73.38. The multi-point (10-fold determination) method was used for the viscosity measurement. All measurements were performed at 20 °C in duplicates.

Supplementary Table S2. Measured solvent viscosity (mPa × s).

Solvent	Dynamic Viscosity (η) at 20 °C mPa×s	Solvent	Dynamic Viscosity (η) at 20 °C mPa×s
2-Me-THF	0.65	NBP/2-Me-THF (6:4)	1.24
Cyrene/2-Me-THF (2:8)	0.72	NBP/2-Me-THF (8:2)	1.68
Cyrene/2-Me-THF (4:6)	1.27	NBP/DOL (2:8)	0.77
Cyrene/2-Me-THF (6:4)	2.37	NBP/DOL (4:6)	1.02
Cyrene/2-Me-THF (8:2)	4.85	NBP/DOL (6:4)	1.35
DMF	0.83	NBP/DOL (8:2)	1.95
DMSO	2.08	NBP/EtOAc (1:9)	0.63
DMSO/2-Me-THF (1:9)	0.65	NBP/EtOAc (2:8)	0.82
DMSO/2-Me-THF (2:8)	0.74	NBP/EtOAc (4:6)	0.88
DMSO/2-Me-THF (3:7)	0.78	NBP/EtOAc (6:4)	1.21
DMSO/2-Me-THF (4:6)	0.92	NBP/EtOAc (8:2)	1.88
DMSO/2-Me-THF (6:4)	1.21	NFM/2-Me-THF (2:8)	0.67
DMSO/2-Me-THF (8:2)	1.59	NFM/2-Me-THF (4:6)	1.30
DMSO/DOL (1:9)	0.78	NFM/2-Me-THF (6:4)	2.03
DMSO/DOL (2:8)	0.81	NFM/2-Me-THF (8:2)	3.98
DMSO/DOL (3:7)	0.84	NFM/DOL (2:8)	0.86
DMSO/DOL (4:6)	0.94	NFM/DOL (4:6)	1.29
DMSO/DOL (6:4)	1.19	NFM/DOL (6:4)	2.08
DMSO/DOL (8:2)	1.54	NFM/DOL (8:2)	3.75
DMSO/EtOAc (1:9)	0.66	NFM/EtOAc (2:8)	0.75
DMSO/EtOAc (2:8)	0.76	NFM/EtOAc (4:6)	1.17
DMSO/EtOAc (3:7)	0.78	NFM/EtOAc (6:4)	2.04
DMSO/EtOAc (4:6)	0.89	NFM/EtOAc (8:2)	4.58
DMSO/EtOAc (5:5)	0.98	PC/2-Me-THF (2:8)	0.66
DMSO/EtOAc (6:4)	1.13	PC/2-Me-THF (4:6)	0.97
DMSO/EtOAc (7:3)	1.35	PC/2-Me-THF (6:4)	1.32
DMSO/EtOAc (8:2)	1.56	PC/2-Me-THF (8:2)	1.83
DMSO/EtOAc (9:1)	1.78	THP	0.86
DOL	0.78	TOU	1.57
EtOAc	0.63	TOU/EtOAc (3:7)	0.71

NBP	3.30	TOU/EtOAc (4:6)	0.75
NBP/2-Me-THF (2:8)	0.66	TOU/EtOAc (5:5)	0.81
NBP/2-Me-THF (4:6)	0.87		

2.5 Solvent polarity

The solvent polarities were determined using a Shimadzu UVmini-1240 UV-VIS spectrophotometer, in Hellma Quartz SUPRASIL ® 10 mm precision cells at room temperature (25 °C) and normal pressure. The solvent polarities $E_T(30)$ (kcal mol^{-1}) were calculated using equation (2), where h is Planck's constant, c is the speed of light in a vacuum, N_A is the Avogadro constant, ν_{max} is the frequency at the maximum of the highest absorbing wavelength and λ_{max} is the highest absorbing wavelength.

$$E_T(30)\ (kcal\ mol^{-1}) = hcN_A\nu_{max} = 28591/\lambda_{max} \quad (2)$$

For each solvent system, 2,6-diphenyl-4-(2,4,6-triphenylpyridin-1-ium-1-yl)phenolate (Reichardt's Dye; 5 mg) was dissolved in the designated solvent (10 mL). An aliquot was then further diluted until the absorbance of λ_{max} reached 0.6-0.8 AU (this was generally achieved at a concentration of 0.05 mg/mL). λ_{max} was measured between 400 nm and 800 nm.[1, 2] The concentration dependency of Reichardt's dye on the absorption was investigated in DMF between 0.012-0.05 mg dye per mL solvent (Supplementary Table S3). Since no change in the absorption was observed in the investigated concentration range it was presumed that the dye concentration had no to low effect on the $\lambda_{max.}$ The measured solvent polarities $E_T(30)$ (kcal mol^{-1}) are listed in Supplementary Table S4.

Supplementary Table S3. Measured polarities of DMF at various concentrations of Reichardt's dye.

Concentration (mg/mL)	$E_T(30)$ (kcal/mol)	λ_{Max} avg. (nm)	Wavelength 1 (nm)	Wavelength 2 (nm)	Wavelength 3 (nm)
0.05	43.38	659.1	659.6	659.4	652.2
0.025	43.30	660.3	659.2	661.4	660.4
0.012	43.33	659.8	660.0	660.0	659.4

Supplementary Table S4. Measured polarities (and reference values)[2] of solvents and binary solvent mixtures.

Solvent	$E_T(30)$ reference (kcal/mol)[2]	$E_T(30)$ measured (kcal/mol)	λ_{Max} avg. (nm)	Wavelength 1 (nm)	Wavelength 2 (nm)	Wavelength 3 (nm)
DMF	43.2	43.38	659.1	659.6	659.4	658.2
NBP	NA	41.98	681.0	680.4	681.0	681.6

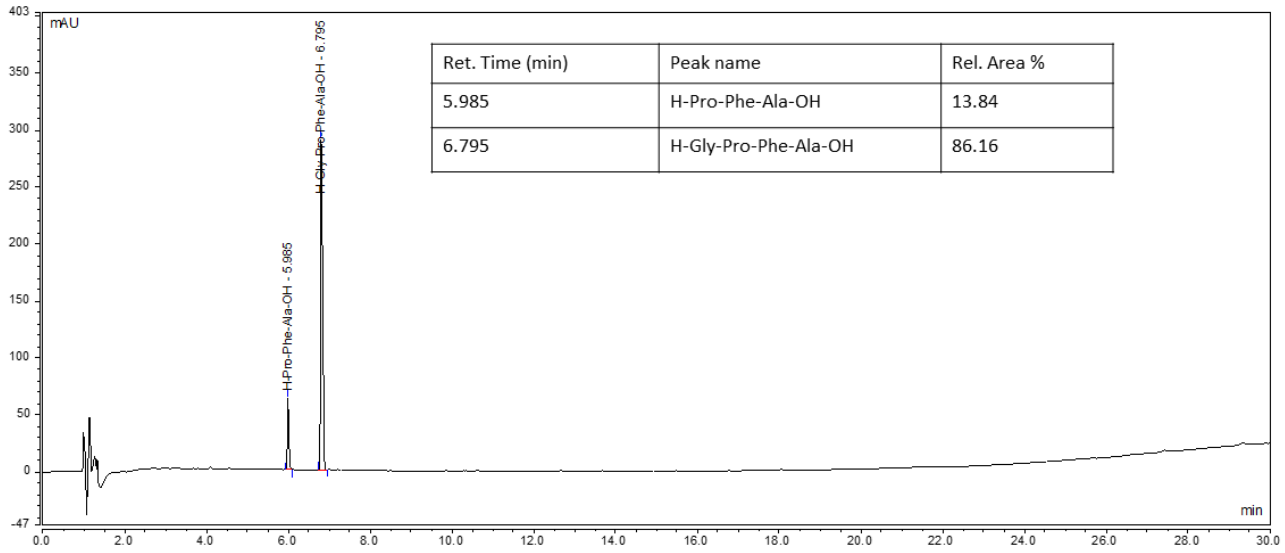
THP	36.2	36.23	789.1	789.4	789.6	788.4
2-Me-THF	36.5	36.34	786.7	787.0	786.2	787.0
DOL	43.1	39.53	723.3	723.0	723.4	723.4
DMSO	45.1	44.86	637.3	638.4	637.4	636.2
EtOAc	38.1	38.33	745.9	744.0	746.0	747.8
NFM/EtOAc (2:8)	NA	41.12	695.3	696.8	695.0	694.0
NFM/EtOAc (4:6)	NA	41.89	682.5	683.0	682.0	682.6
NFM/EtOAc (6:4)	NA	42.53	672.3	671.0	672.4	673.6
NFM/EtOAc (8:2)	NA	42.82	667.7	670.0	667.0	666.2
NBP/EtOAc (1:9)	NA	38.97	733.7	735.0	729.0	737.0
NBP/EtOAc (2:8)	NA	39.14	730.5	732.4	729.4	729.8
NBP/EtOAc (4:6)	NA	40.25	710.4	710.4	710.6	710.2
NBP/EtOAc (6:4)	NA	41.27	692.7	694.2	692.0	692.0
NBP/EtOAc (8:2)	NA	41.69	685.9	687.2	682.2	688.2
NBP/2-Me-THF (2:8)	NA	37.79	756.5	753.2	754.4	762.0
NBP/2-Me-THF (4:6)	NA	39.51	723.6	723.8	726.0	721.0
NBP/2-Me-THF (6:4)	NA	40.33	709.0	708.2	710.6	708.2
NBP/2-Me-THF (8:2)	NA	41.32	691.9	695.2	690.2	690.4
DMSO/EtOAc (1:9)	NA	41.29	692.4	692.4	691.4	693.4
DMSO/EtOAc (2:8)	NA	42.26	676.6	676.4	676.2	677.2
DMSO/EtOAc (3:7)	NA	43.01	664.8	663.4	665.8	665.2
DMSO/EtOAc (4:6)	NA	43.48	657.5	659.0	656.0	657.6
DMSO/EtOAc (5:5)	NA	43.94	650.7	650.2	651.0	651.0
DMSO/EtOAc (6:4)	NA	44.27	645.9	647.2	645.2	645.2
DMSO/EtOAc (7:3)	NA	44.55	641.8	641.8	641.8	641.8
DMSO/EtOAc (8:2)	NA	44.70	639.6	639.6	639.6	639.6
DMSO/EtOAc (9:1)	NA	44.90	636.7	636.2	635.6	638.4
DMSO/DOL (1:9)	NA	41.21	693.9	694.0	694.6	693.0
DMSO/DOL (2:8)	NA	42.22	677.3	677.4	676.6	677.8
DMSO/DOL (3:7)	NA	42.90	666.5	665.2	667.0	667.4
DMSO/DOL (4:6)	NA	43.38	659.1	657.8	658.4	661.0
DMSO/DOL (6:4)	NA	44.20	646.8	647.0	646.6	646.8
DMSO/DOL (8:2)	NA	44.67	640.0	640.2	639.6	640.2
DMSO/2-Me-THF (2:8)	NA	41.95	681.6	680.8	682.0	682.0
DMSO/2-Me-THF (3:7)	NA	42.84	667.3	666.0	668.0	668.0
DMSO/2-Me-THF (4:6)	NA	43.17	662.3	662.8	658.6	665.6
DMSO/2-Me-THF (6:4)	NA	43.87	651.7	652.4	651.0	651.8
DMSO/2-Me-THF (8:2)	NA	44.61	640.9	641.2	641.2	640.2
NBP/DOL (2:8)	NA	40.84	700.0	700.0	701.0	699.0
NBP/DOL (4:6)	NA	41.11	695.4	694.6	696.0	695.6
NBP/DOL (6:4)	NA	41.49	689.1	689.6	688.6	689.2

NBP/DOL (8:2)	NA	41.86	683	683.0	682.0	684.0
NFM/DOL (2:8)	NA	40.89	699.2	699.4	698.8	699.4
NFM/DOL (4:6)	NA	41.84	683.4	681.8	685.0	683.4
NFM/DOL (6:4)	NA	42.34	675.2	678.2	675.2	672.2
NFM/DOL (8:2)	NA	42.98	665.3	665.2	663.8	666.8
NFM/2-Me-THF (2:8)	NA	40.18	711.5	710.2	710.4	714.0
NFM/2-Me-THF (4:6)	NA	41.21	693.8	693.2	694.0	694.2
NFM/2-Me-THF (6:4)	NA	41.96	681.5	681.6	681.0	681.8
NFM/2-Me-THF (8:2)	NA	42.60	671.1	667.0	672.2	674.2

2.6 Peptide bond formation kinetics

Fmoc-Gly-OH (1.18 g, 3.97 mmol, 1.3 equiv.) and Oxyma (1.30 g, 9.15 mmol, 3 equiv.) were dissolved in the indicated solvent (50 mL). DIC (1.42 mL, 9.15 mmol, 3 equiv.) was added. The clear solution was then directly added to the reactor containing pre-swelled peptide resin H-Pro-Phe-Ala-2-CTR (5.0 g, loading = 0.61 mmol/g). The progress of the peptide bond formation was monitored by analysing a reaction suspension (1 mL) at set time points (t = 0, 1, 2, 3, 5, 8, 12, 16, 20, 30, 60, 90, and 120 min.). The withdrawn resin suspension aliquots were immediately transferred to a fritted syringe (2 mL) and filtered. The resin was further washed with DMF (2 × 2 mL) and subjected to Fmoc-removal using 20 % (v/v) piperidine in DMF (1 × 5 min and 1 × 10 min, respectively). After Fmoc-removal, the resin was filtered and washed with DMF and IPA (3 × 10 mL, alternating), then IPA (3 × 10 mL), and DIPE (1 × 10 mL) respectively. The dried resin was treated with 1 mL of HFIP/DCM (1:4) and agitated for 15 min at ambient temperature. The cleaved peptide solution was transferred to a vial and the solvent was evaporated under vacuum. The peptide residue was then dissolved in MeOH/H_2O (1:1) and subjected to HPLC analysis (Method A).

The relative percentage of tetrapeptide (H-Gly-Pro-Phe-Ala-OH) in comparison with tripeptide (H-Pro-Phe-Ala-OH) provided an insight into the degree of completion for the coupling reaction at a given time point. For example, the peptide retrieved after 5 min for the coupling experiment in 2-Me-THF was analysed by HPLC (Supplementary Figure S2) where the relative percentage of target peptide (H-Gly-Pro-Phe-Ala-OH) indicated the percentage completed coupling reaction after 5 min. Similarly, for all other solvents and solvent mixtures, the percentage completed coupling reaction was calculated at the given time points and the results are tabulated below (Supplementary Tables S5A-C).



Ret. Time (min)	Peak name	Rel. Area %
5.985	H-Pro-Phe-Ala-OH	13.84
6.795	H-Gly-Pro-Phe-Ala-OH	86.16

Supplementary Figure S2. Amino acid coupling kinetics study. HPLC analysis (λ = 200 nm) of retrieved peptide after 5 min coupling reaction in 2-Me-THF.

Supplementary Table S5A. Data for coupling kinetics experiments performed in various solvent systems depicting % completion of the coupling reaction over time.

	% Coupling						
Time (min)	**DMF**	**NBP**	**2-Me-THF**	**THP**	**DOL**	**DMSO**	**DMSO/EtOAc (2:8)**
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	7.8	5.3	42.1	62.7	75.3	0.9	2.9
2	11.6	6.9	54.9	78.0	97.3	1.9	4.1
3	15.4	11.8	66.7	88.4	99.3	3.1	5.1
5	20.7	14.5	86.2	97.0	99.5	4.6	7.6
8	30.0	21.0	97.8	98.6	99.7	6.7	11.7
12	41.3	26.6	99.5	99.0	99.7	9.3	16.4
16	48.6	36.1	99.7	99.3	99.8	11.6	20.2
20	56.4	38.9	99.6	99.3	99.8	14.1	24.4
30	73.9	59.2	NA	99.6	99.8	19.5	34.5
60	98.8	87.5	99.7	99.6	99.8	35.6	61.3
90	99.4	98.7	99.6	99.5	99.8	51.8	80.4
120	99.4	99.4	99.8	99.6	99.9	64.8	NA

Supplementary Table S5B. Data for coupling kinetics experiments performed in various solvent systems depicting % completion of the coupling reaction over time.

	% Coupling						
Time (min)	**NBP/ EtOAc (2:8)**	**NBP/DOL (2:8)**	**NBP/DOL (4:6)**	**NBP/ 2-Me-THF (2:8)**	**NBP/ 2-Me-THF (4:6)**	**NFM/ 2-Me-THF (2:8)**	**NFM/DOL (2:8)**
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	9.3	NA	NA	NA	NA	NA	NA
2	13.9	NA	NA	NA	NA	NA	NA
3	19.1	NA	NA	NA	NA	NA	NA
5	23.2	NA	NA	NA	NA	NA	NA
8	20.9	NA	NA	NA	NA	NA	NA
12	38.0	99.0	87.8	90.1	90.9	75.3	99.3
16	48.3	NA	NA	NA	NA	NA	NA
20	55.3	NA	NA	NA	NA	NA	NA
30	72.2	99.6	96.7	99.7	93.9	85.7	99.8
60	97.0	99.7	99.5	99.8	98.6	99.6	99.8
90	99.6	NA	NA	NA	NA	NA	NA
120	99.6	99.9	99.7	98.6	99.5	99.7	99.9

Supplementary Table S5C. Data for coupling kinetics experiments performed in various solvent systems depicting % completion of the coupling reaction over time.

	% Coupling					
Time (min)	**NFM/DOL (4:6)**	**NFM/EtOAc (2:8)**	**DMSO/DOL (2:8)**	**DMSO/DOL (4:6)**	**DMSO/2-Me-THF (2:8)**	**DMSO/ 2-Me-THF (4:6)**
0	0.0	0.0	0.00	0.0	0.0	0.0
1	20.0	NA	2.6	11.1	7.9	5.6
2	33.4	NA	NA	10.7	3.7	8.6
3	78.7	NA	3.9	16.3	18.5	8.7
5	91.5	NA	6.0	22.4	20.1	16.3
8	95.4	NA	27.3	31.1	30.9	13.5
12	97.2	77.5	66.4	29.3	33.6	20.4
16	98.7	NA	79.1	44.8	40.2	33.0
20	99.4	NA	85.3	42.2	48.9	31.9
30	99.5	93.7	91.4	61.3	68.4	34.5
60	99.5	99.6	99.0	95.9	93.4	54.1
90	99.8	NA	99.1	99.1	99.6	69.7
120	99.6	99.5	98.8	99.0	96.1	82.2

2.7 Fmoc-removal kinetics

A solution of 20% (v/v) piperidine in the designated solvent (10 mL) was added to a reactor (10 mL syringe) containing pre-swelled (2 × in the candidate solvent) Fmoc-Ala-Phe-Phe-Ala-Ramage resin (1.0 g, loading = 0.42 mmol/g). Aliquots of reaction mixture (100 µL) were withdrawn using a fritted syringe at a range of time points (t = 0, 1, 2, 3, 5, 7, 9, 12, 16, 20, 25, 30, 40, 50, 90 min), and diluted with MeOH to a final volume of 20 mL. The absorbance (A) of solution at λ = 289.8 nm was determined using a cuvette of 1 cm path length (L). The concentration (c) of dibenzofulvene-piperidine adduct was calculated using equation (3), and the extinction coefficient ε_{DMF} = 6089 L × mol^{-1} cm^{-1}.[3]

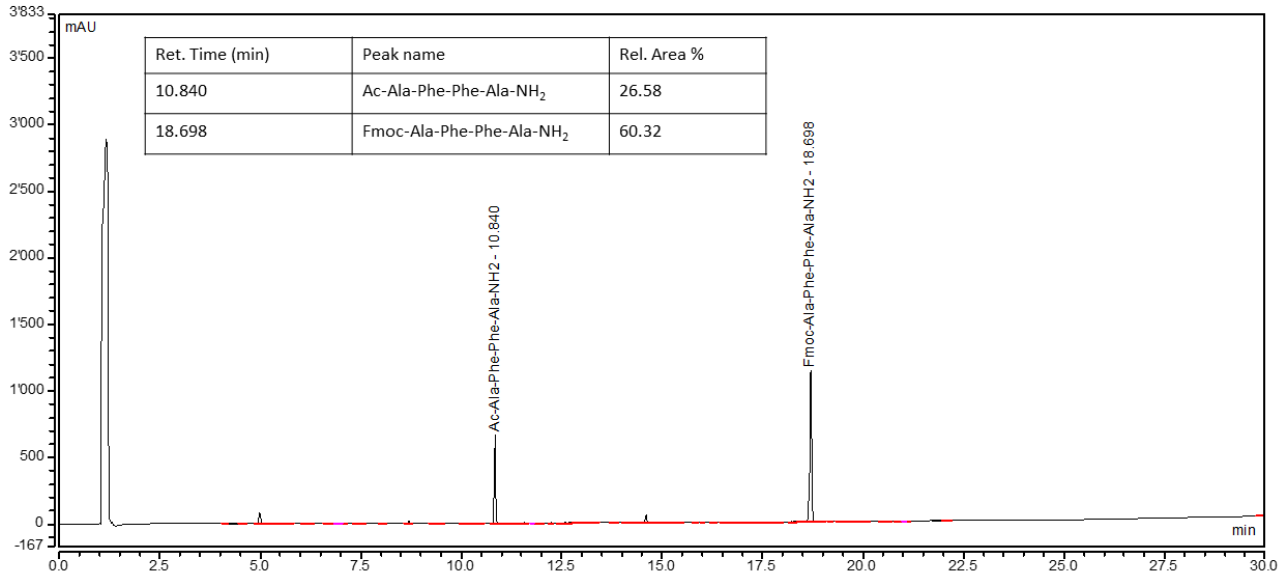
$$A_{289.8\ nm} = \varepsilon \times c \times L \quad (3)$$

After 90 min, the peptidyl resin was washed with DMF (10 mL, 4 × 1 min) and the free amino groups were acetylated using 10 mL of a DMF solution containing Ac_2O and pyridine. The peptidyl resin was washed with 10 mL solvent per washing step (in sequence 3 × 5 min alternating with DMF and IPA, 3 × 1 min with IPA, 3 × 1 min with MeOH and 3 × 1 min with DIPE) and dried. The peptide cleavage was performed by treating 100 mg of peptidyl resin with 1 mL of TFA/H_2O (95:5, v/v) mixture for 2 hrs at ambient temperature. 50 µL of cleavage solution was diluted with AcOH (1 mL) and directly subjected for HPLC analysis (Method A). The relative % of Ac-Ala-Phe-Phe-Ala-NH_2 (in comparison to Fmoc-Ala-Phe-Phe-Ala-NH_2 provided the endpoint % Fmoc-removal ($X_{endpoint}$) in the candidate solvent. As an example, the endpoint HPLC-analysis of Fmoc-removal (%) in NBP is provided in Supplementary Figure S3.

The Fmoc-removal (%) in the candidate solvent for given time point ($X_{solvent}$) is calculated using the following formula (4):

$$X_{solvent} = X_{endpoint} \times C_{solvent} \ / \ C_{endpoint} \quad (4)$$

$C_{solvent}$ is the concentration of DBF-piperidine adduct in indicated solvent at given time point, and $C_{endpoint}$ is the concentration of DBF-piperidine adduct after 90 minutes. The results from Fmoc-removal kinetics experiments are summarized in Table S6.



Supplementary Figure S3. HPLC analysis (λ = 220 nm) of cleaved crude peptide to determine endpoint Fmoc-removal (%) after 90 min for experiment performed in NBP with relative area (%) for Ac-Ala-Phe-Phe-Ala-NH_2 and Fmoc-Ala-Phe-Phe-Ala-NH_2.

It is assumed that Fmoc-Ala-Phe-Phe-Ala-NH_2 (B) absorbs twice as much as Ac-Ala-Phe-Phe-Ala-NH_2 (A) at λ = 220 nm. The endpoint % Fmoc-removal ($X_{endpoint}$) was calculated using the formula (5):

$$X_{endpoint}\ (\%) = A / [(B/2) + A] \times 100 \qquad (5)$$

Accordingly, the endpoint Fmoc-removal (%) in NBP was calculated as 46.8%.

The determined % Fmoc-removal for all evaluated solvents systems at all time points are listed below in Supplementary Tables S6.

Supplementary Table S6A. Data for Fmoc-removal kinetics experiments determined in various solvent systems as described above.

	% Fmoc-removal						
Time (min)	**DMF**	**NBP**	**2-Me-THF**	**THP**	**DOL**	**DMSO**	**DMSO/ 2-Me-THF (4:6)**
0	0.0	0.0	0.0	0.0	0.0	0.0	0.3
1	99.3	11.9	0.2	NA	NA	72.2	74.2
2	100.0	5.9	0.5	NA	NA	NA	92.8
3	96.3	18.0	0.7	NA	NA	94.3	91.6
5	94.5	18.0	1.0	NA	38.2	98.8	97.6
7	95.2	18.4	1.3	NA	NA	96.3	92.5
9	95.6	19.0	1.6	NA	NA	100.0	100.0
12	94.7	19.0	2.0	NA	61.0	99.3	93.0
16	96.0	20.	2.6	NA	NA	96.3	91.9
20	95.2	21.7	3.3	NA	74.4	98.6	91.0
25	94.9	22.7	4.2	NA	NA	99.5	88.7
30	95.8	24.9	5.5	NA	NA	100.0	96.2
40	95.9	26.7	7.3	NA	NA	99.5	90.8
50	97.8	30.8	9.8	NA	NA	99.8	89.4
90	97.8	46.8	19.2	23.4	94.6	99.7	89.2

Supplementary Table S6B. Data for Fmoc-removal kinetics experiments determined in various solvent systems as described above.

	% Fmoc-removal						
Time (min)	**DMSO/DOL (4:6)**	**DMSO/ EtOAc (2:8)**	**NBP/ EtOAc (2:8)**	**NBP/ EtOAc (4:6)**	**NBP/ DOL (2:8)**	**NBP/DOL (4:6)**	**NBP/2-Me-THF (2:8)**
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	72.4	59.5	14.5	1.3	7.5	11.7	1.6
2	87.2	71.7	21.2	2.2	19.6	39.9	1.7
3	87.8	81.4	27.4	3.1	29.4	70.3	1.7
5	94.3	84.2	33.9	4.7	50.2	87.0	2.2
7	100.0	89.9	40.9	6.1	64.1	93.2	3.1
9	95.4	92.9	46.3	7.8	71.9	94.5	3.6
12	89.5	94.7	54.1	9.0	79.9	98.1	2.2
16	87.6	97.8	57.4	11.9	89.0	98.8	5.2
20	88.8	97.8	70.6	15.4	99.7	98.2	5.5
25	96.7	97.7	79.7	19.1	NA	99.0	3.3
30	96.9	99.9	81.1	21.8	95.8	99.2	3.6
40	NA	99.0	86.1	27.0	95.4	100.0	4.3
50	97.8	98.3	97.2	58.6	92.	NA	5.2
90	98.7	97.6	98.9	69.9	98.0	96.9	9.2

Supplementary Table S6C. Data for Fmoc-removal kinetics experiments determined in various solvent systems as described above.

	% Fmoc-removal					
Time (min)	**NFM/2-Me-THF (2:8)**	**NFM/DOL (2:8)**	**NFM/DOL (4:6)**	**NFM/EtOAc (2:8)**	**NBP/2-Me-THF (4:6)**	**DMSO/EtOAc (6:4)**
0	0.0	0.0	0.0	0.0	0.0	0.0
1	NA	23.3	49.0	1.9	1.5	88.3
2	NA	45.6	66.0	12.3	2.7	96.8
3	NA	58.8	83.3	19.7	3.1	89.3
5	NA	73.8	92.2	44.6	4.1	95.6
7	NA	86.3	95.1	59.9	4.6	99.0
9	2.4	91.6	96.5	64.9	5.2	98.8
12	NA	NA	97.7	71.8	5.6	98.4
16	NA	96.5	98.1	76.2	6.4	94.5
20	3.1	97.4	99.2	80.1	6.8	98.9
25	NA	96.4	100.0	83.9	7.3	100.0
30	NA	99.8	99.4	86.8	8.2	97.9
40	NA	98.5	95.8	90.1	9.0	97.4
50	NA	96.0	NA	90.6	9.8	97.9
90	12.1	97.4	91.3	94.7	13.8	97.9

2.8 Solid-phase peptide synthesis (SPPS)

2.8.1 Manual SPPS of Ac-LVAYAG-NH_2 on 1.5 mmol scale

Fmoc-Rink-Amide-resin (2.00 g, 1.48 mmol, loading = 0.74 mmol/g) was swelled in the indicated solvent (22 mL) for 1 h in a 50 mL modified round bottom flask with a frit. A solution of 20% (v/v) piperidine in the indicated solvent (22 mL) was added to the resin, which was agitated for 2 × 15 min. The solvent was then drained, and the resin washed with solvent (2 × 22 mL), IPA (22 mL), and solvent again (22 mL). DIC (4 equiv.), Oxyma Pure (3 equiv.), and Fmoc-AA-OH (3 equiv.) were dissolved in the indicated solvent (18 mL) and transferred to the resin, which was agitated for 30 min. A Kaiser test was carried out according to the manufacturer's instructions. If the Kaiser test was positive, the reaction was continued for 30 min and subsequently capped with a solution of solvent/Ac_2O/DIPEA (120:4.73:10.45, 20 mL), for 2 × 5 min. The resin was then washed with IPA (22 mL) and solvent (22 mL). Approximately 10 mg peptide resin was cleaved with 400 µL TFA/TIPS/H_2O (95/2.5/2.5, v/v/v) for 1 h. Afterwards MeCN (1.2 mL) and 7.0 M NH_4OAc (0.68 mL) was added. The solution was filtered and directly injected on the HPLC (Method B) and the purity was assessed at 214 nm (Supplementary Table S7).

Supplementary Table S7. Peptide purities for the SPPS of Ac-LVAYAG-NH_2

Solvent	Purity[a]
DMF	98%
DOL	67%
DMSO/EtOAc (1:9)	71%
DMSO/EtOAc (2:8)	87%
DMSO/EtOAc (3:7)	90%
DMSO/EtOAc (4:6)	97%
DMSO/EtOAc (5:5)	99%
DMSO/DOL (2:8)	70%
DMSO/DOL (3:7)	95%
DMSO/DOL (4:6)	99%
DMSO/2-Me-THF (3:7)	98%
DMSO/2-Me-THF (4:6)	97%
NBP/DOL (2:8)	82%
NBP/DOL (4:6)	79%
NBP/2-Me-THF (4:6)	78%
NFM/DOL (2:8)	83%
NFM/2-Me-THF (2:8)	58%

[a]Purity determined by HPLC analysis at 214 nm

2.8.2 Manual SPPS of Aib-enkephalin (Ac-Y-Aib-Aib-FL-OH) on 0.42 mmol scale

Fmoc-Leu-Wang-resin (0.52 g, 0.42 mmol, loading = 0.81 mmol/g) was swelled in the indicated solvent (5 mL) for 1 h in a 10 mL fritted syringe. A solution of 20% (v/v) piperidine in the indicated solvent (5 mL) was added to the resin, which was agitated for 2 × 15 min. The solvent was then drained, and the resin washed with solvent (2 × 5 mL), IPA (5 mL), and solvent again (5 mL). DIC (4 equiv.), Oxyma Pure (3 equiv.), and Fmoc-AA-OH (3 equiv.) were dissolved in the indicated solvent (5 mL) and transferred to the resin, which was agitated for 60 min. The resin was then washed with IPA (5 mL) and solvent (5 mL). After final deprotection the peptide was capped with a solution of DMF/Ac_2O/DIPEA (120:4.73:10.45, v/v/v; 5 mL) for 2 × 5 min. Approximately 10 mg peptide resin was cleaved with 400 µL TFA/TIPS/H_2O (95/2.5/2.5, v/v/v) for 1 h. Afterwards MeCN (1.2 mL) and 7.0 M NH_4OAc (0.68 mL) was added. The solution was filtered and directly injected on the LC-MS (Method C) and the purity was assessed at 214 nm (Supplementary Table S8).

Supplementary Table S8. Peptide purities for the SPPS of Aib-enkephalin.

Solvent	Purity[a]
DMF	48%
DMSO/EtOAc (3:7)	41%
DMSO/EtOAc (4:6)	46%
DMSO/EtOAc (5:5)	35%
DMSO/DOL (3:7)	43%
DMSO/DOL (4:6)	44%
DMSO/2-Me-THF (3:7)	53%
DMSO/2-Me-THF (4:6)	41%

[a]Purity determined by HPLC analysis at 214 nm

2.8.3 Automated SPPS of the Aib-enkephalin, Jung-Redemann, Thymosin α1, and Dasiglucagon amide peptides on 0.15 mmol scale

- Aib-enkephalin (Ac-Y-Aib-Aib-FL-NH_2)
- Jung-Redemann (H-WFTTLISTIM-NH_2)
- Thymosin α1 (Ac-SDAAVDTSSEITTKDLKEKKEVVEEAEN-NH_2)
- Dasiglucagon amide (H-HSQGTFTSDYSKYLD-Aib-ARAEEFVKWLEST-NH_2)

Each peptide sequence was synthesized in duplicate or triplicate on an automated peptide synthesiser (Symphony X, Gyros Protein Technologies) using a Rink Amide AM polystyrene resin (loading = 0.74 mmol/g, mesh 100-200) on 0.15 mmol scale. Fmoc-protected amino acids were dissolved in a 0.33 M solution of Oxyma Pure in the indicated solvent system, to a final amino acid concentration of 0.33 M. Fmoc-removal was carried out using a solution of 20% (v/v) piperidine in the indicated solvent system (4 mL) for 2 × 15 min. The drained resin was then sequentially washed with DCM (2 mL to wash down the resin from the reactor side), IPA (2 mL) and solvent (9 × 4 mL). Peptide bond formation was carried out in cycles using amino acid derivatives, Oxyma Pure and DIC (4 equiv. each) for 80 min, with a second addition of DIC (4 equiv.) after 20 min. In the synthesis of Dasiglucagon amide, Aib was double coupled. After coupling, the resin was washed with solvent (3 × 4 mL), DCM (2 mL), IPA (2 mL) and allowed to re-swell in the solvent (4 mL) for 5 min before proceeding to the next coupling cycle. The last step of the synthesis was a final Fmoc-removal. After each synthesis, the dry resin was weighed and either capped with a solution of DMF/Ac_2O/DIPEA (120:4.73:10.45, 4 mL) (2 × 10 min for Aib-enkephalin and Thymosin α1) before test cleavage of the resin, or directly test cleaved (Jung-Redemann and Dasiglucagon amide). Approximately 10 mg peptide resin was cleaved with 400 µL TFA/TIPS/H_2O (95/2.5/2.5, v/v/v) for 1 h. Afterwards MeCN (1.2 mL) and 7.0 M NH_4OAc (0.68 mL) was added. Aib-enkephalin and Jung-Redemann were analysed by UPLC (Method E). Thymosin α1 was analysed by LC-MS (Method C). Dasiglucagon amide was analysed by LC-MS (Method D).

2.8.4 Automated SPPS of Dasiglucagon amide on 5 mmol scale

- Dasiglucagon amide (H-HSQGTFTSDYSKYLD-Aib-ARAEEFVKWLEST-NH_2)

Each peptide sequence was synthesized on an automated peptide synthesiser (CSBIO CS536XT) using a Rink Amide AM polystyrene resin (loading = 0.74 mmol/g, mesh 100-200), on a 5.0 mmol scale. Fmoc-

protected amino acids were dissolved in a 0.30 M solution of Oxyma Pure in the indicated solvent or solvent mixture, to a final amino acid concentration of 0.30 M.

Fmoc-removal was carried out using a solution of 20% (v/v) piperidine in the indicated solvent system (100 mL) for 2 × 15 min. The drained resin was then sequentially washed with solvent (3 × 100 mL), IPA (100 mL) and solvent (100 mL). Peptide bond formation was carried out in cycles using amino acid (3 equiv.), Oxyma Pure (3 equiv.) and DIC (3 equiv.) for 80 min, with a second addition of DIC (1 equiv.) after 20 min. The coupling of the Aib residue was performed twice. After coupling, the resin was washed with solvent (100 mL), IPA (100 mL) and with solvent (2 × 100 mL) to allow the resin to re-swell before proceeding to the next coupling cycle. The last step of the synthesis was a final Fmoc-removal. Approximately 10 mg peptide resin was cleaved with 400 µL TFA/TIPS/H_2O (95/2.5/2.5, v/v/v) for 1 h. Afterwards MeCN (1.2 mL) and 7.0 M NH_4OAc (0.68 mL) was added. The solution was filtered and analysed by LC-MS (Method F).

2.8.5 Optimised automated SPPS of Dasiglucagon amide on 0.45 mmol scale

- Dasiglucagon amide (H-HSQGTFTSDYSKYLD-Aib-ARAEEFVKWLEST-NH_2)

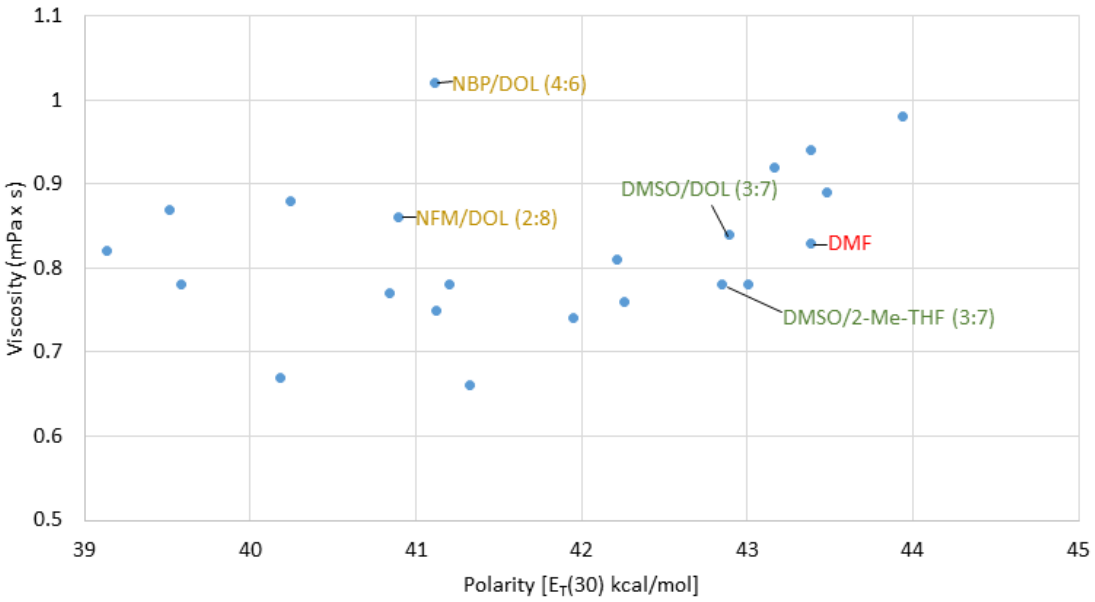
The synthesis was performed in duplicate on an automated peptide synthesiser (Symphony X, Gyros Protein Technologies) using a Rink Amide AM polystyrene resin (loading = 0.74 mmol/g, mesh 100-200) on 0.45 mmol scale. Fmoc-protected amino acids were dissolved in a 0.27 M solution of Oxyma Pure in the indicated solvent system, to a final amino acid concentration of 0.27 M. Fmoc-removal was carried out using a solution of 20% (v/v) piperidine in the indicated solvent or mixture (12 mL) for 2 × 15 min. The drained resin was then sequentially washed with DCM (6 mL to wash down the resin from the reactor side), IPA (6 mL) and solvent (9 × 12 mL). Peptide bond formation was carried out in cycles using amino acid, Oxyma Pure and DIC (3 equiv. each) for 80 min, with a second addition of DIC (1 equiv.) after 20 min. After coupling, the resin was washed with solvent (3 × 12 mL), DCM (6 mL), IPA (6 mL) and allowed to re-swell in the solvent (12 mL) for 5 min before proceeding to the next coupling cycle. The last step of the synthesis was a final Fmoc-removal. After each synthesis, the dry resin was weighed before test cleavage of the resin. For the optimised synthesis, coupling of His-1, Asp-15, Arg-18 and Glu-20 were repeated with a fresh set of AAD and coupling reagents. Aib was double coupled in all cases. Approximately 10 mg peptide resin was cleaved with 400 µL TFA/TIPS/H_2O (95/2.5/2.5, v/v/v) for 1 h. Afterwards MeCN (1.2 mL) and 7.0 M NH_4OAc (0.68 mL) was added. The solution was filtered and analysed by LC-MS (Method D).

2.8.6 Automated SPPS of Bivalirudin on 7.5 mmol scale

- Bivalirudin (H-fPRPGGGGNGDFEEIPEEYL-OH)

The synthesis of Bivalirudin was carried out on 7.5 mmol scale using an automated synthesizer (Sonata, Gyros Protein Technologies). H-Leu-2-CTR resin (9.26 g, loading = 0.81 mmol/g) was pre-swelled in the indicated binary solvent mixture. Fmoc-protected amino acid derivatives (2 equiv.) were pre-activated for 5 min using DIC/Oxyma (2 equiv. each) and the resin was treated with the reagent mixture for 90 min, except for tyrosine which was coupled using a TBTU/DIPEA (2 and 4 equiv. respectively) activation protocol. At the end of each coupling reaction, the resin was drained and capped using a solution (150 mL) of Ac_2O and collidine in the candidate solvent. The resin was then filtered and washed with the indicated solvent. Fmoc-removal was carried out using a solution of 20% (v/v) piperidine in the corresponding solvent (10 + 5 min). After the final cycle, the resin was washed with the indicated solvent (150 mL) and IPA (150 mL) and dried under vacuum before the resin weight gain was calculated. The dry resin (100 mg) was treated with TFA/H_2O (95:5, v/v; 1 mL) for 2 h at ambient temperature. The cleavage solution was filtered, and the crude peptide was precipitated as a white to off-white powder using cold DIPE. The obtained crude peptide was subjected to HPLC and LC-MS analysis (Method A).

The binary solvent mixtures for SPPS of Bivalirudin were selected by inspecting the space occupied by the solvent mixtures with respect to DMF on the polarity-viscosity plot as shown in Supplementary Figure S4.



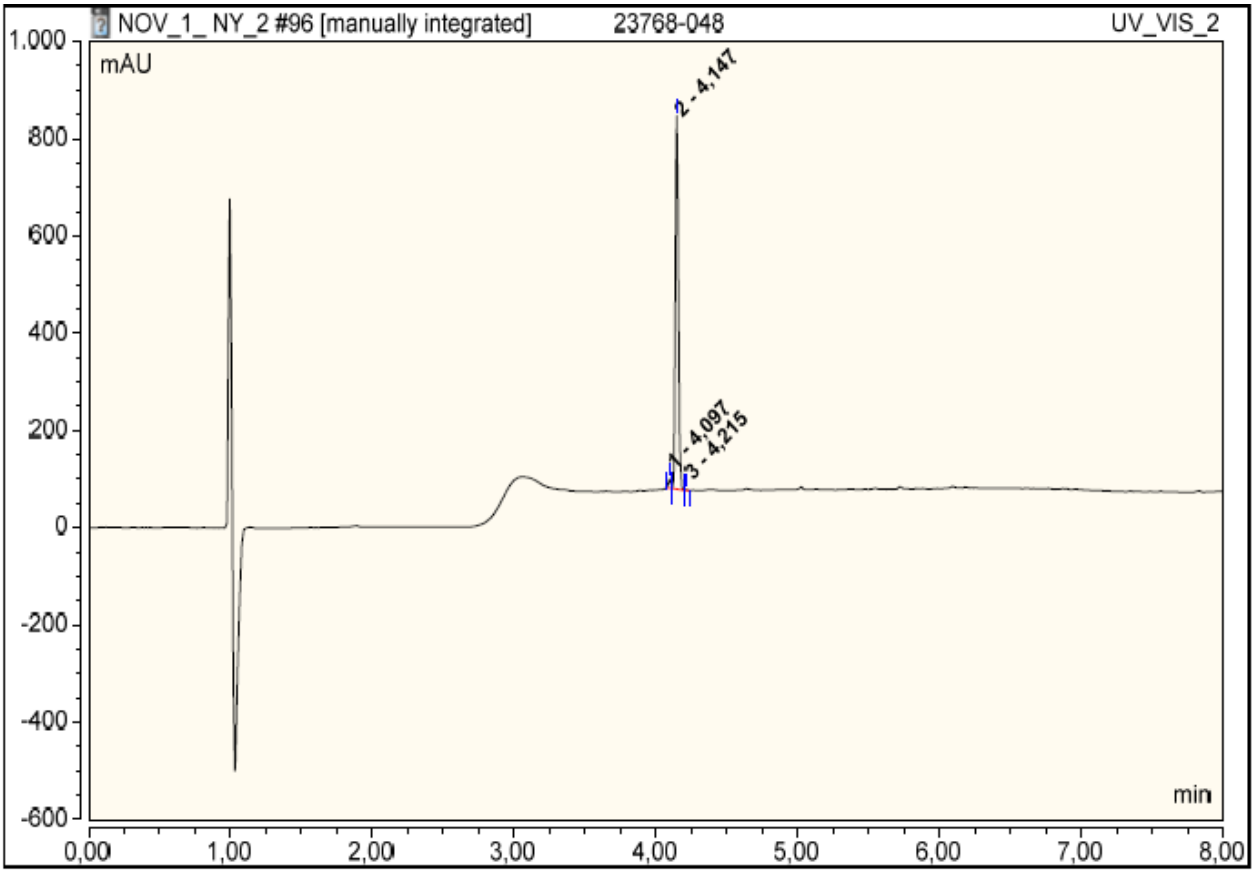
Supplementary Figure S4. The polarity-viscosity plot for a selection of binary solvent for SPPS of Bivalirudin (7.5 mmol).

3. Chromatograms

3.1 Manual SPPS of Ac-LVAYAG-NH_2 on 1.5 mmol scale

Manual SPPS of Ac-LVAYAG-NH_2 in DMF

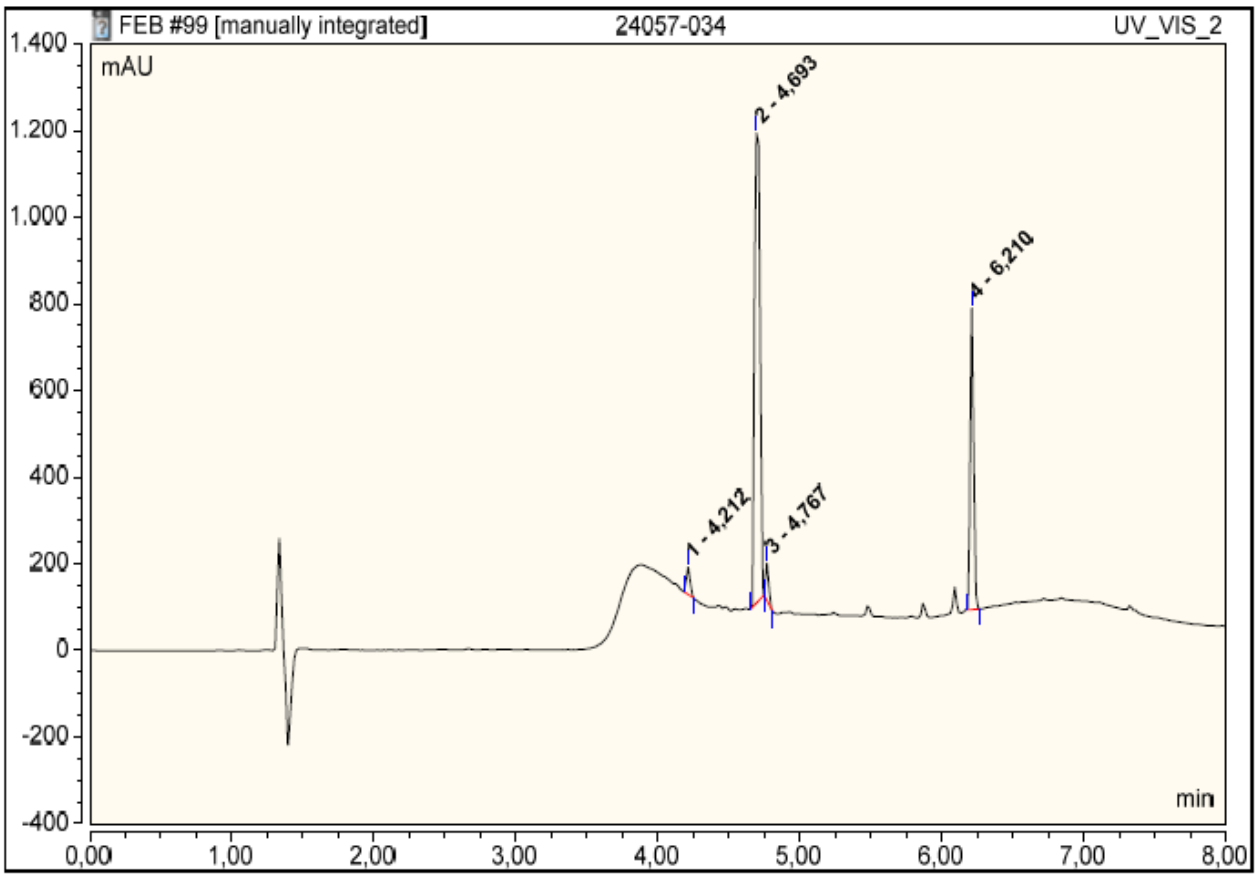
1.5 mmol scale, HPLC method B



Peak no.	R_T (min)	Compound	Relative area
1	4.097	Ac-Leu-Val-Tyr-Ala-Gly-NH_2	1.85%
2	4.147	Target molecule: Ac-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	97.95%
3	4.215	Ac-Leu-Val-Ala-Tyr-Ala-NH_2	0.2%

Manual SPPS of Ac-LVAYAG-NH_2 in DOL

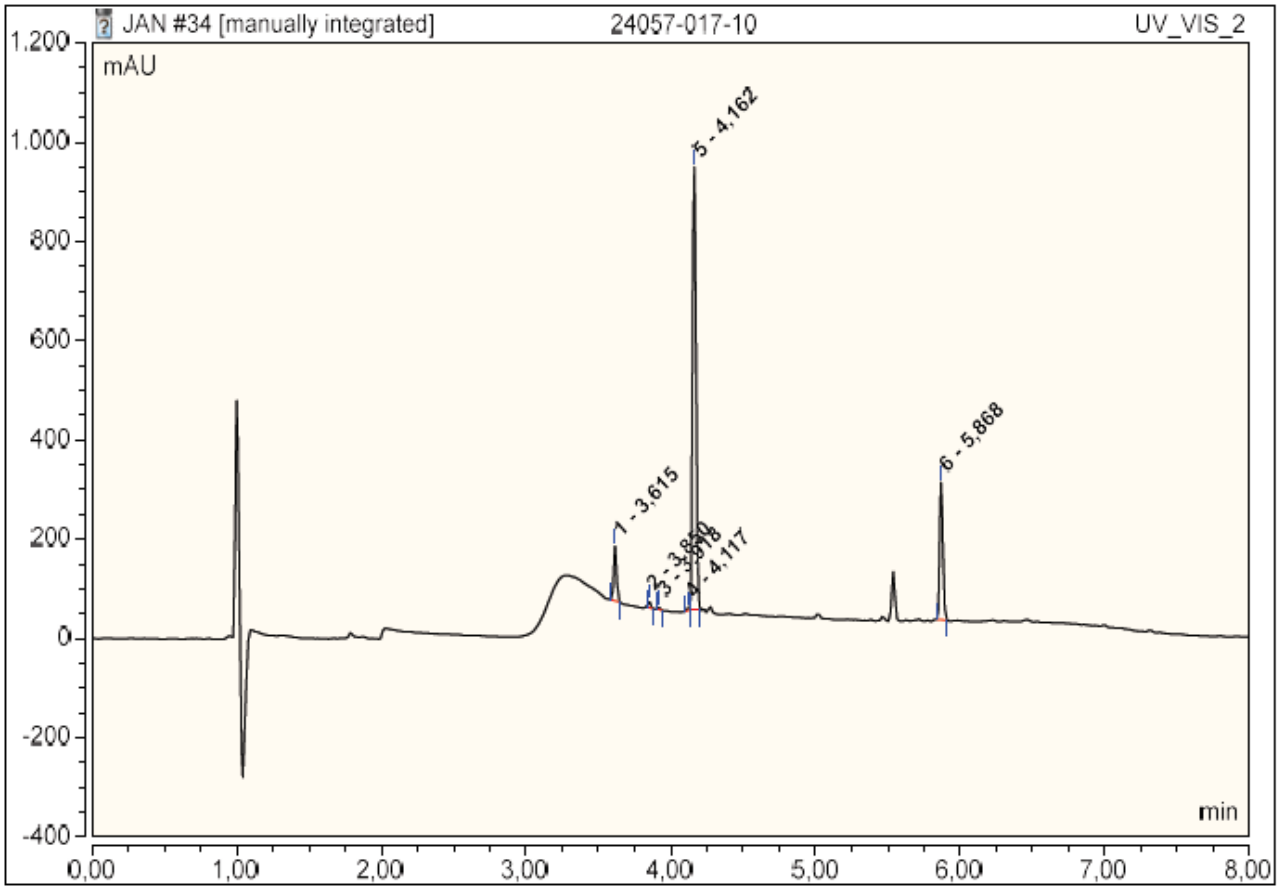
1.5 mmol scale, HPLC method B



Peak no.	R_T (min)	Compound	Relative area
1	4.212	Ac-Val-Ala-Tyr-Ala-Gly-NH_2	2.48%
2	4.693	Target molecule: Ac-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	67.16%
3	4.767	Ac-Leu-Val-Ala-Tyr-Ala-NH_2	3.01%
4	6.210	Fmoc-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	27.36%

Manual SPPS of Ac-LVAYAG-NH_2 in DMSO/EtOAc (1:9)

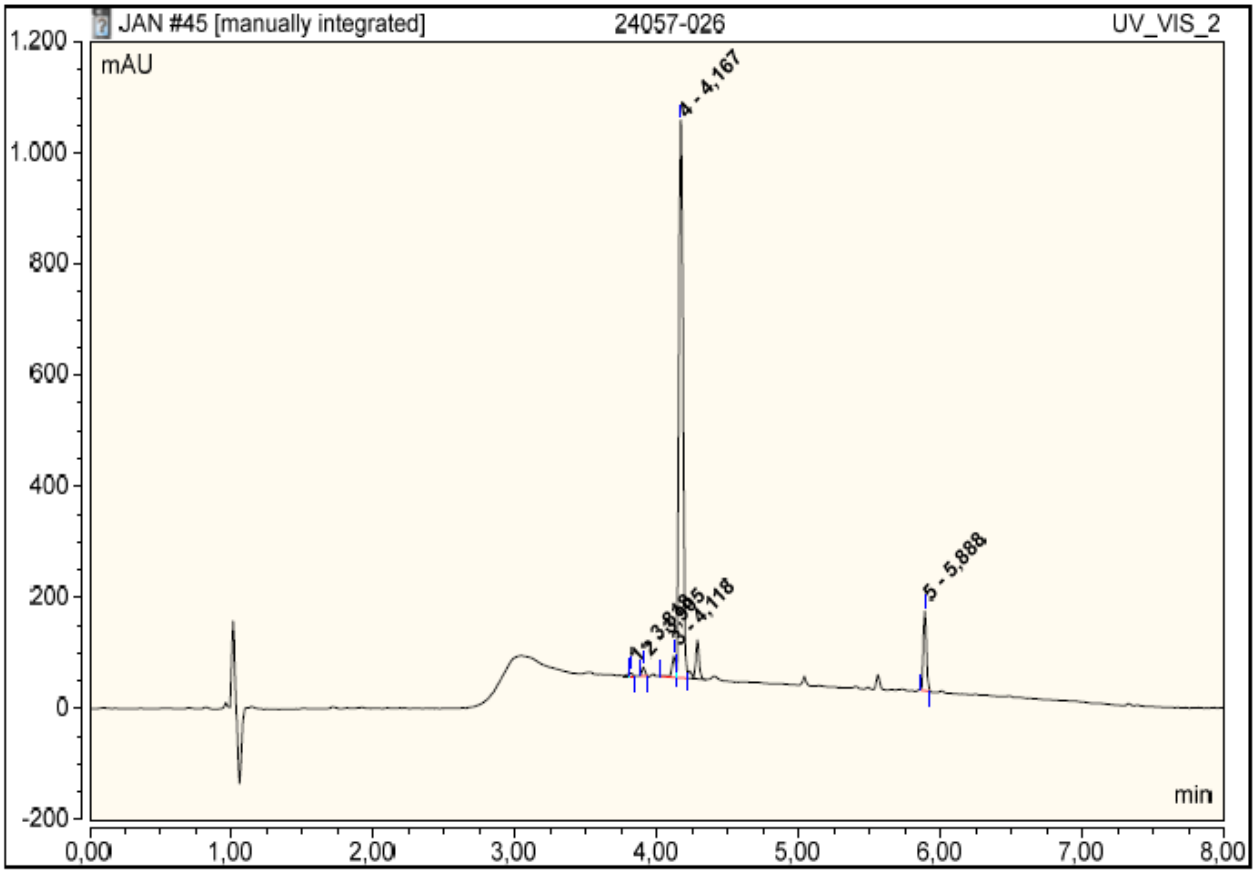
1.5 mmol scale, HPLC method B



Peak no.	R_T (min)	Compound	Relative area
1	3.615	Ac-Val-Ala-Tyr-Ala-Gly-NH_2	7.40%
2	3.850	Ac-Leu-Val-Ala-Ala-Gly-NH_2	0.74%
3	3.918	Ac-Leu-Ala-Tyr-Ala-Gly-NH_2	0.28%
4	4.117	Ac-Leu-Val-Tyr-Ala-Gly-NH_2	0.27%
5	4.162	Target molecule: Ac-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	71.37%
6	5.868	Fmoc-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	19.95%

Manual SPPS of Ac-LVAYAG-NH_2 in DMSO/EtOAc (2:8)

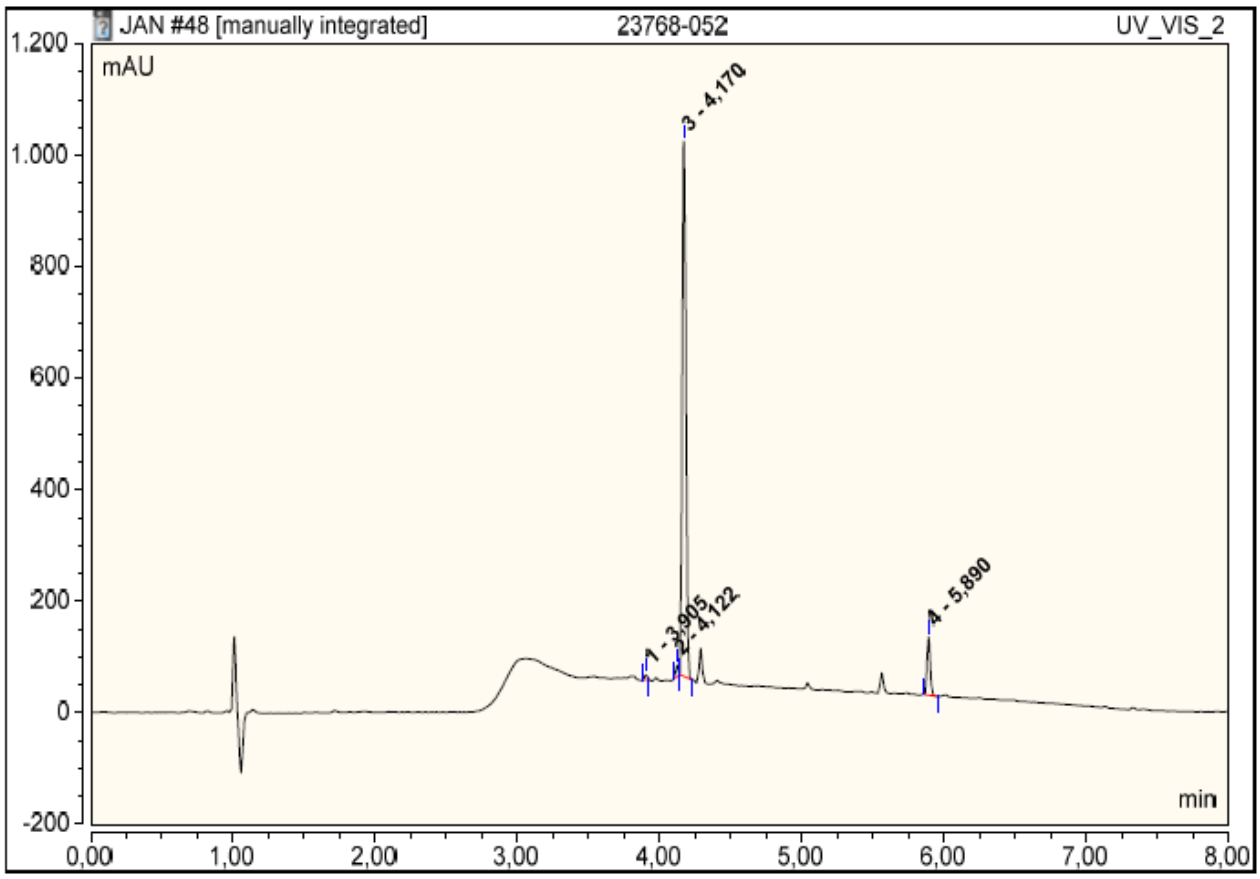
1.5 mmol scale, HPLC method B



Peak no.	R_T (min)	Compound	Relative area
1	3.818	Ac-Leu-Val-Ala-Ala-Gly-NH_2	0.40%
2	3.905	Ac-Leu-Ala-Tyr-Ala-Gly-NH_2	0.94%
3	4.118	Ac-Leu-Val-Ala-Tyr-Ala-Gly-Gly-NH_2	2.58%
4	4.167	Target molecule: Ac-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	86.77%
5	5.888	Fmoc-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	9.32%

Manual SPPS of Ac-LVAYAG-NH_2 in DMSO/EtOAc (3:7)

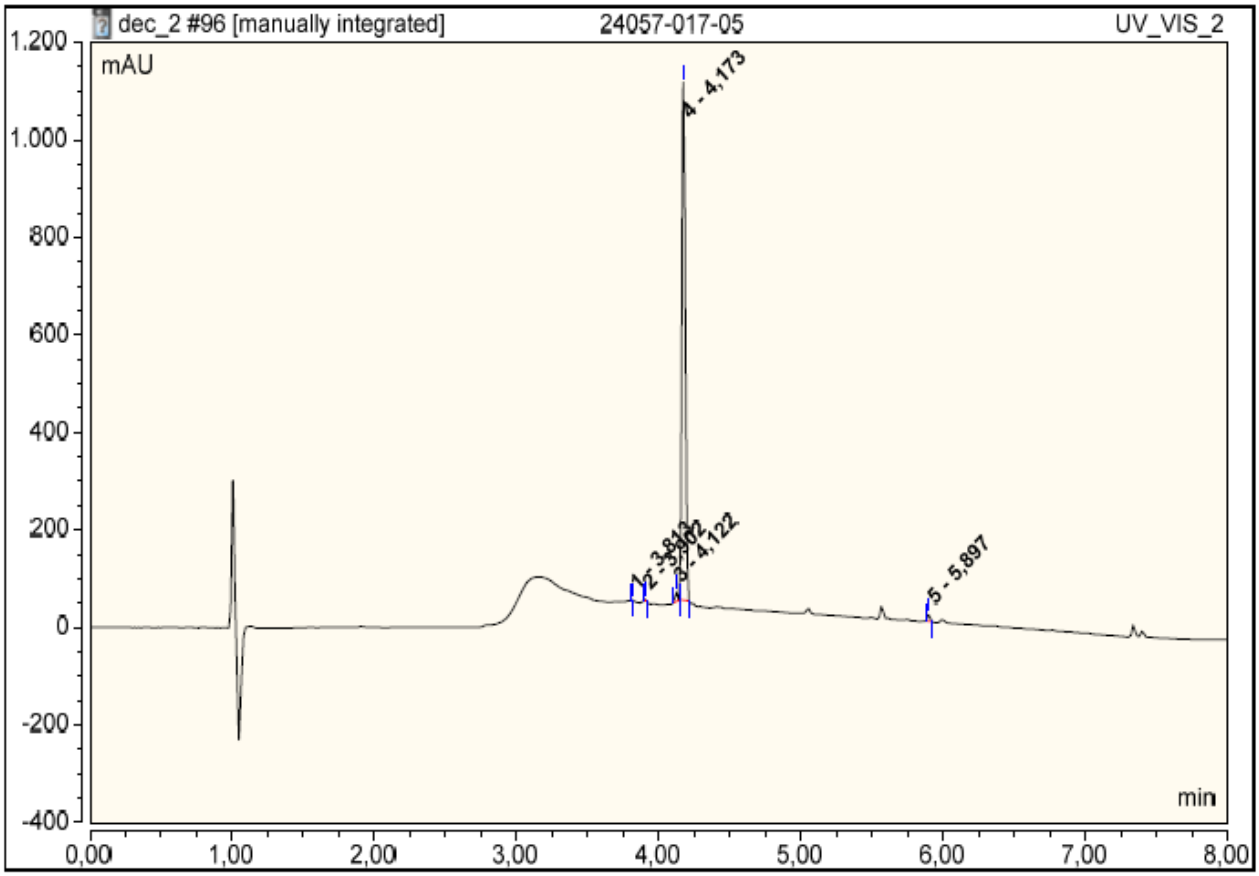
1.5 mmol scale, HPLC method B



Peak no.	R_T (min)	Compound	Relative area
1	3.905	Ac-Leu-Ala-Tyr-Ala-Gly-NH_2	0.70%
2	4.122	Ac-Leu-Val-Ala-Tyr-Ala-Gly-Gly-NH_2	1.27%
3	4.170	Target molecule: Ac-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	89.84%
4	5.890	Fmoc-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	8.19%

Manual SPPS of Ac-LVAYAG-NH_2 in DMSO/EtOAc (4:6)

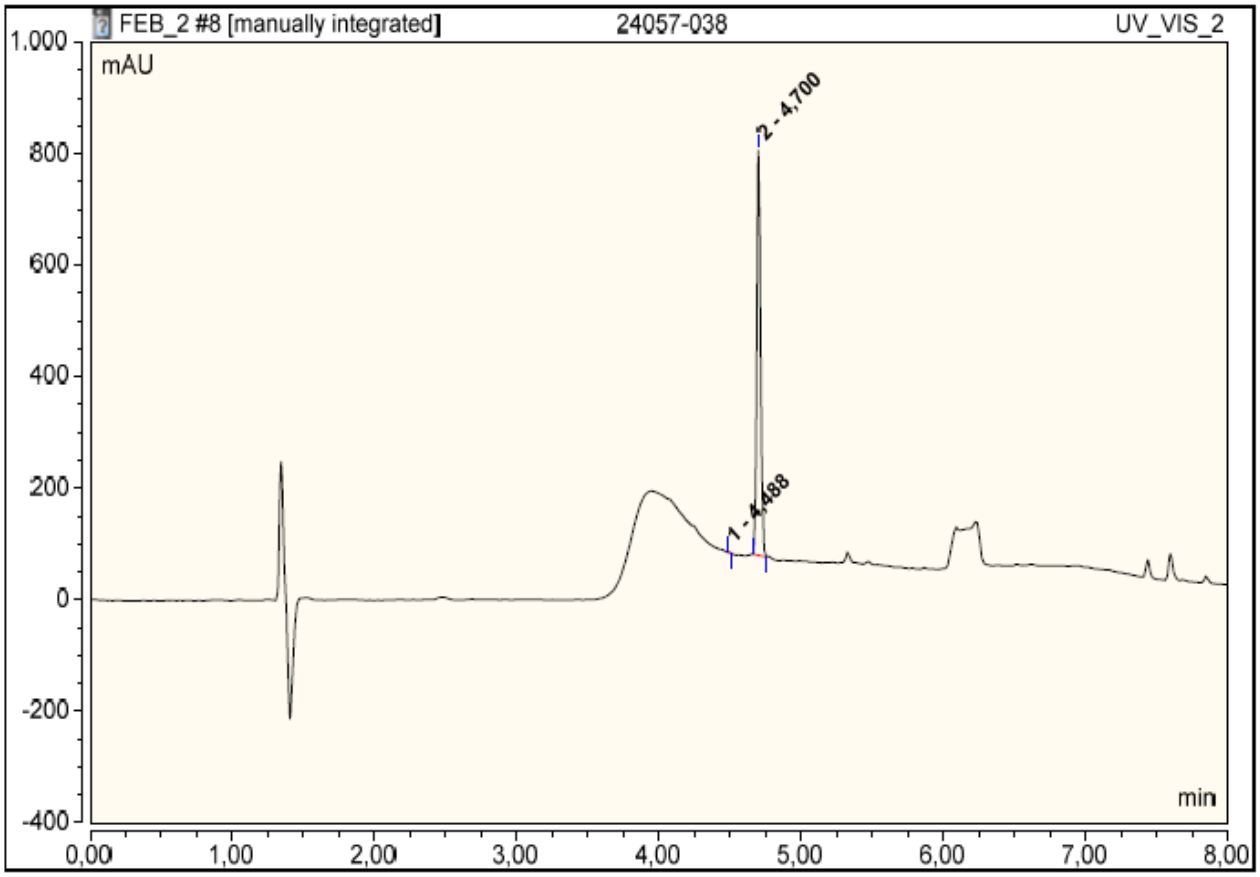
1.5 mmol scale, HPLC method B



Peak no.	R_T (min)	Compound	Relative area
1	3.813	Ac-Leu-Val-Ala-Ala-Gly-NH_2	0.03%
2	3.902	Ac-Leu-Ala-Tyr-Ala-Gly-NH_2	0.15%
3	4.122	Ac-Leu-Val-Tyr-Ala-Gly-NH_2	1.37%
4	4.173	Target molecule: Ac-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	97.47%
5	5.897	Fmoc-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	0.98%

Manual SPPS of Ac-LVAYAG-NH_2 in DMSO/EtOAc (5:5)

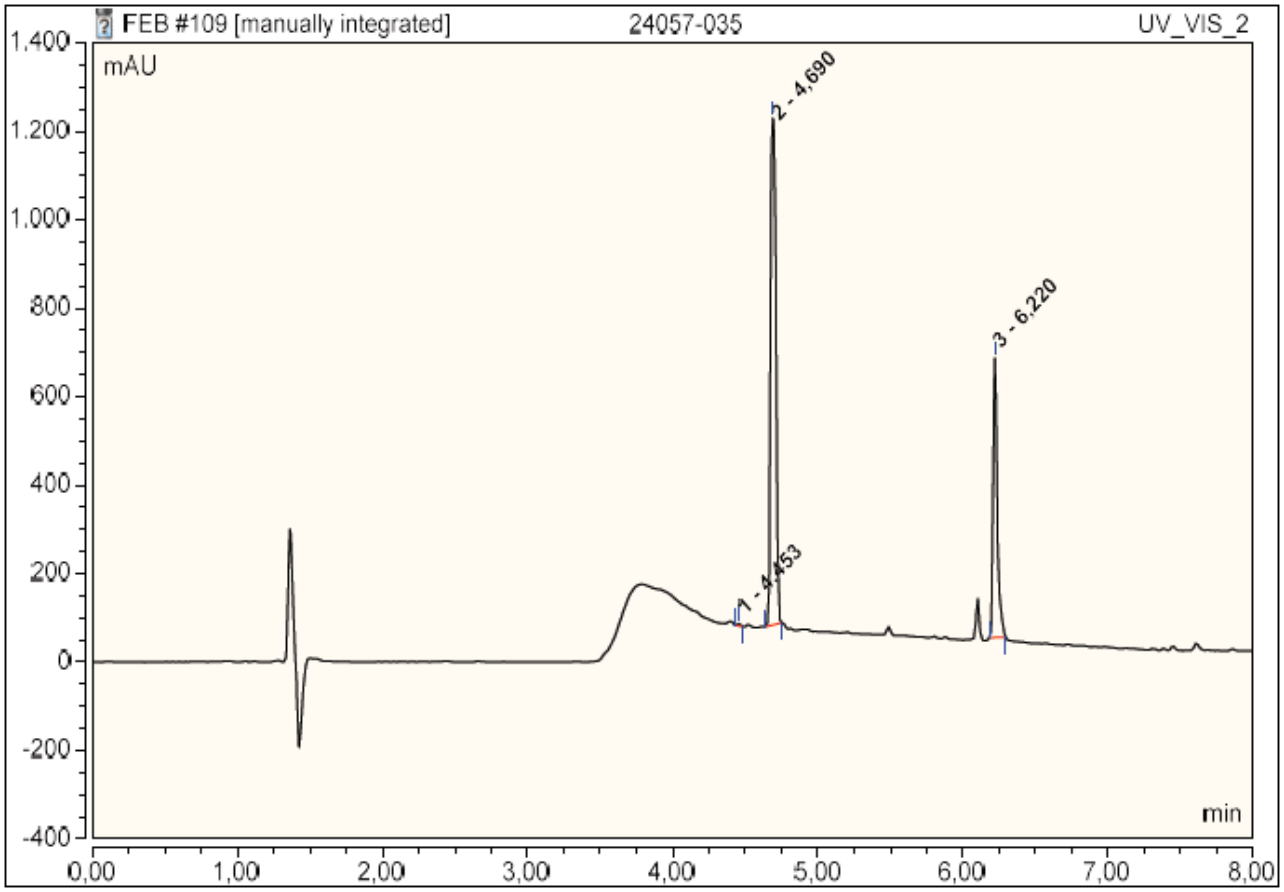
1.5 mmol scale, HPLC method B



Peak no.	R_T (min)	Compound	Relative area
1	4.488	Ac-Leu-Val-Ala-Ala-Gly-NH_2	0.12%
2	4.700	Target molecule: Ac-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	99.88%

Manual SPPS of Ac-LVAYAG-NH_2 in DMSO/DOL (2:8)

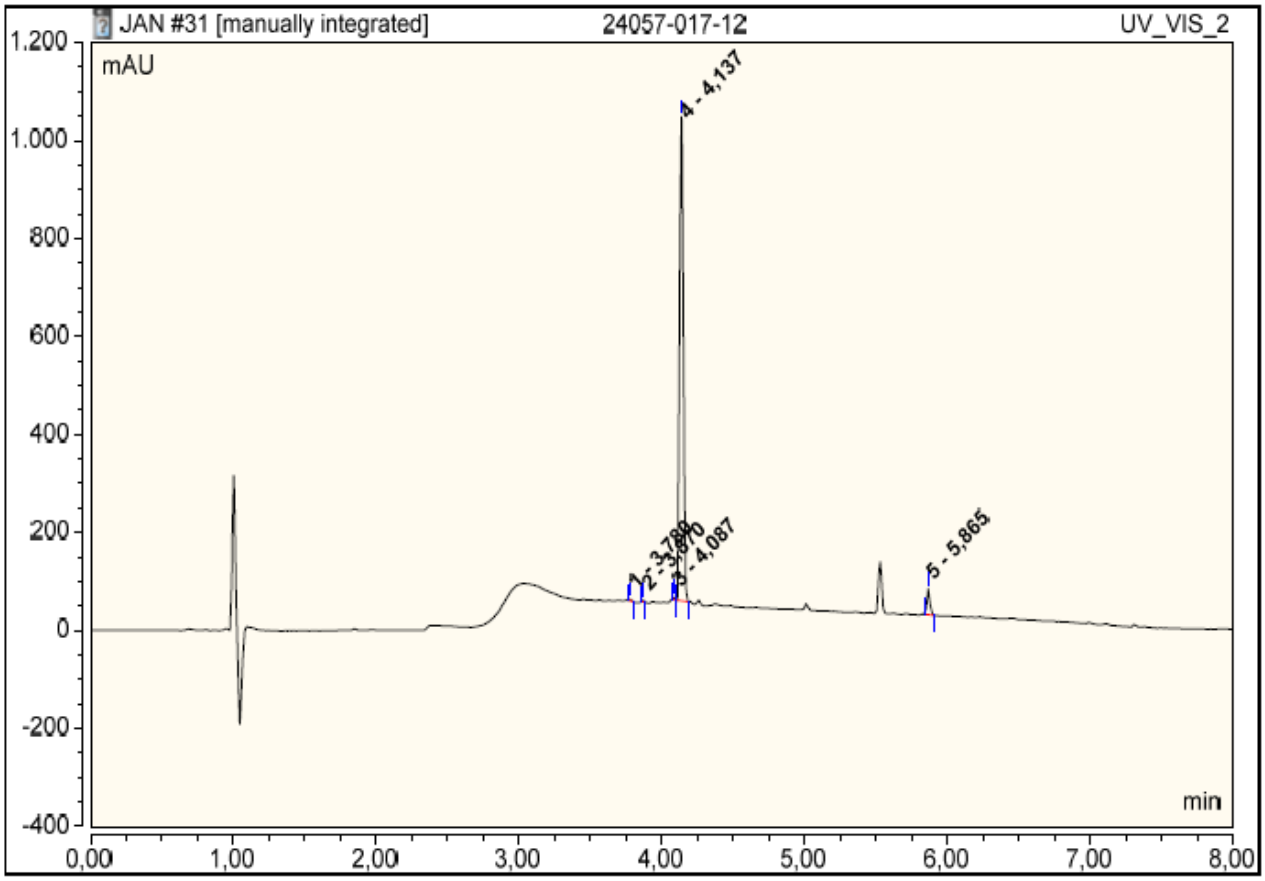
1.5 mmol scale, HPLC method B



Peak no.	R_T (min)	Compound	Relative area
1	4.453	Ac-Leu-Val-Ala-Ala-Gly-NH_2	0.20%
2	4.690	Target molecule: Ac-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	70.15%
3	6.220	Fmoc-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	29.64%

Manual SPPS of Ac-LVAYAG-NH_2 in DMSO/DOL (3:7)

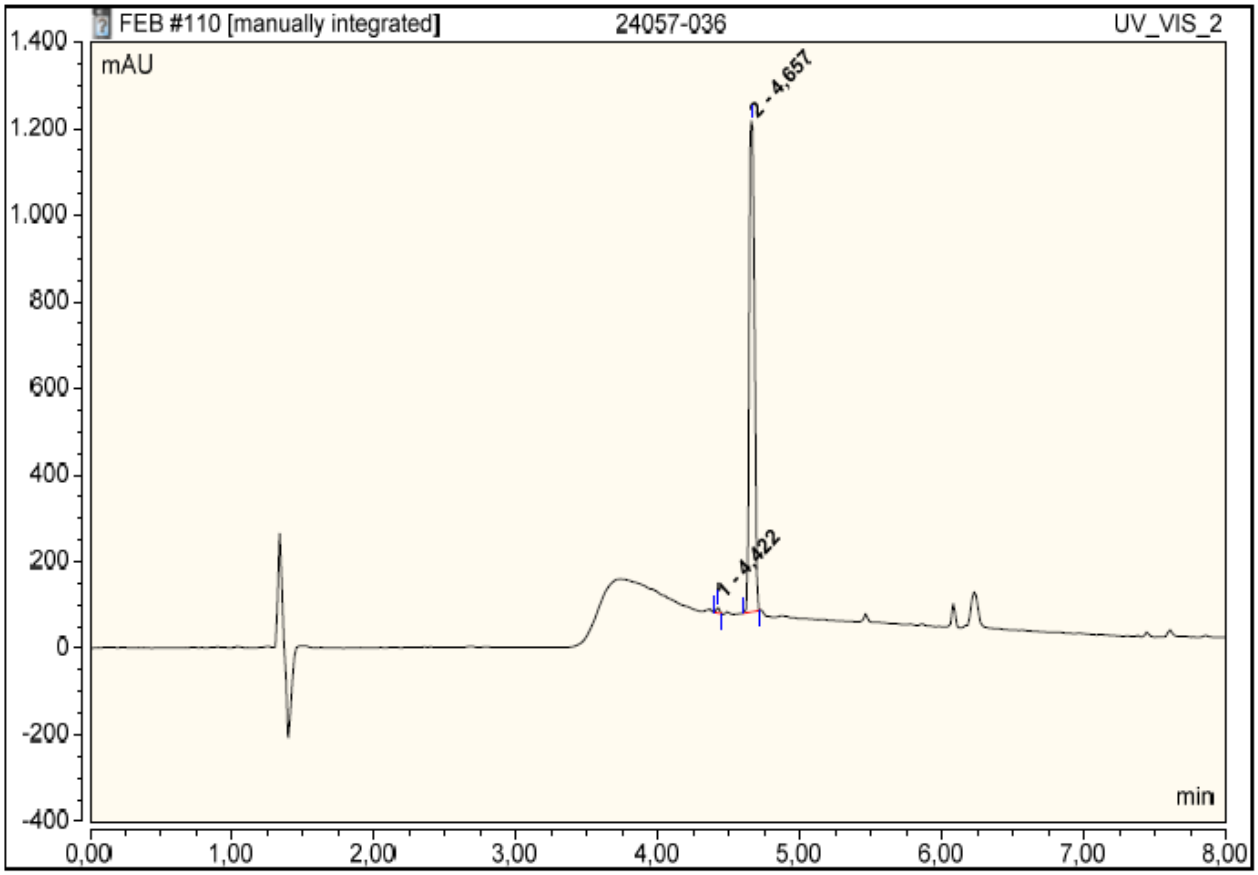
1.5 mmol scale, HPLC method B



Peak no.	R_T (min)	Compound	Relative area
1	3.780	Ac-Leu-Val-Ala-Ala-Gly-NH_2	0.21%
2	3.670	Ac-Leu-Ala-Tyr-Ala-Gly-NH_2	0.21%
3	4.087	Ac-Leu-Val-Tyr-Ala-Gly-NH_2	0.34%
4	4.137	Target molecule: Ac-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	95.13%
5	5.865	Fmoc-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	4.10%

Manual SPPS of Ac-LVAYAG-NH_2 in DMSO/DOL (4:6)

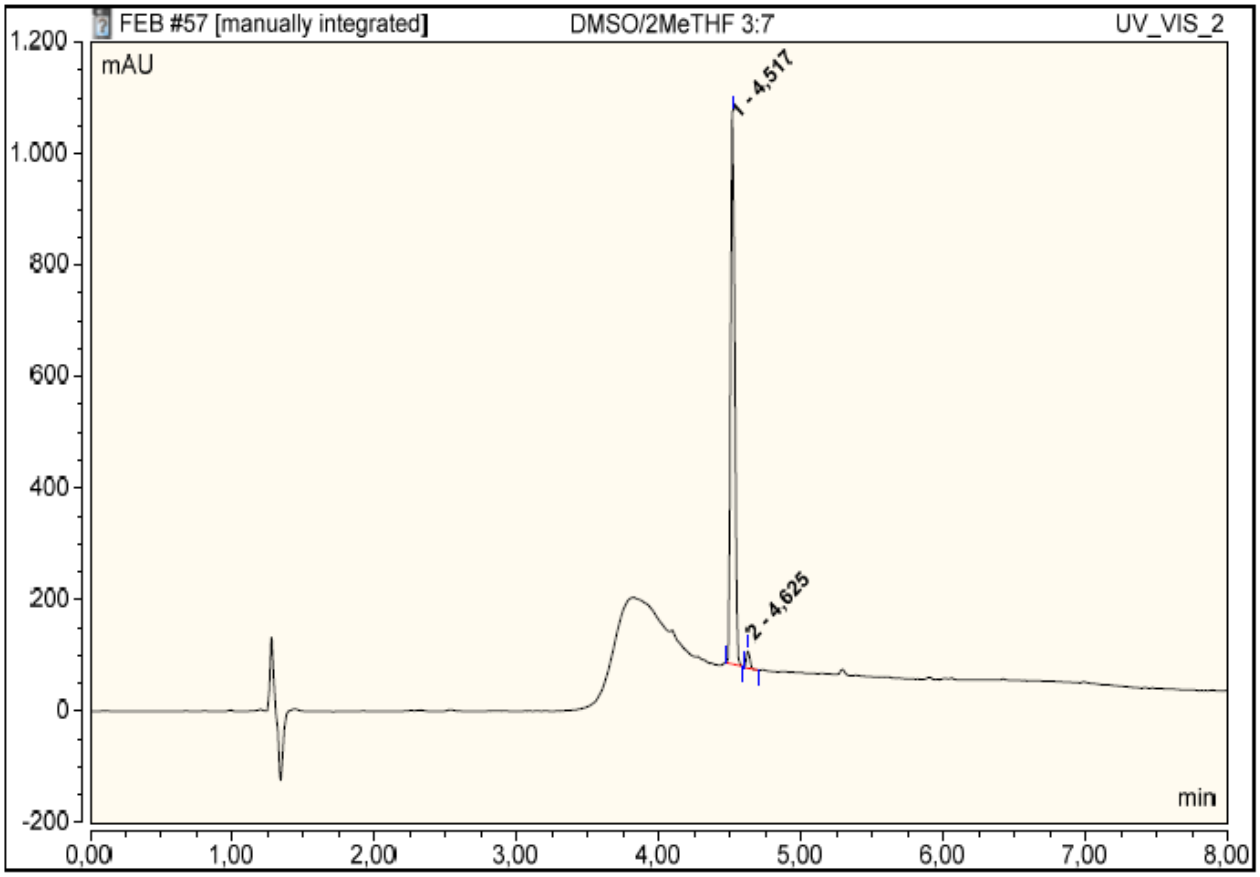
1.5 mmol scale, HPLC method B



Peak no.	R_T (min)	Compound	Relative area
1	4.422	Ac-Leu-Val-Ala-Ala-Gly-NH_2	0.68%
2	4.657	Target molecule: Ac-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	99.32%

Manual SPPS of Ac-LVAYAG-NH_2 in DMSO/2-Me-THF (3:7)

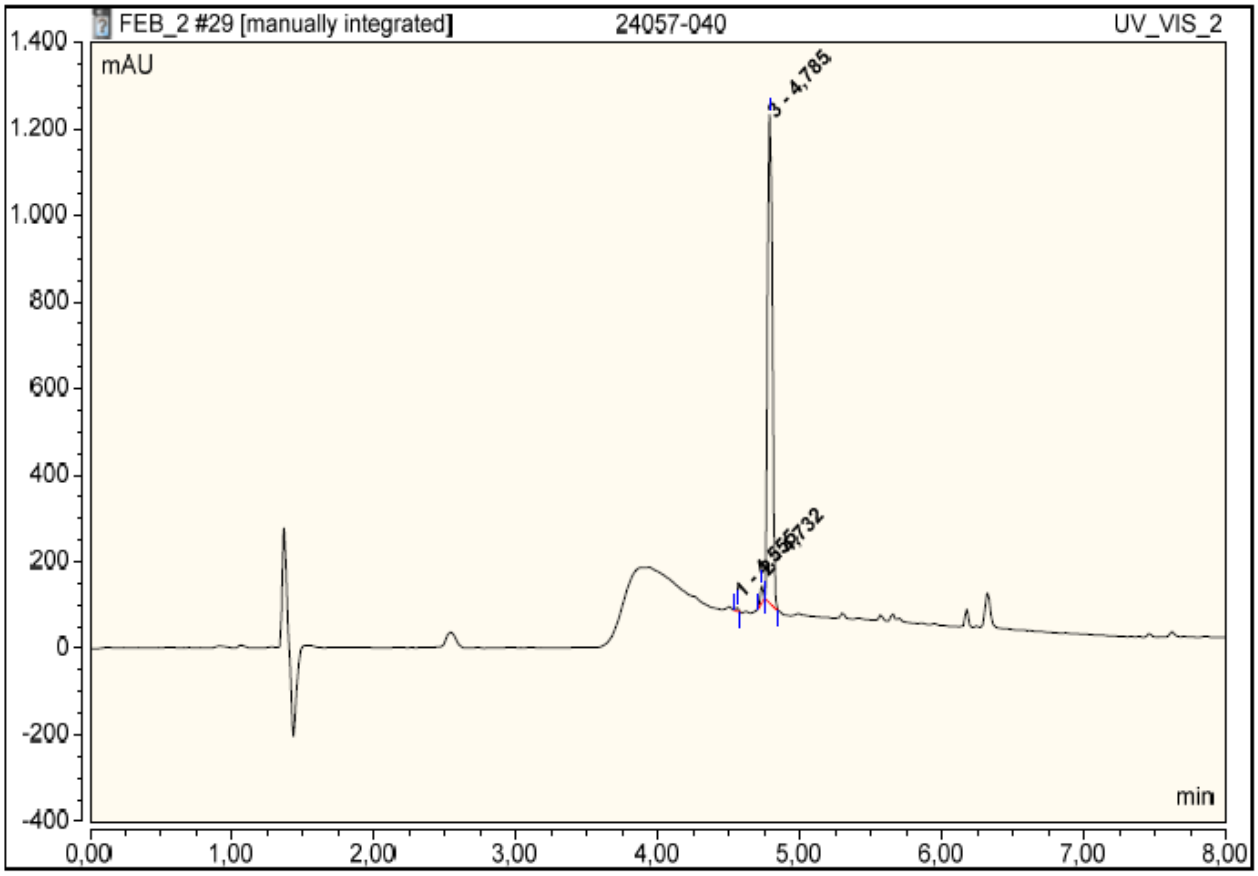
1.5 mmol scale, HPLC method B



Peak no.	R_T (min)	Compound	Relative area
1	4.517	Target molecule: Ac-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	97.69%
2	4.625	Ac-Leu-Val-Ala-Tyr-Ala-NH_2	2.31%

Manual SPPS of Ac-LVAYAG-NH_2 in DMSO/2-Me-THF (4:6)

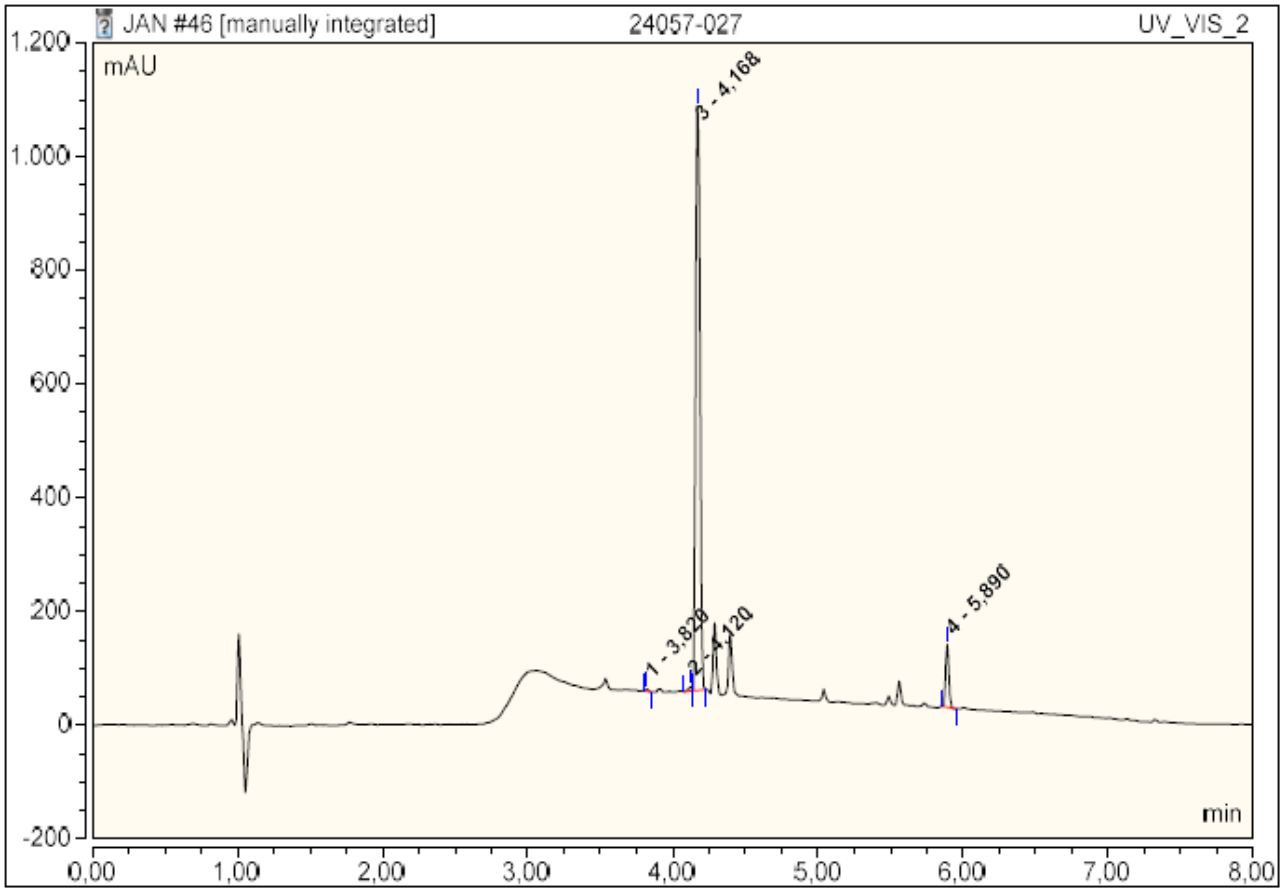
1.5 mmol scale, HPLC method B



Peak no.	R_T (min)	Compound	Relative area
1	4.555	Ac-Leu-Val-Ala-Ala-Gly-NH_2	0.49%
2	4.732	Ac-Leu-Val-Ala-Tyr-Ala-NH_2	2.03%
3	4.785	Target molecule: Ac-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	97.48%

Manual SPPS of Ac-LVAYAG-NH_2 in NBP/DOL (2:8)

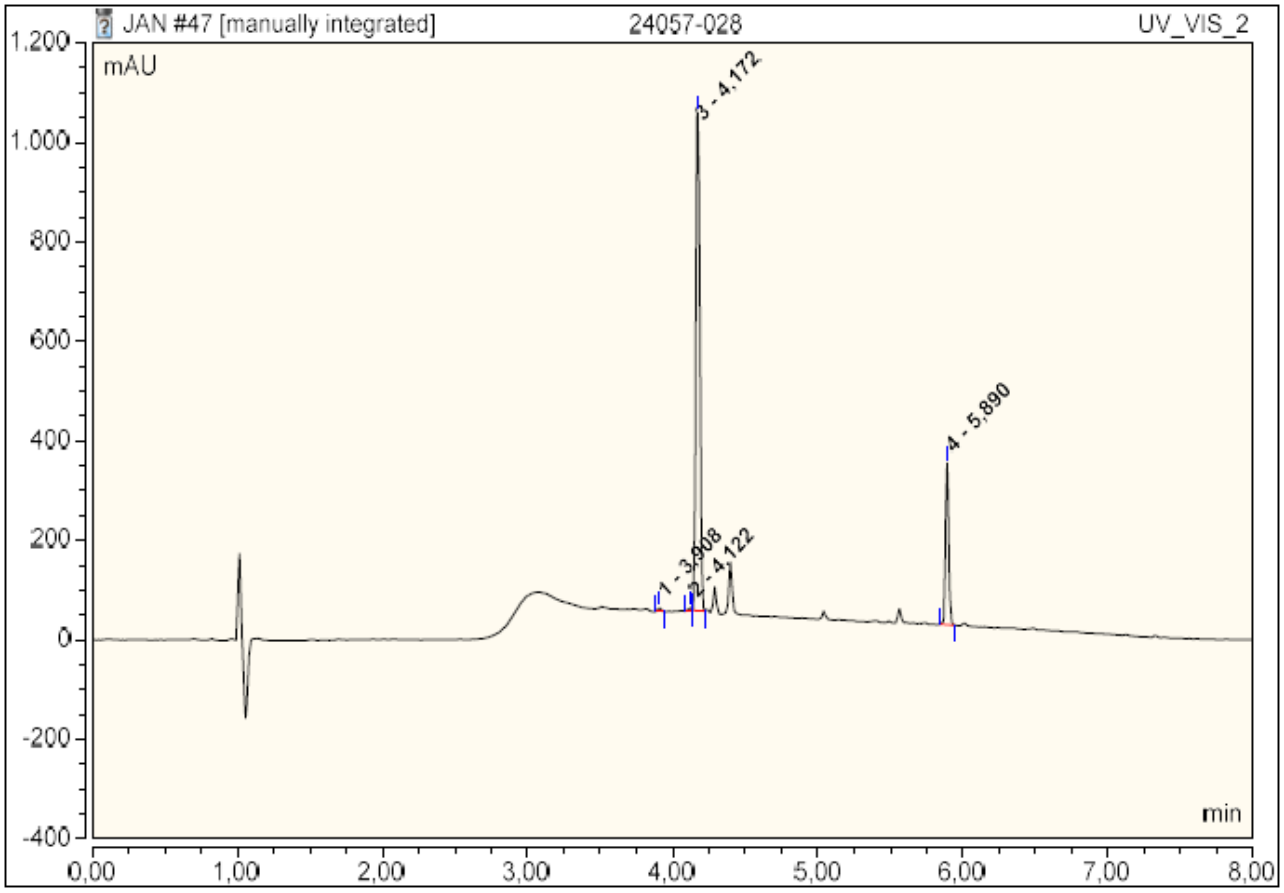
1.5 mmol scale, HPLC method B



Peak no.	R_T (min)	Compound	Relative area
1	3.820	Ac-Leu-Val-Ala-Ala-Gly-NH_2	0.23%
2	4.120	Ac-Leu-Val-Tyr-Ala-Gly-NH_2	0.52%
3	4.168	Target molecule: Ac-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	82.28%
4	5.890	Fmoc- Leu-Val-Ala-Tyr-Ala-Gly-NH_2	16.96%

Manual SPPS of Ac-LVAYAG-NH_2 in NBP/DOL (4:6)

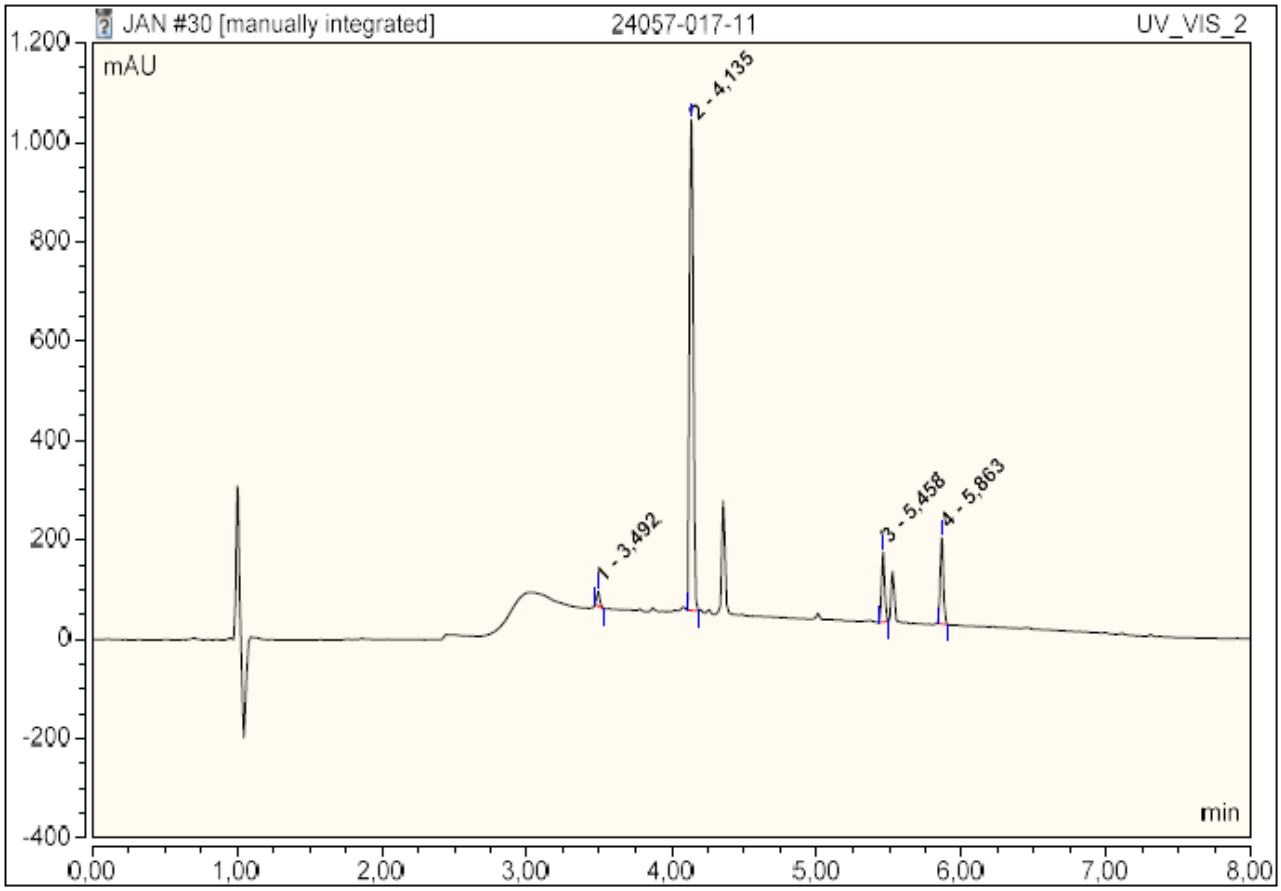
1.5 mmol scale, HPLC method B



Peak no.	R_T (min)	Compound	Relative area
1	3.908	Ac-Leu-Ala-Tyr-Ala-Gly-NH_2	0.46%
2	4.122	Ac-Leu-Val-Tyr-Ala-Gly-NH_2	0.36%
3	4.172	Target molecule: Ac-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	79.13%
4	5.890	Fmoc- Leu-Val-Ala-Tyr-Ala-Gly-NH_2	20.05%

Manual SPPS of Ac-LVAYAG-NH_2 in NBP/2-Me-THF (4:6)

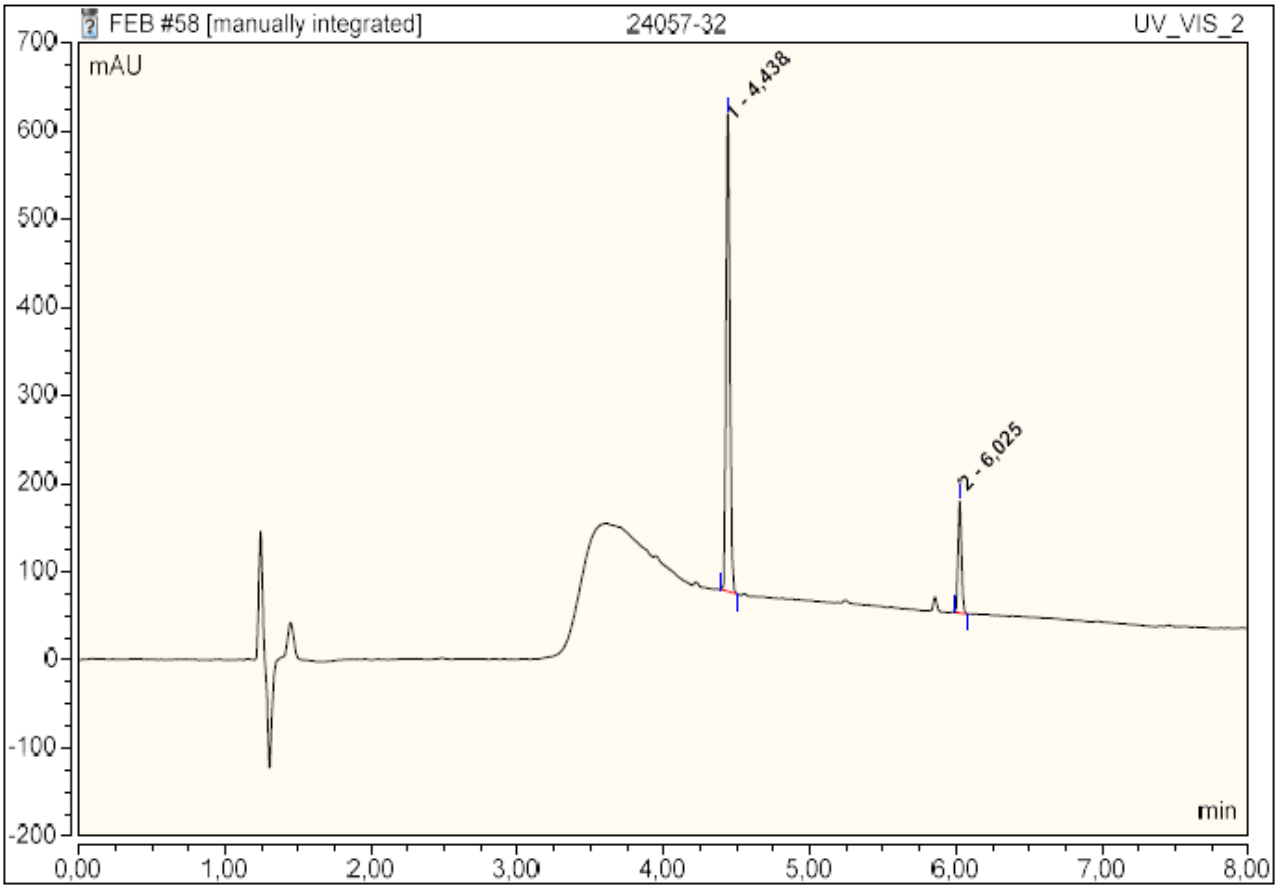
1.5 mmol scale, HPLC method B



Peak no.	R_T (min)	Compound	Relative area
1	3.492	Ac-Val-Ala-Tyr-Ala-Gly-NH_2	1.85%
2	4.135	Target molecule: Ac-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	77.67%
3	5.458	Fmoc-Val-Ala-Tyr-Ala-Gly-NH_2	9.07%
4	5.863	Fmoc-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	11.41%

Manual SPPS of Ac-LVAYAG-NH_2 in NFM/DOL (2:8)

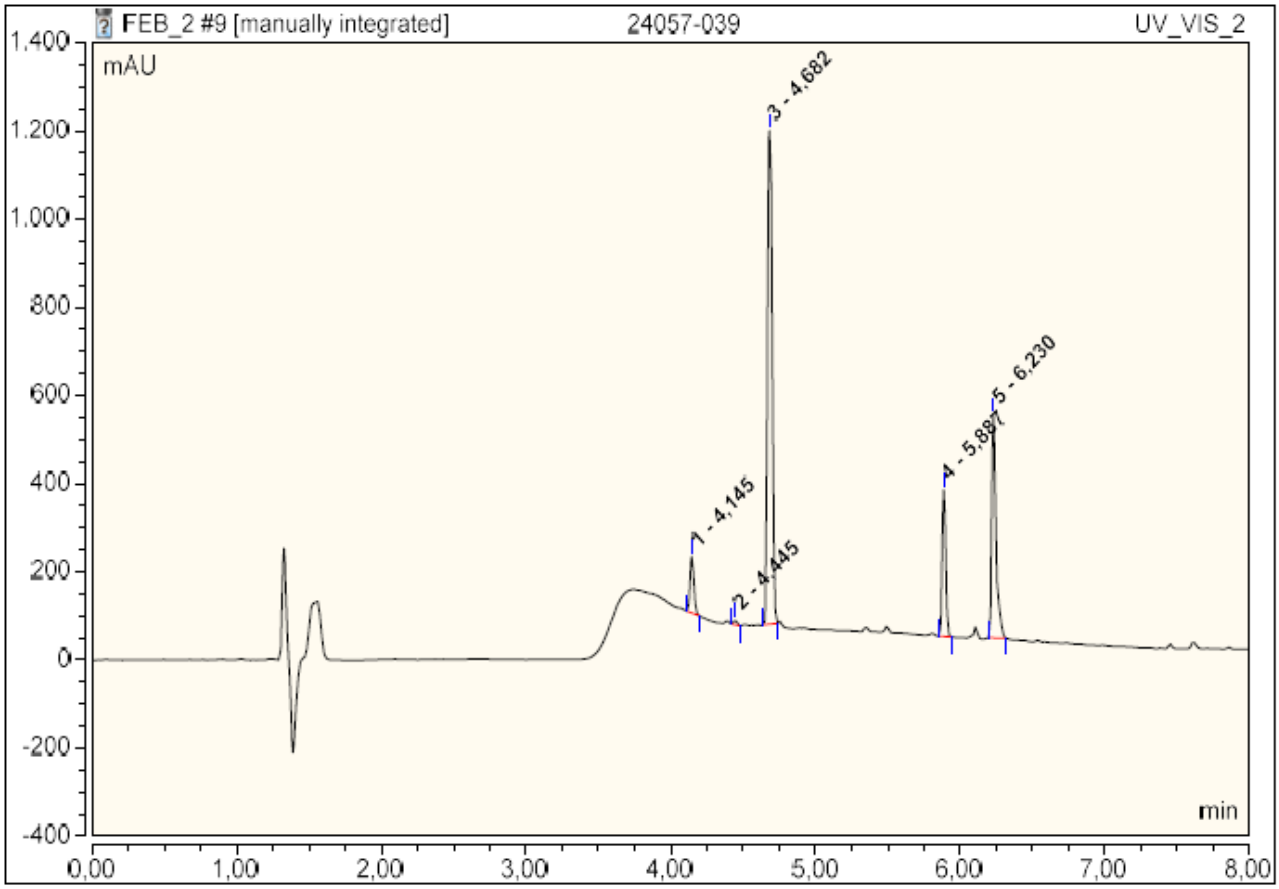
1.5 mmol scale, HPLC method B



Peak no.	R_T (min)	Compound	Relative area
1	4.438	Target molecule: Ac-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	82.63%
2	6.025	Fmoc-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	17.37%

Manual SPPS of Ac-LVAYAG-NH_2 in NFM/2-Me-THF (2:8)

1.5 mmol scale, HPLC method B

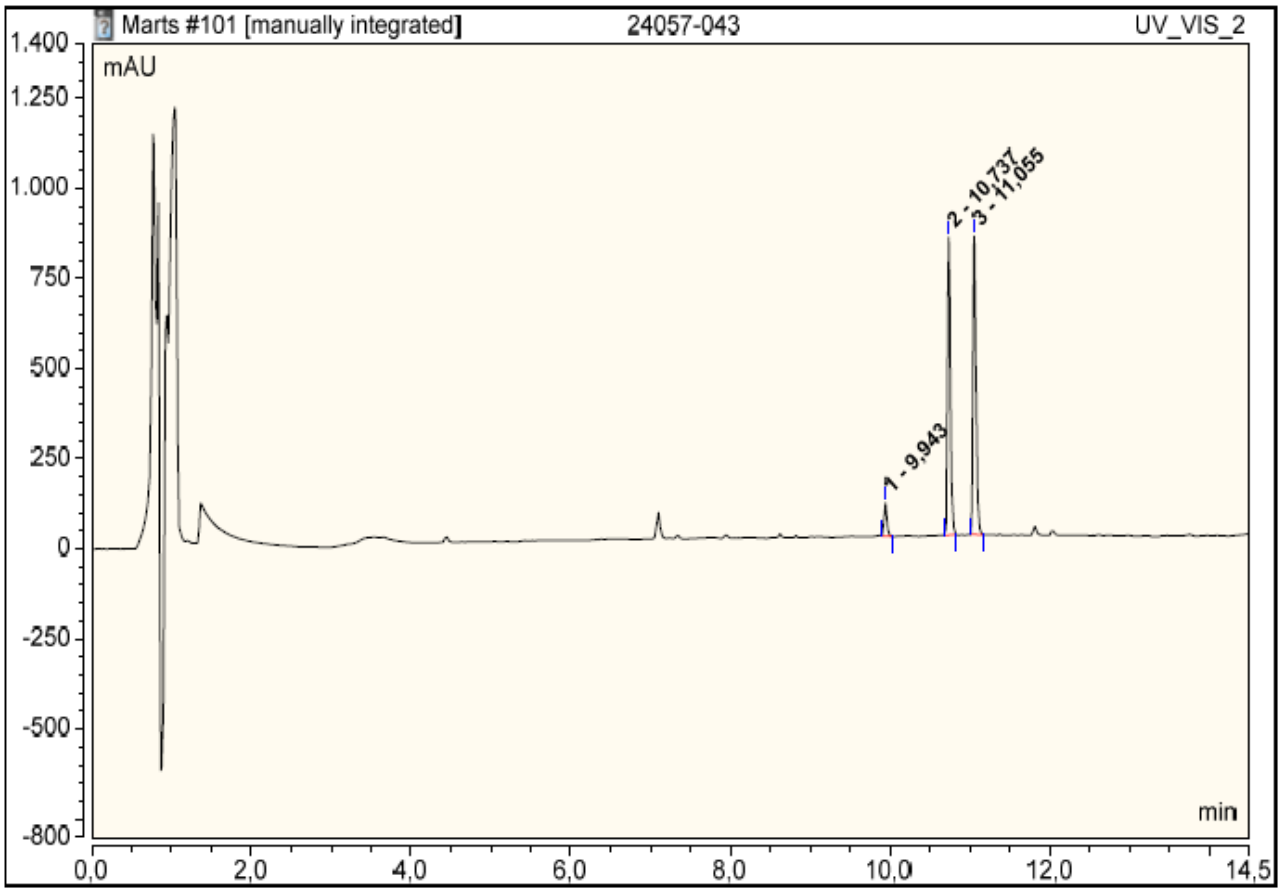


Peak no.	R_T (min)	Compound	Relative area
1	4.145	Ac-Val-Ala-Tyr-Ala-Gly-NH_2	5.19%
2	4.445	Ac-Leu-Val-Ala-Ala-Gly-NH_2	0.37%
3	4.682	Target molecule: Ac-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	58.39%
4	5.887	Fmoc-Val-Ala-Tyr-Ala-Gly-NH_2	12.69%
5	6.230	Fmoc-Leu-Val-Ala-Tyr-Ala-Gly-NH_2	23.36%

3.2 Manual SPPS of Aib-Enkephalin (Ac-Y-Aib-Aib-FL-OH) on 0.42 mmol scale

Manual SPPS of Ac-Y-Aib-Aib-FL-OH in DMF

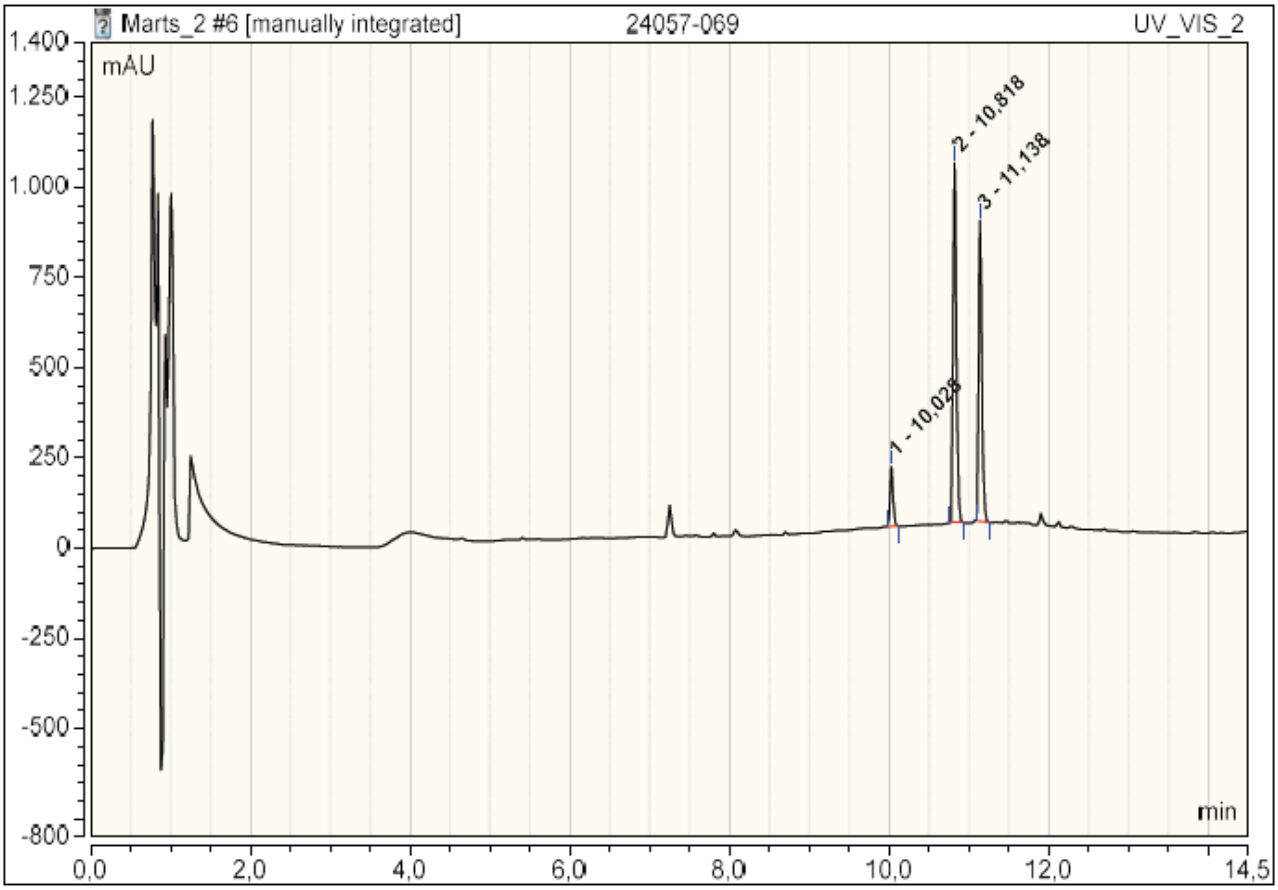
0.42 mmol scale, HPLC method C



Peak no.	R_T (min)	Compound	Relative area
1	9.943	Ac-Aib-Phe-Leu-OH	4.88%
2	10.737	Ac-Tyr-Aib-Phe-Leu-OH	47.03%
3	11.055	Target molecule: Ac-Tyr-Aib-Aib-Phe-Leu-OH	48.08%

Manual SPPS of Ac-Y-Aib-Aib-FL-OH in DMSO/EtOAc (3:7)

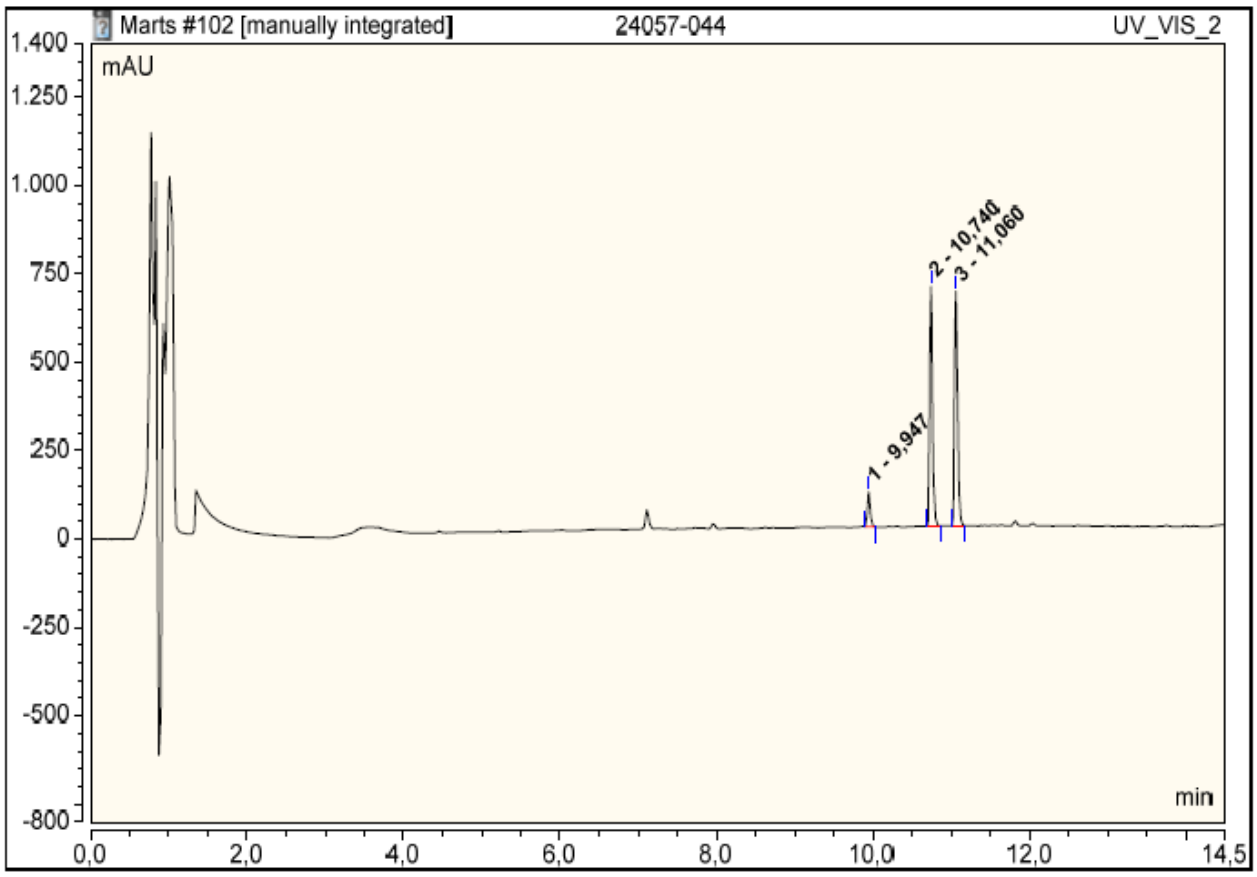
0.42 mmol scale, HPLC method C



Peak no.	R_T (min)	Compound	Relative area
1	10.028	Ac-Aib-Phe-Leu-OH	7.63%
2	10.818	Ac-Tyr-Aib-Phe-Leu-OH	51.31%
3	11.138	Target molecule: Ac-Tyr-Aib-Aib-Phe-Leu-OH	41.06%

Manual SPPS of Ac-Y-Aib-Aib-FL-OH in DMSO/EtOAc (4:6)

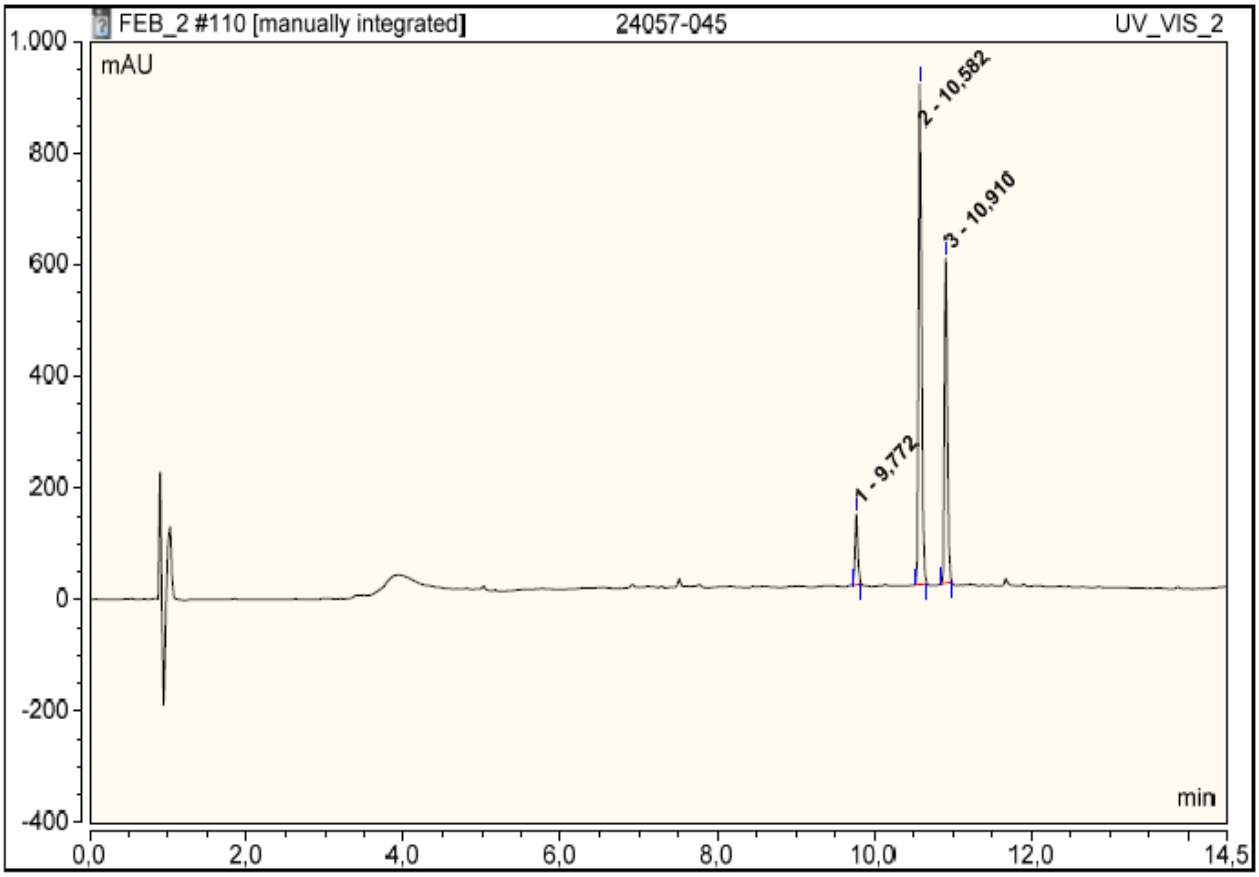
0.42 mmol scale, HPLC method C



Peak no.	R_T (min)	Compound	Relative area
1	9.947	Ac-Aib-Phe-Leu-OH	6.50%
2	10.740	Ac-Tyr-Aib-Phe-Leu-OH	47.07%
3	11.060	Target molecule: Ac-Tyr-Aib-Aib-Phe-Leu-OH	46.43%

Manual SPPS of Ac-Y-Aib-Aib-FL-OH in DMSO/EtOAc (5:5)

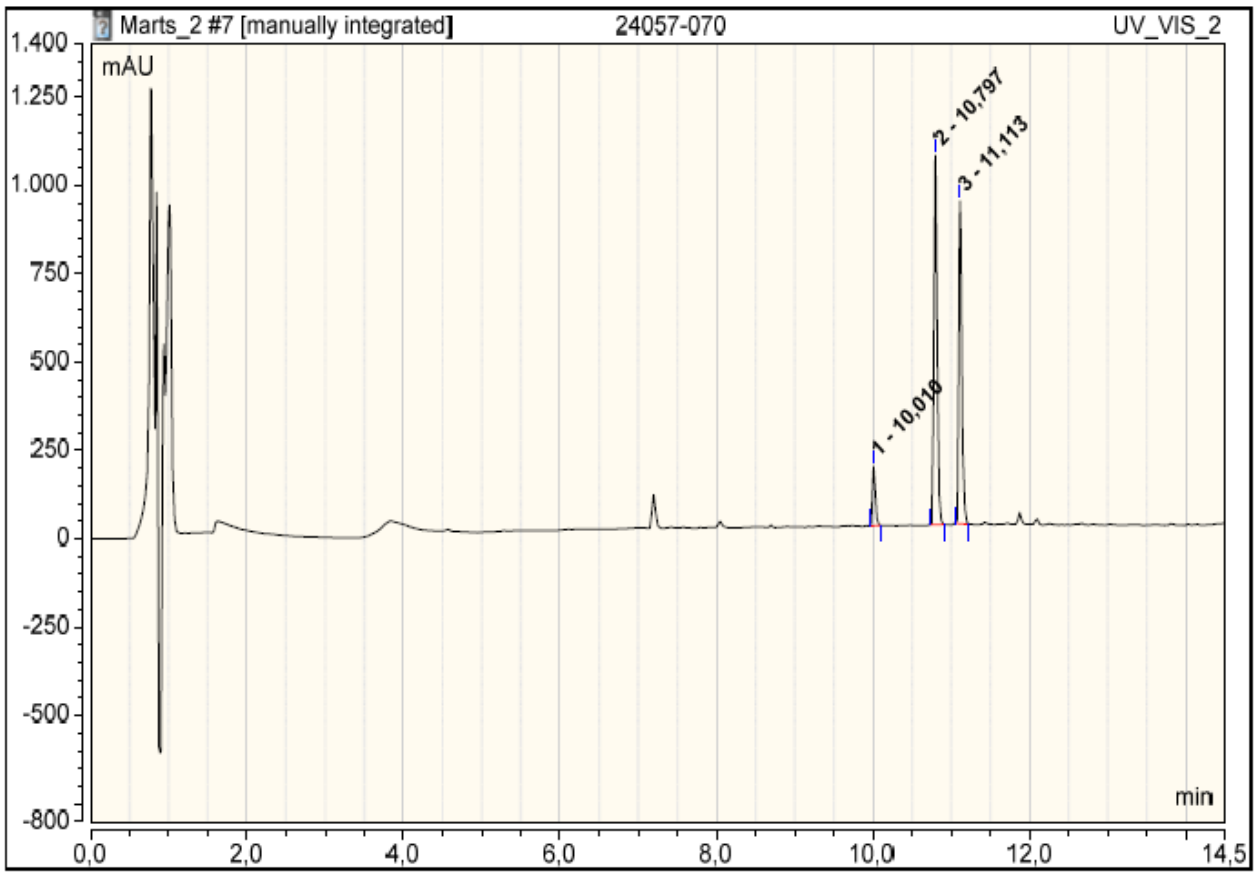
0.42 mmol scale, HPLC method C



Peak no.	R_T (min)	Compound	Relative area
1	9.772	Ac-Aib-Phe-Leu-OH	7.15%
2	10.582	Ac-Tyr-Aib-Phe-Leu-OH	58.01%
3	10.910	Target molecule: Ac-Tyr-Aib-Aib-Phe-Leu-OH	34.84%

Manual SPPS of Ac-Y-Aib-Aib-FL-OH in DMSO/DOL (3:7)

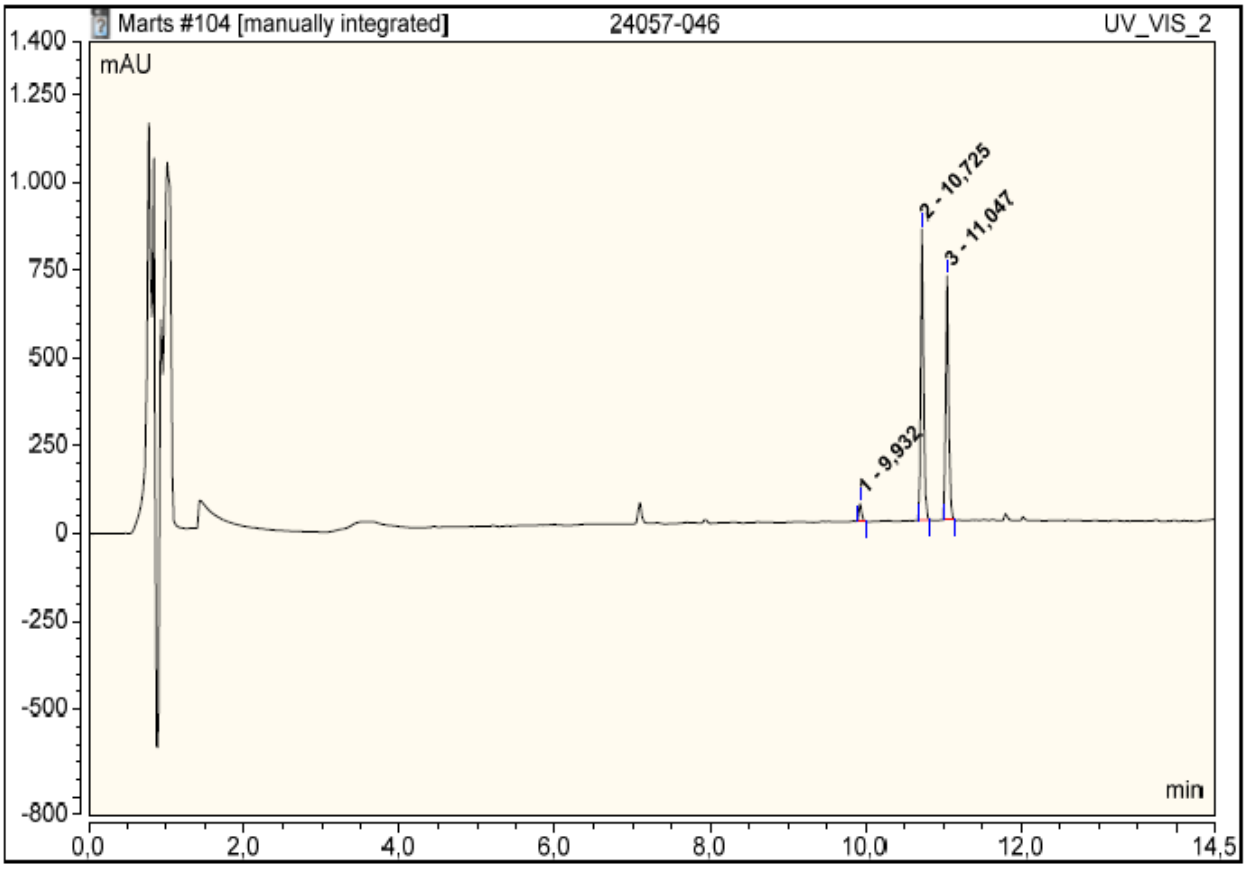
0.42 mmol scale, HPLC method C



Peak no.	R_T (min)	Compound	Relative area
1	10.010	Ac-Aib-Phe-Leu-OH	7.04%
2	10.797	Ac-Tyr-Aib-Phe-Leu-OH	50.14%
3	11.113	Target molecule: Ac-Tyr-Aib-Aib-Phe-Leu-OH	42.82%

Manual SPPS of Ac-Y-Aib-Aib-FL-OH in DMSO/DOL (4:6)

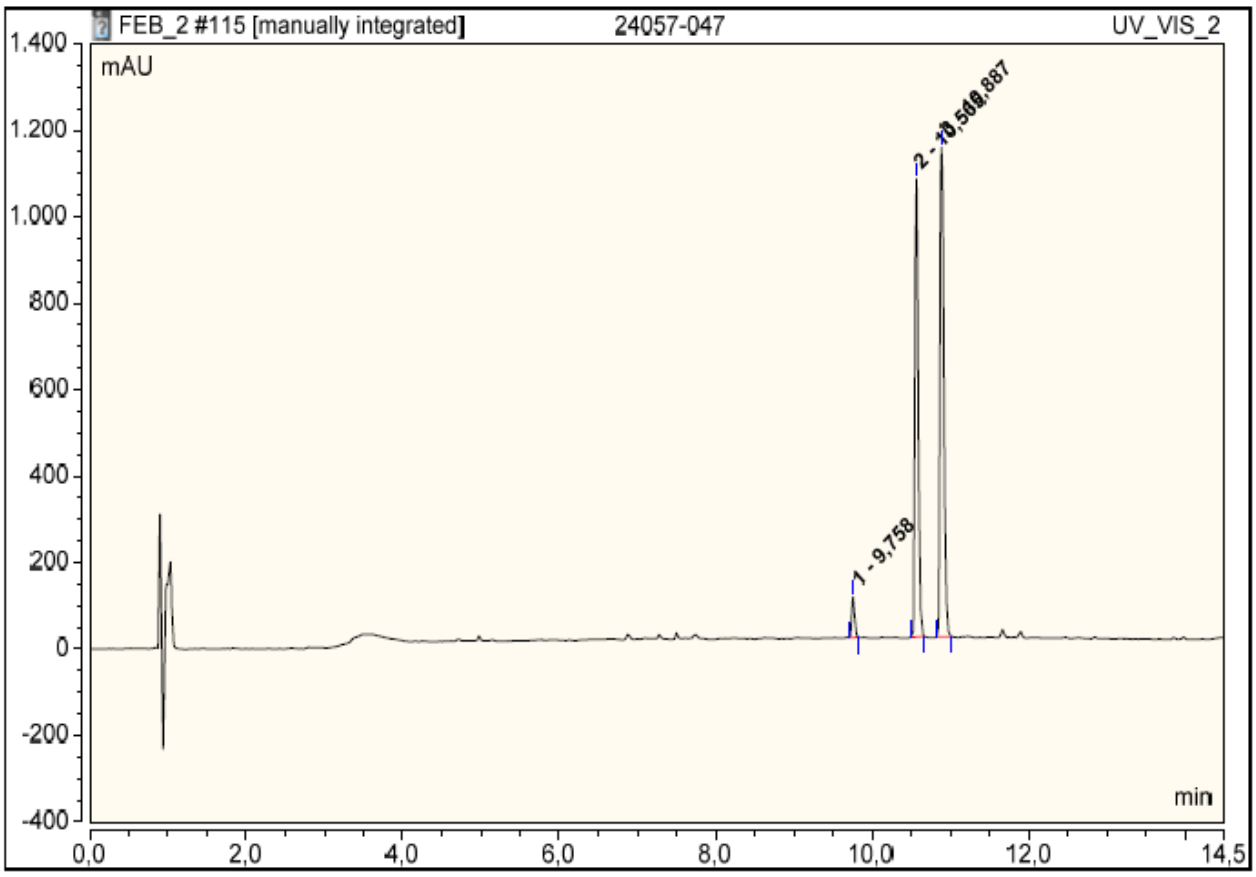
0.42 mmol scale, HPLC method C



Peak no.	R_T (min)	Compound	Relative area
1	9.932	Ac-Aib-Phe-Leu-OH	2.94%
2	10.725	Ac-Tyr-Aib-Phe-Leu-OH	53.06%
3	11.047	Target molecule: Ac-Tyr-Aib-Aib-Phe-Leu-OH	44.00%

Manual SPPS of Ac-Y-Aib-Aib-FL-OH in DMSO/2-MeTHF (3:7)

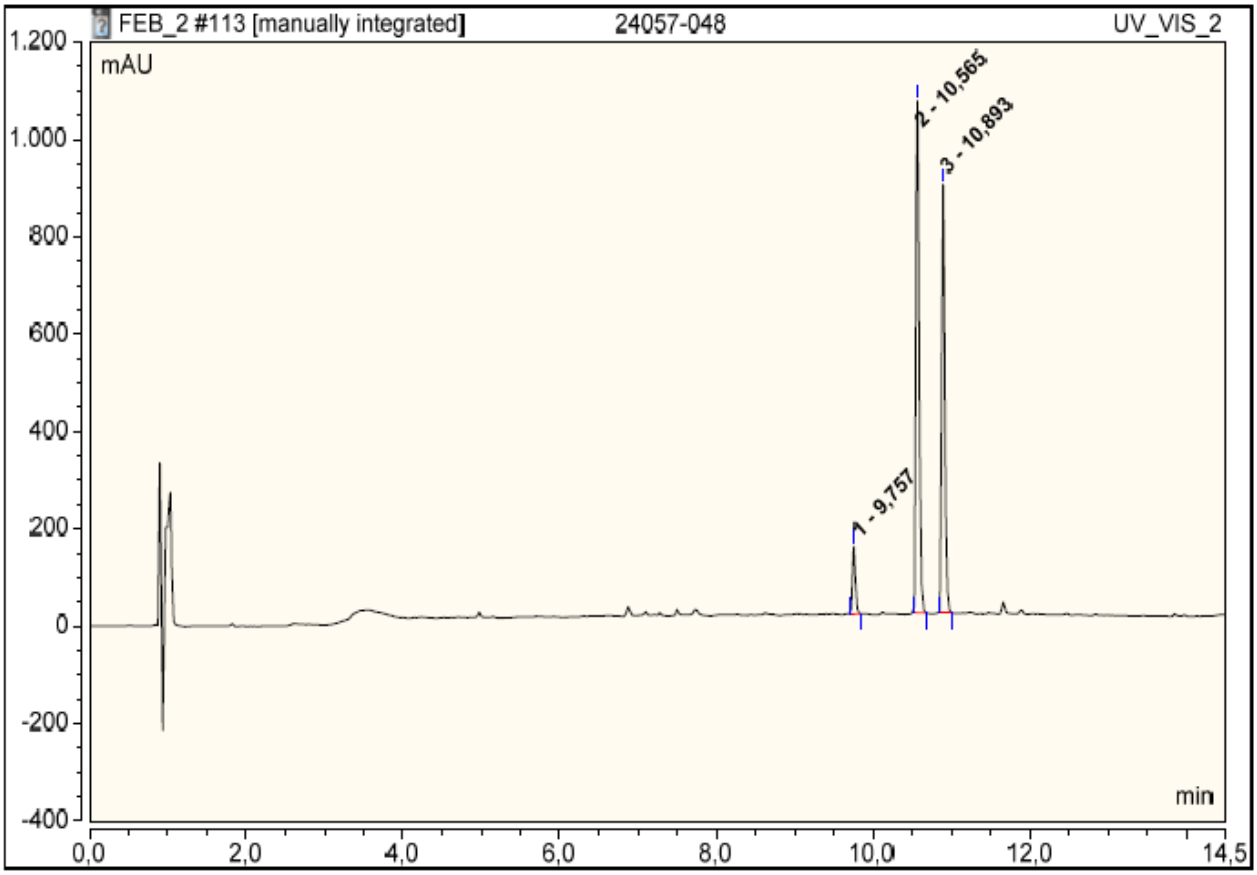
0.42 mmol scale, HPLC method C



Peak no.	R_T (min)	Compound	Relative area
1	9.758	Ac-Aib-Phe-Leu-OH	3.07%
2	10.565	Ac-Tyr-Aib-Phe-Leu-OH	43.50%
3	10.887	Target molecule: Ac-Tyr-Aib-Aib-Phe-Leu-OH	53.42%

Manual SPPS of Ac-Y-Aib-Aib-FL-OH in DMSO/2-Me-THF (4:6)

0.42 mmol scale, HPLC method C

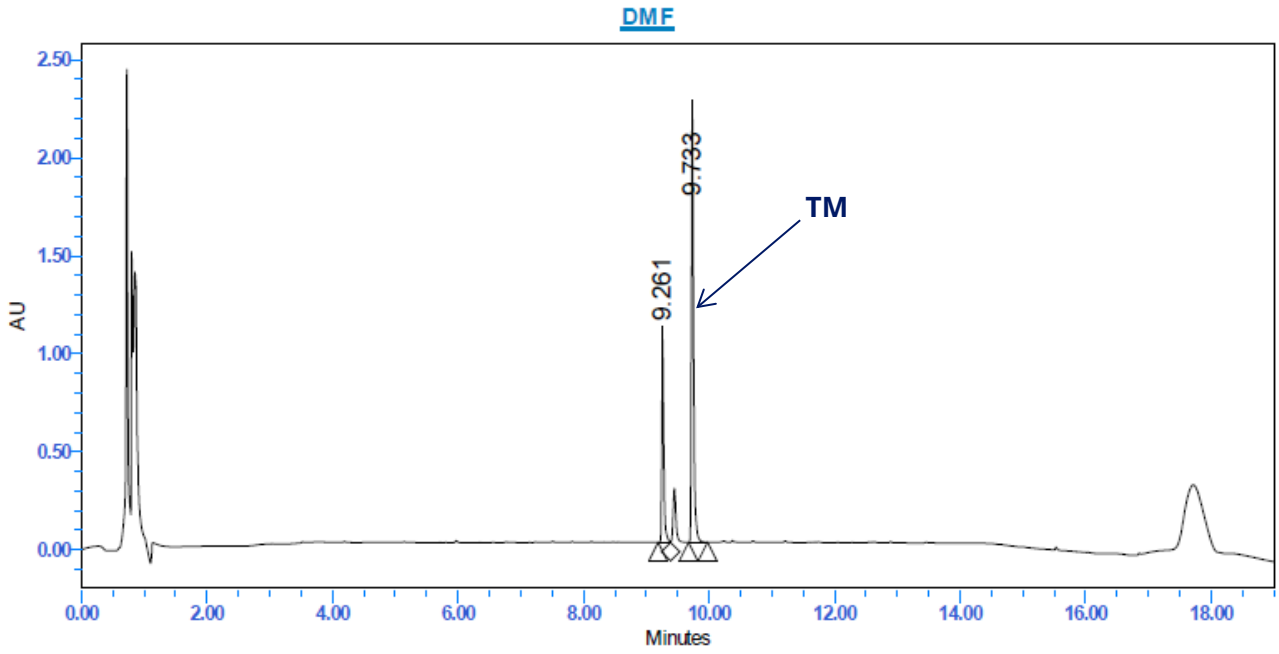


Peak no.	R_T (min)	Compound	Relative area
1	9.757	Ac-Aib-Phe-Leu-OH	5.74%
2	10.565	Ac-Tyr-Aib-Phe-Leu-OH	53.06%
3	10.883	Target molecule: Ac-Tyr-Aib-Aib-Phe-Leu-OH	41.20%

3.3 Automated SPPS of Aib-enkephalin (Ac-Y-Aib-Aib-FL-NH_2) on 0.15 mmol scale

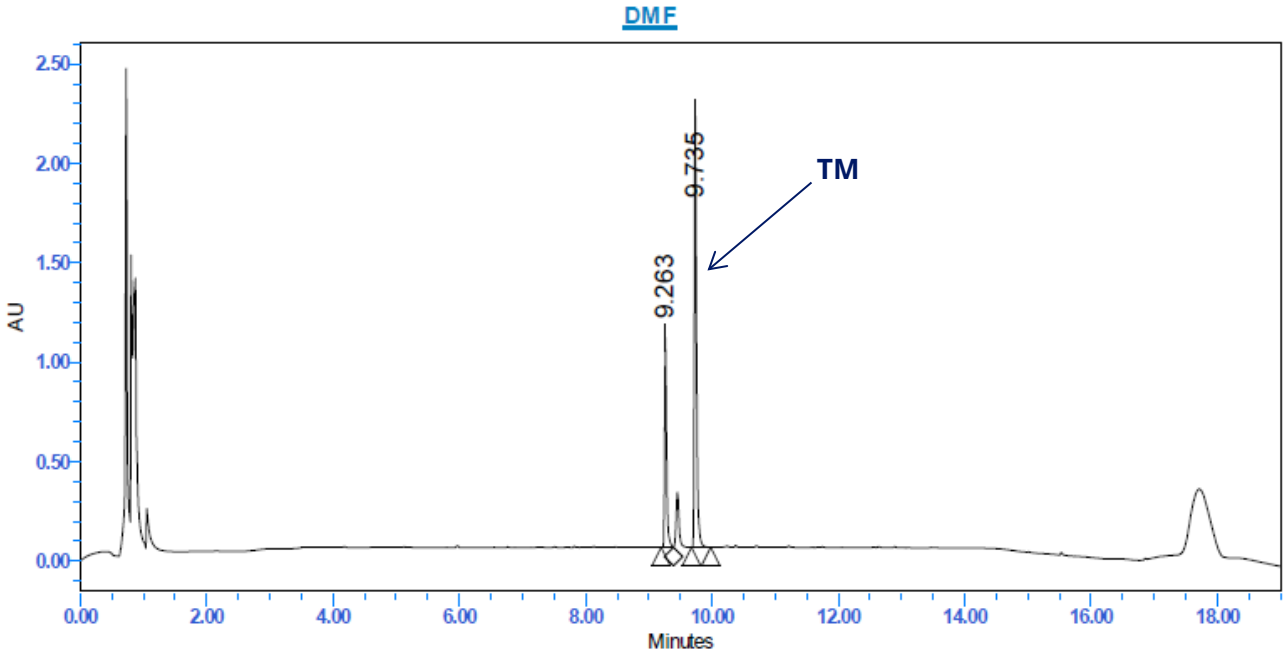
Automated SPPS of Aib-enkephalin (Ac-Y-Aib-Aib-FL-NH_2) in DMF, repeated three times

0.15 mmol scale, HPLC method E



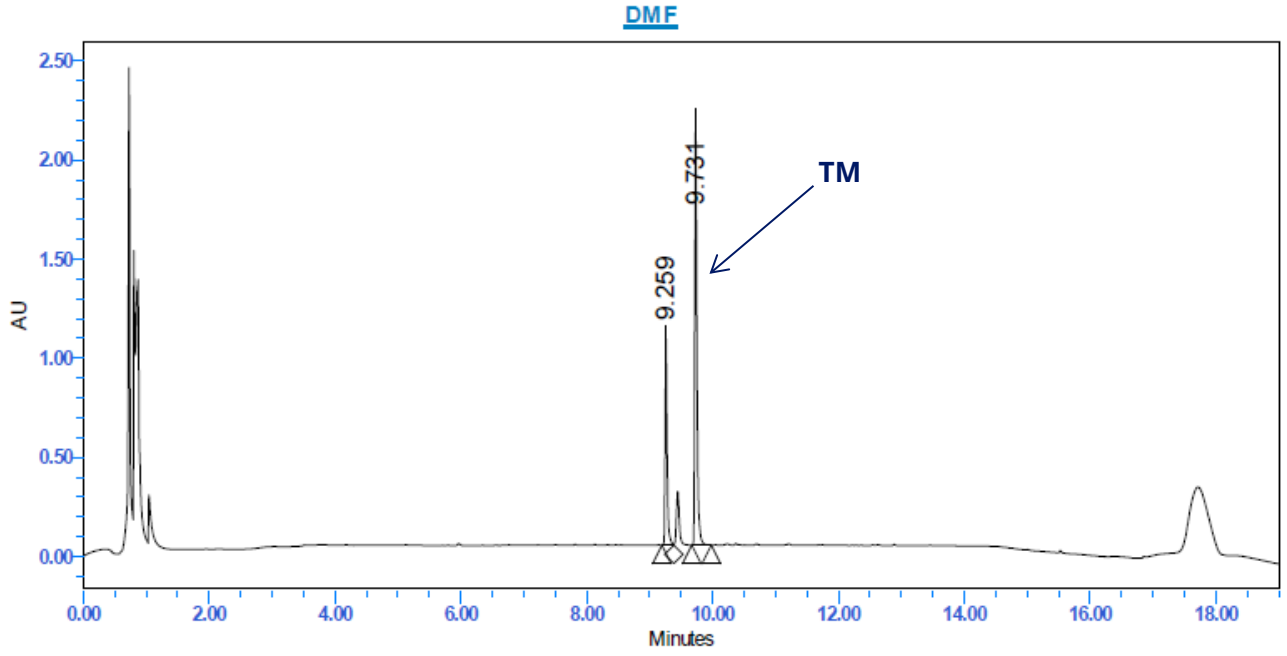
Peak Results

	Name	RT	Area	Height	Amount	% Area
1		9.261	2259166	1034517		30.09
2		9.733	5249804	2196197		69.91



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		9.263	2291769	1054299		30.65
2		9.735	5185120	2185046		69.35

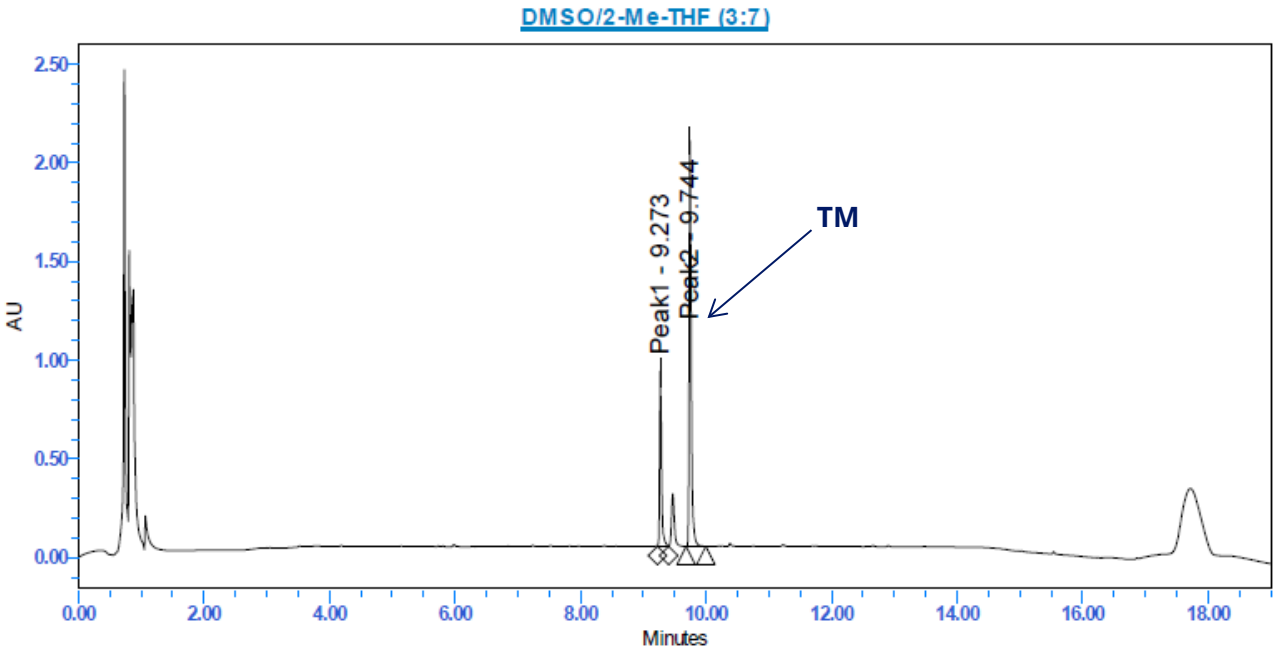


Peak Results

	Name	RT	Area	Height	Amount	% Area
1		9.259	2265985	1042759		31.03
2		9.731	5035822	2144858		68.97

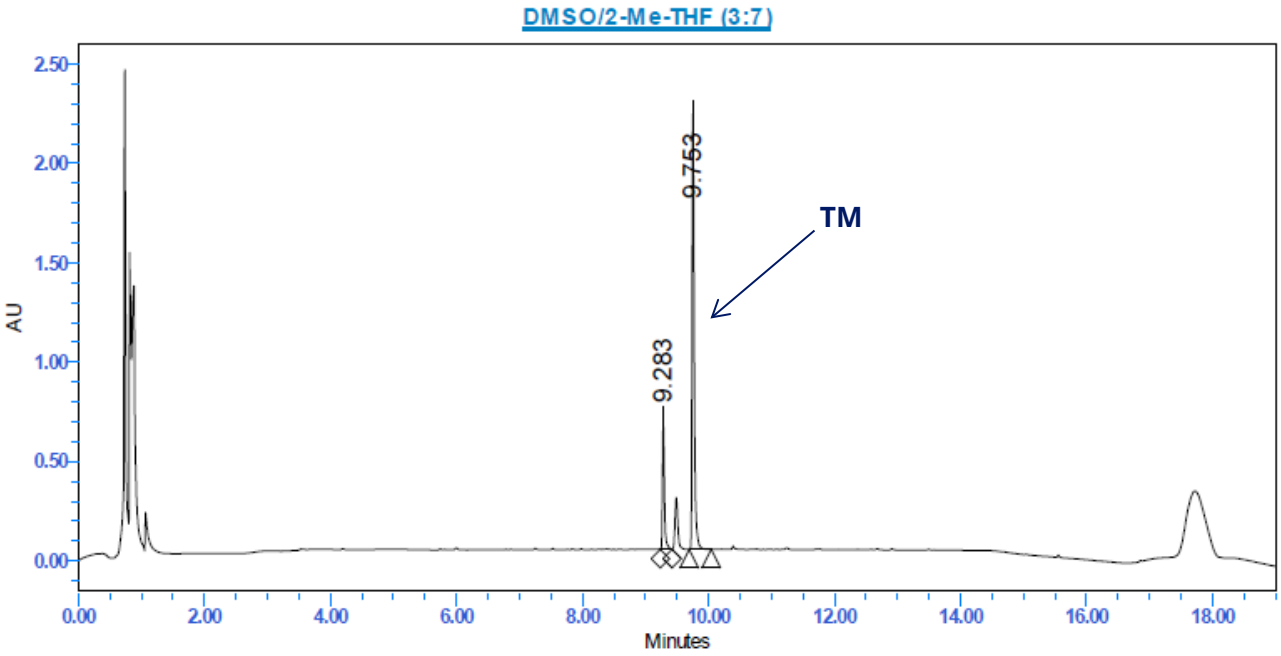
Automated SPPS of Aib-enkephalin (Ac-Y-Aib-Aib-FL-NH_2) in DMSO/2-Me-THF (3:7), repeated two times

0.15 mmol scale, HPLC method E



Peak Results

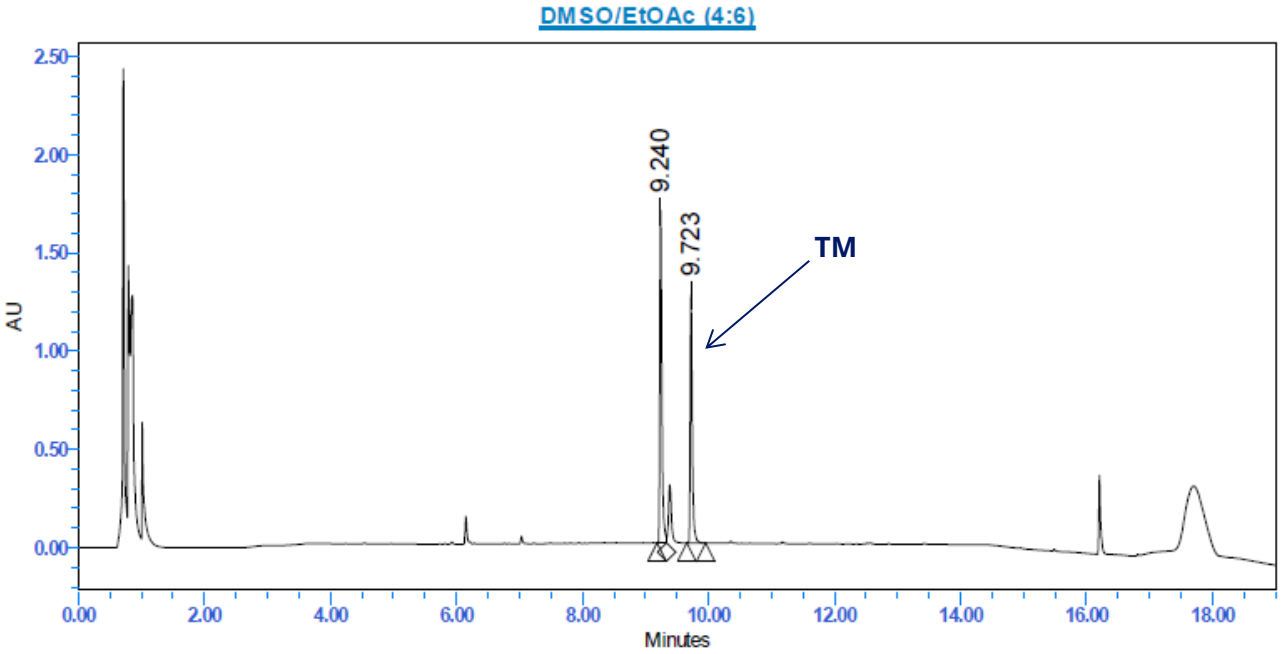
	Name	RT	Area	Height	Amount	% Area
1	Peak1	9.273	1907590	887427		28.80
2	Peak2	9.744	4716706	2054011		71.20



Peak Results

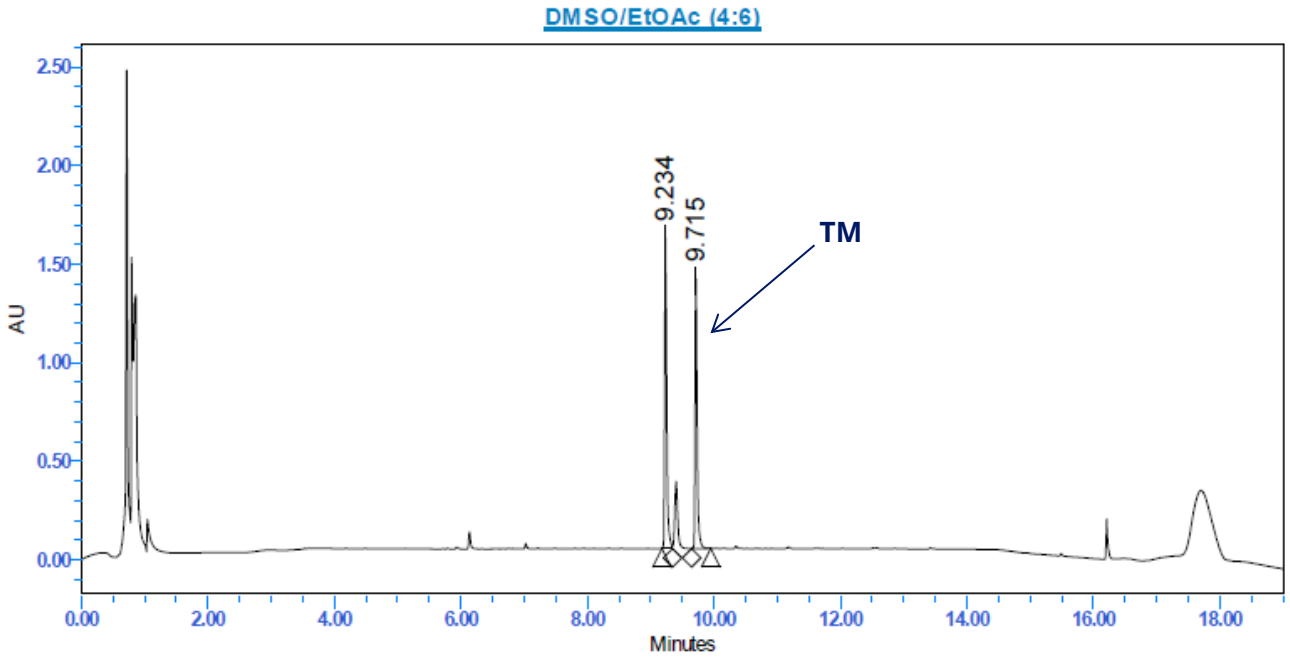
	Name	RT	Area	Height	Amount	% Area
1		9.283	1376144	646091		21.17
2		9.753	5123674	2193809		78.83

Automated SPPS of Aib-enkephalin (Ac-Y-Aib-Aib-FL-NH_2) in DMSO/EtOAc (4:6), repeated two times 0.15 mmol scale, HPLC method E



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		9.240	3814557	1693520		57.45
2		9.723	2825161	1263867		42.55

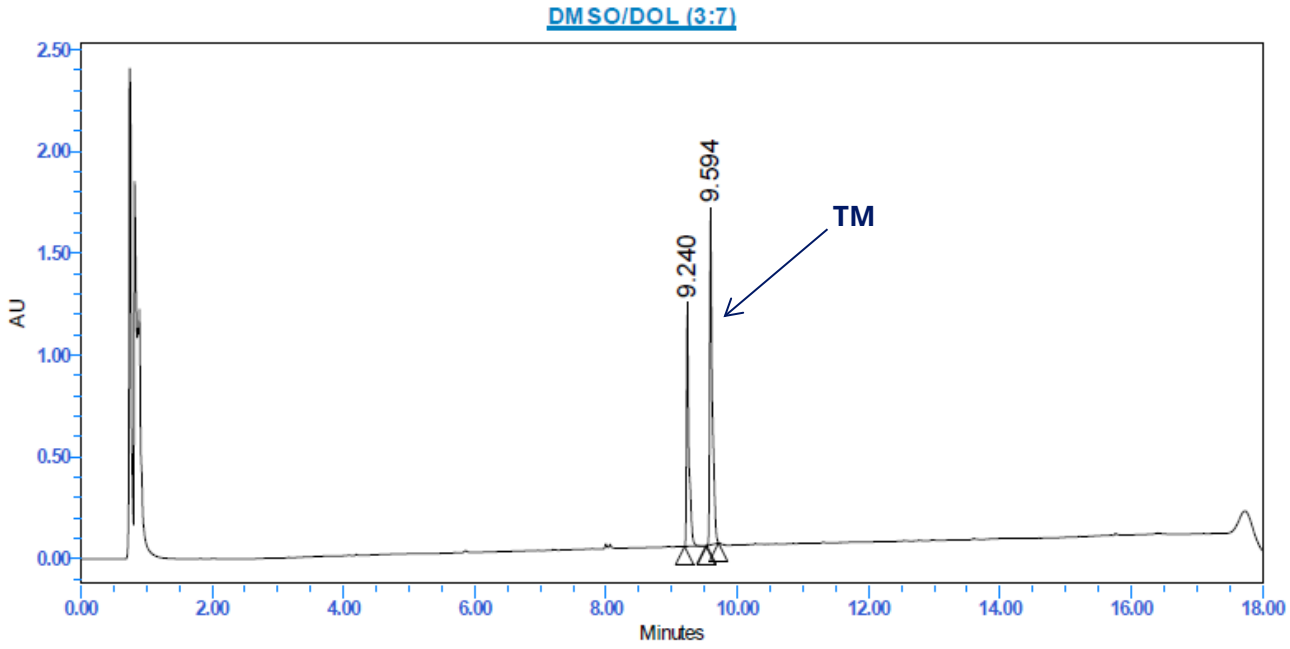


Peak Results

	Name	RT	Area	Height	Amount	% Area
1		9.234	3532066	1579111		53.61
2		9.715	3056711	1369512		46.39

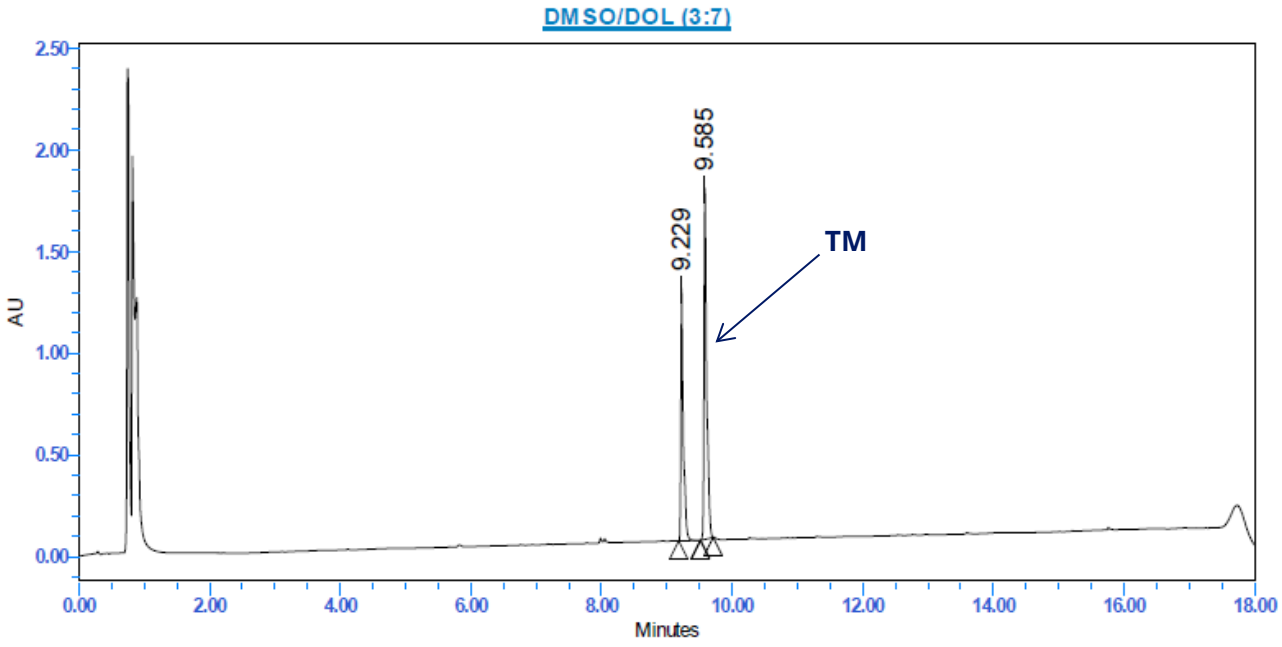
Automated SPPS of Aib-enkephalin (Ac-Y-Aib-Aib-FL-NH_2) in DMSO/DOL (3:7), repeated three times

0.15 mmol scale, HPLC method E



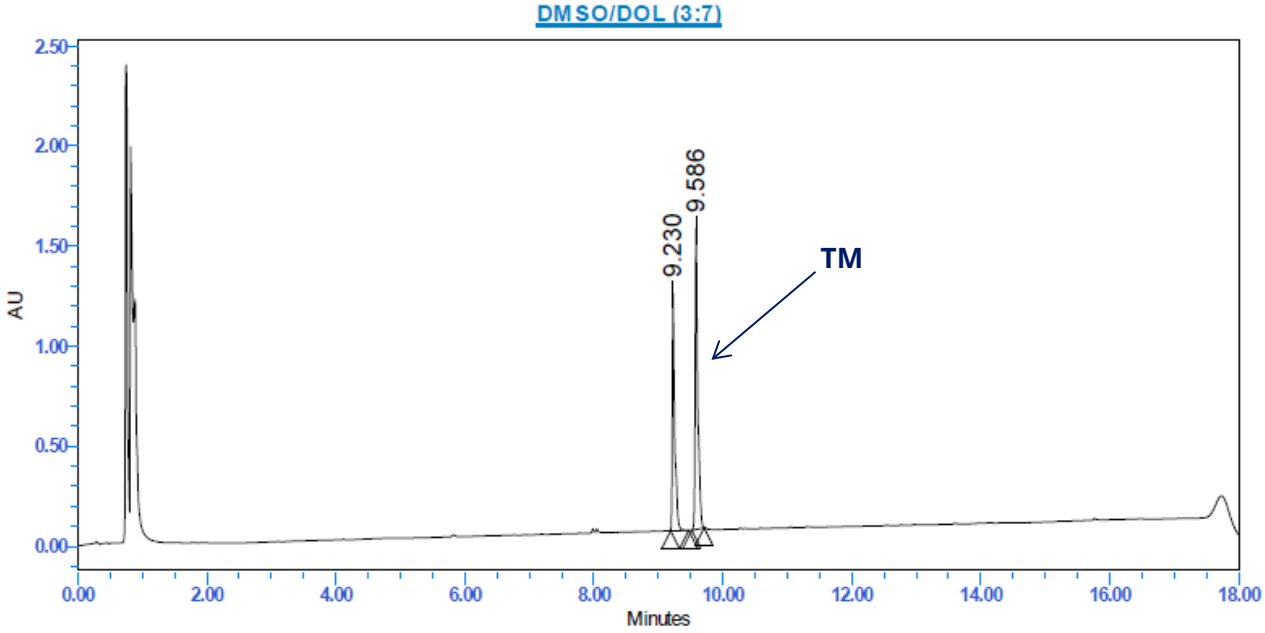
Peak Results

	Name	RT	Area	Height	Amount	% Area
1		9.240	2891921	1133898		39.49
2		9.594	4431816	1590811		60.51



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		9.229	3184767	1240412		39.88
2		9.585	4800462	1725965		60.12



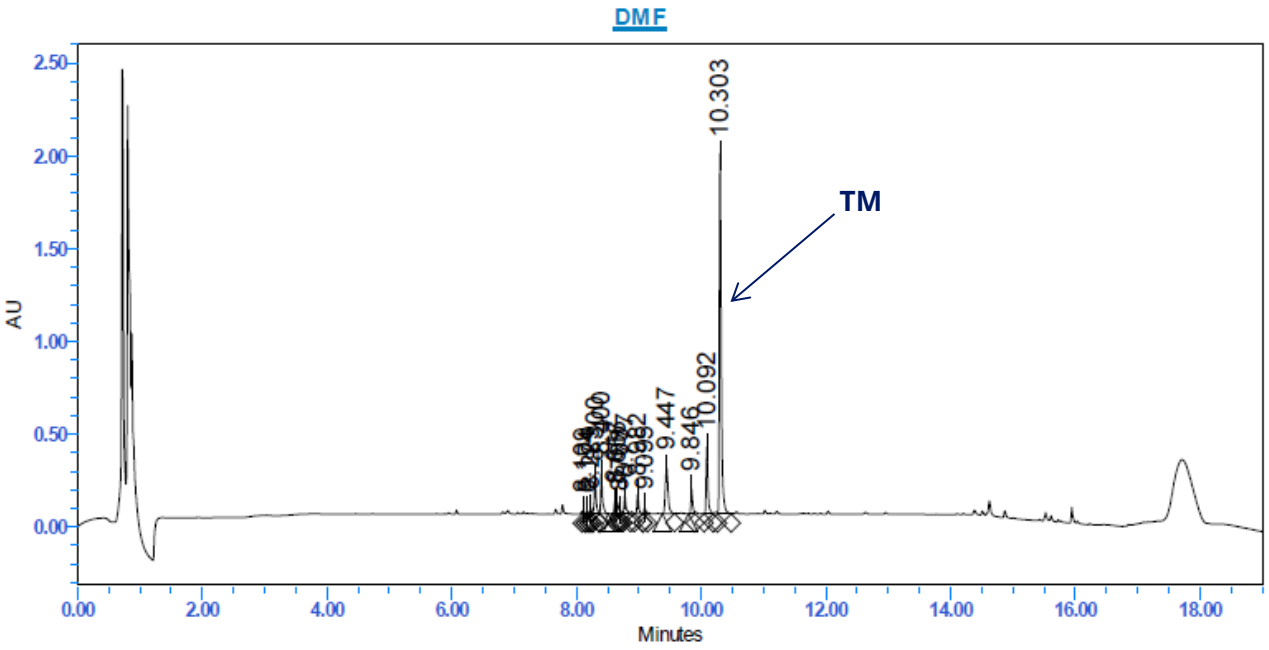
Peak Results

	Name	RT	Area	Height	Amount	% Area
1		9.230	3031578	1186191		42.31
2		9.586	4132942	1507105		57.69

3.4 Automated SPPS of Jung-Redemann (H-WFTTLISTIM-NH_2) on 0.15 mmol scale

Automated SPPS of Jung-Redemann (H-WFTTLISTIM-NH_2) in DMF, repeated three times

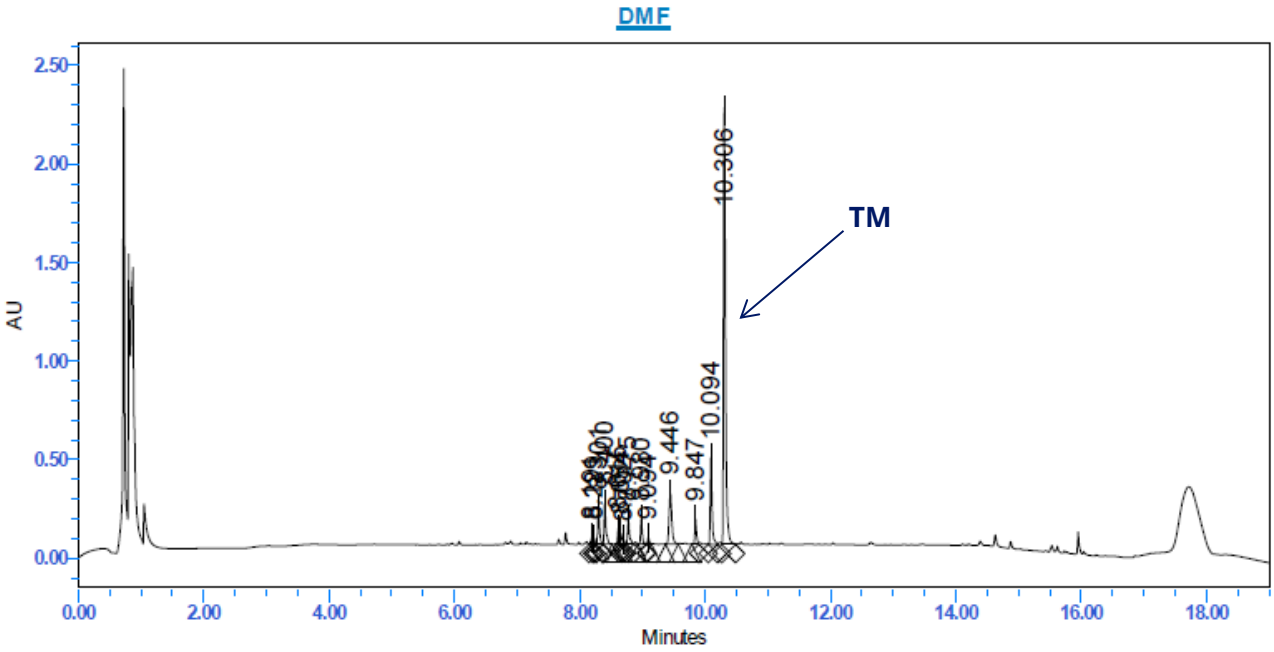
0.15 mmol scale, HPLC method E



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		8.109	34496	19014		0.46
2		8.174	25746	15222		0.34
3		8.229	49106	30644		0.66
4		8.300	381327	190882		5.11
5		8.400	418724	217319		5.61
6		8.617	106120	63483		1.42
7		8.656	128652	59712		1.72
8		8.703	37382	20582		0.50
9		8.777	205527	98590		2.75
10		8.982	200434	104627		2.68

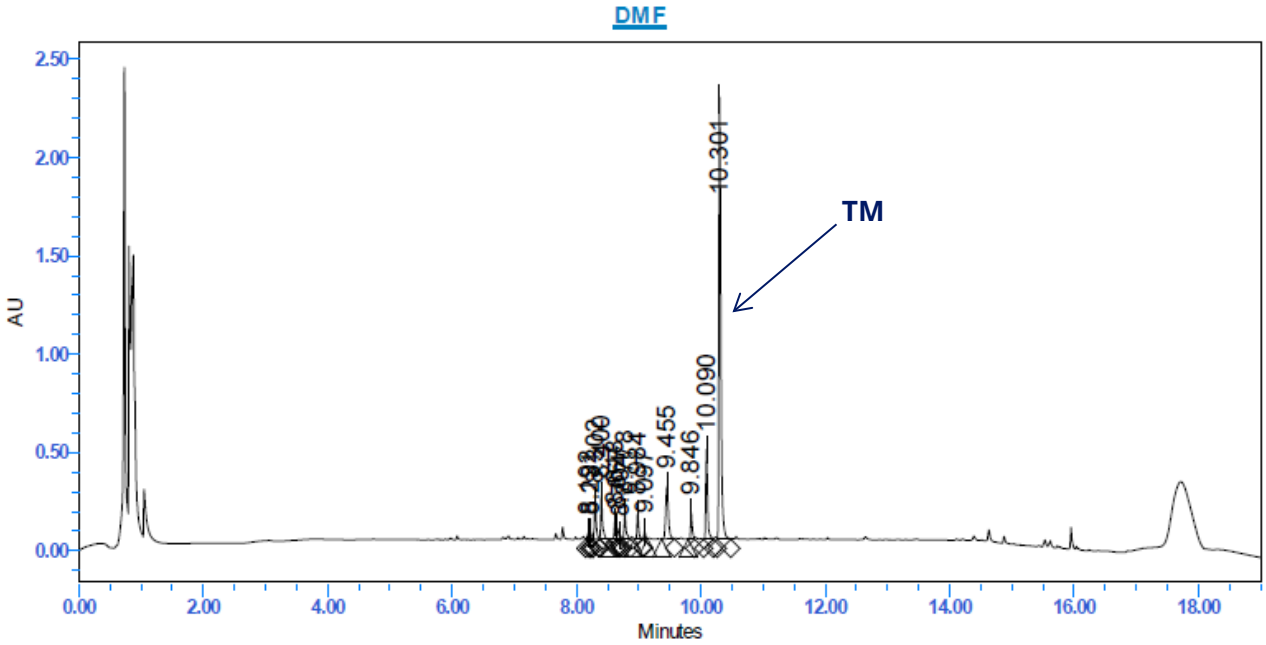
	Name	RT	Area	Height	Amount	% Area
11		9.095	64649	33192		0.87
12		9.447	747843	244498		10.02
13		9.846	252849	135547		3.39
14		10.092	706640	358829		9.46
15		10.303	4107327	1942785		55.01



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		8.191	60631	32325		0.72
2		8.229	54993	30134		0.65
3		8.301	356998	179892		4.22
4		8.400	398577	205338		4.72
5		8.615	132089	78585		1.56
6		8.654	121061	54822		1.43
7		8.702	37332	21052		0.44
8		8.775	260956	127259		3.09
9		8.980	226079	124219		2.68
10		9.094	54408	32327		0.64

	Name	RT	Area	Height	Amount	% Area
11		9.446	787325	256477		9.32
12		9.847	231513	126592		2.74
13		10.094	856818	438575		10.14
14		10.306	4872378	2216608		57.65



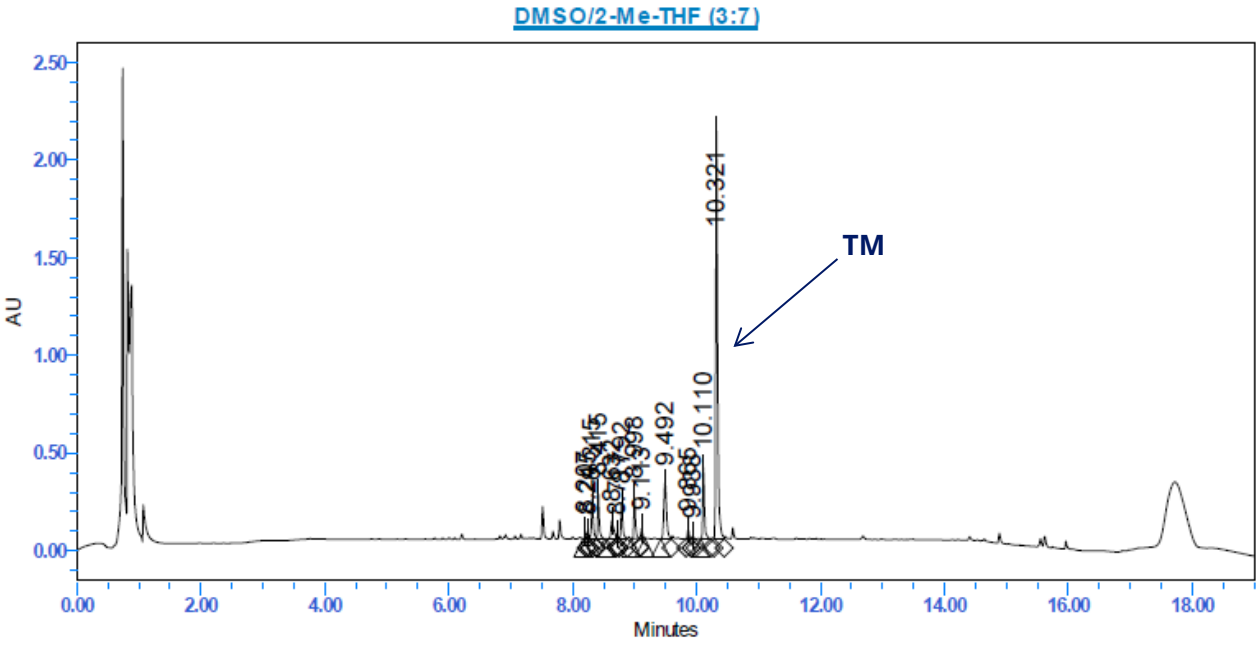
Peak Results

	Name	RT	Area	Height	Amount	% Area
1		8.192	63154	33925		0.73
2		8.231	59485	32664		0.68
3		8.302	381764	193034		4.39
4		8.400	418529	217579		4.81
5		8.618	151200	84733		1.74
6		8.657	114802	54911		1.32
7		8.704	36476	20100		0.42
8		8.778	271339	133233		3.12
9		8.984	228848	126404		2.63
10		9.097	56214	33677		0.65

	Name	RT	Area	Height	Amount	% Area
11		9.455	807845	264874		9.28
12		9.846	245056	134080		2.82
13		10.090	887888	455997		10.20
14		10.301	4980822	2248950		57.23

Automated SPPS of Jung-Redemann (H-WFTTLISTIM-NH_2) in DMSO/2-Me-THF (3:7), repeated two times

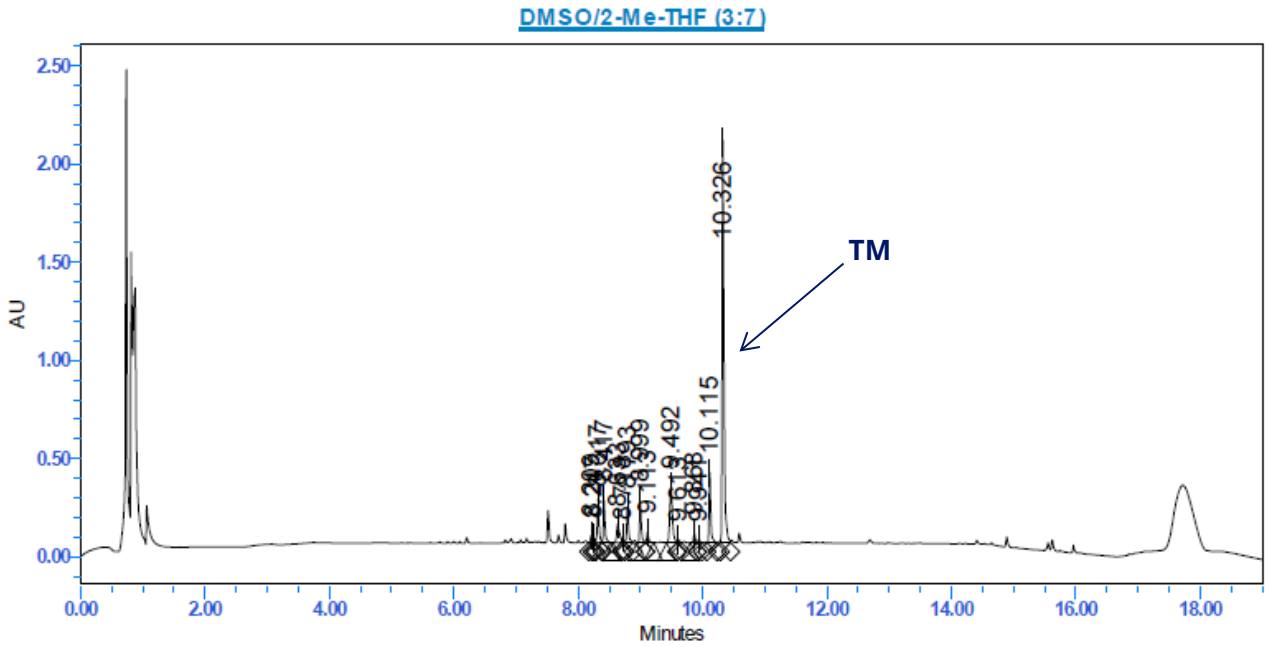
0.15 mmol scale, HPLC method E



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		8.207	58706	38321		0.72
2		8.245	54639	31032		0.67
3		8.315	417362	205904		5.09
4		8.415	410923	230618		5.02
5		8.632	295356	94930		3.61
6		8.717	47955	26363		0.59
7		8.792	363919	185614		4.44
8		8.998	385297	211611		4.70
9		9.113	86563	50050		1.06
10		9.492	875050	285332		10.68

	Name	RT	Area	Height	Amount	% Area
11		9.865	91516	49000		1.12
12		9.938	31745	16655		0.39
13		10.110	698694	366033		8.53
14		10.321	4374852	2089189		53.40



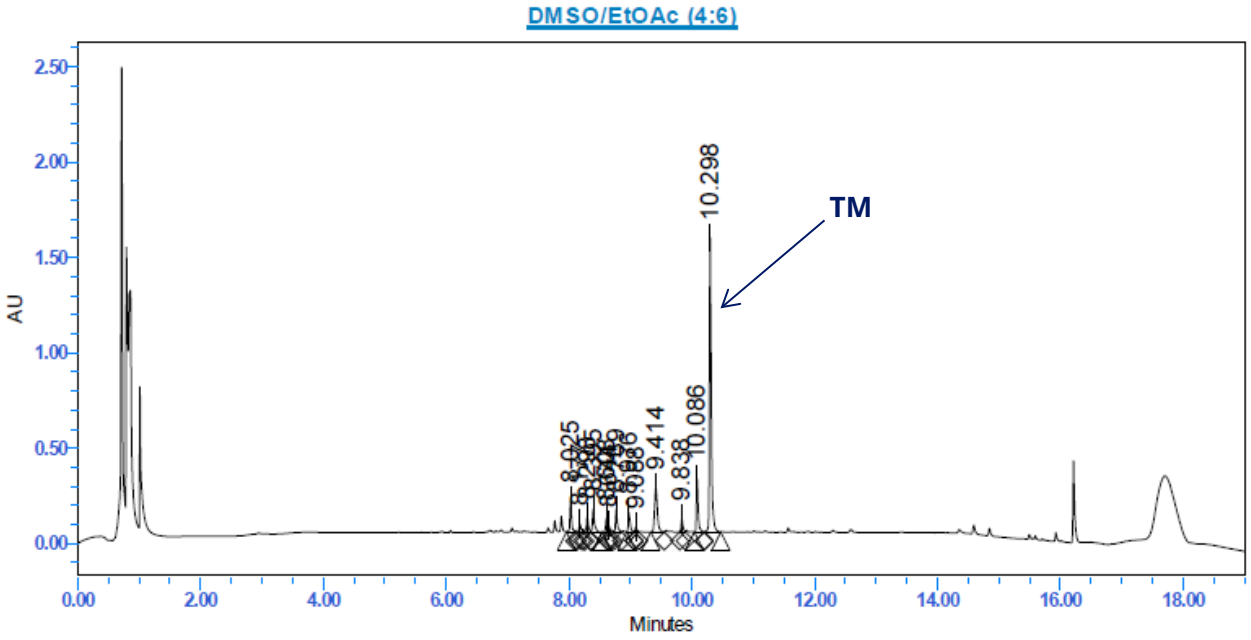
Peak Results

	Name	RT	Area	Height	Amount	% Area
1		8.209	56251	36485		0.70
2		8.247	50477	29002		0.63
3		8.317	389943	190675		4.87
4		8.417	381760	214755		4.77
5		8.633	289628	93480		3.62
6		8.718	44665	25011		0.56
7		8.793	355709	181228		4.45
8		8.999	389491	212230		4.87
9		9.113	98203	51538		1.23
10		9.492	868175	282461		10.85

	Name	RT	Area	Height	Amount	% Area
11		9.613	32988	15582		0.41
12		9.868	92760	47907		1.16
13		9.941	35696	18750		0.45
14		10.115	681691	356867		8.52
15		10.326	4232435	2046907		52.91

Automated SPPS of Jung-Redemann (H-WFTTLISTIM-NH_2) in DMSO/EtOAc (4:6), repeated two times

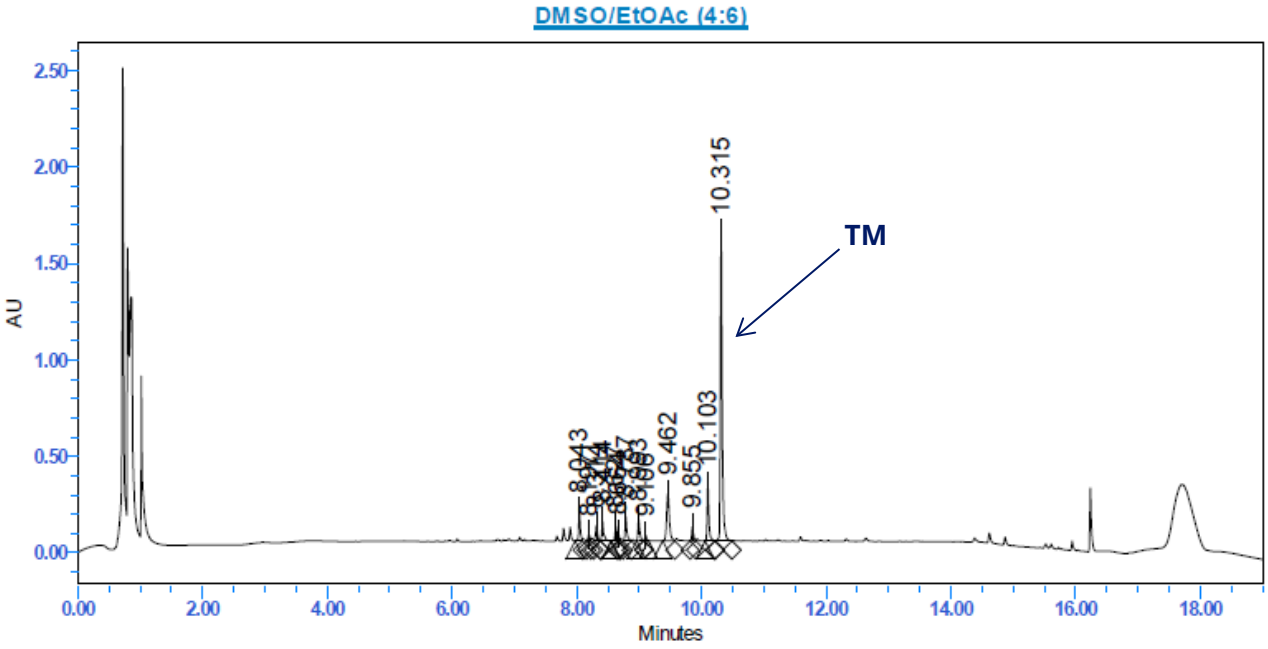
0.15 mmol scale, HPLC method E



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		8.025	306544	165412		4.98
2		8.177	91466	42478		1.48
3		8.298	173746	79057		2.82
4		8.395	276992	120423		4.50
5		8.608	140916	71640		2.29
6		8.644	64116	35302		1.04
7		8.769	234613	116272		3.81
8		8.976	212946	102264		3.46
9		9.088	48475	26212		0.79
10		9.414	741827	236606		12.04

	Name	RT	Area	Height	Amount	% Area
11		9.838	132640	68447		2.15
12		10.086	552492	279749		8.97
13		10.298	3183498	1545010		51.68



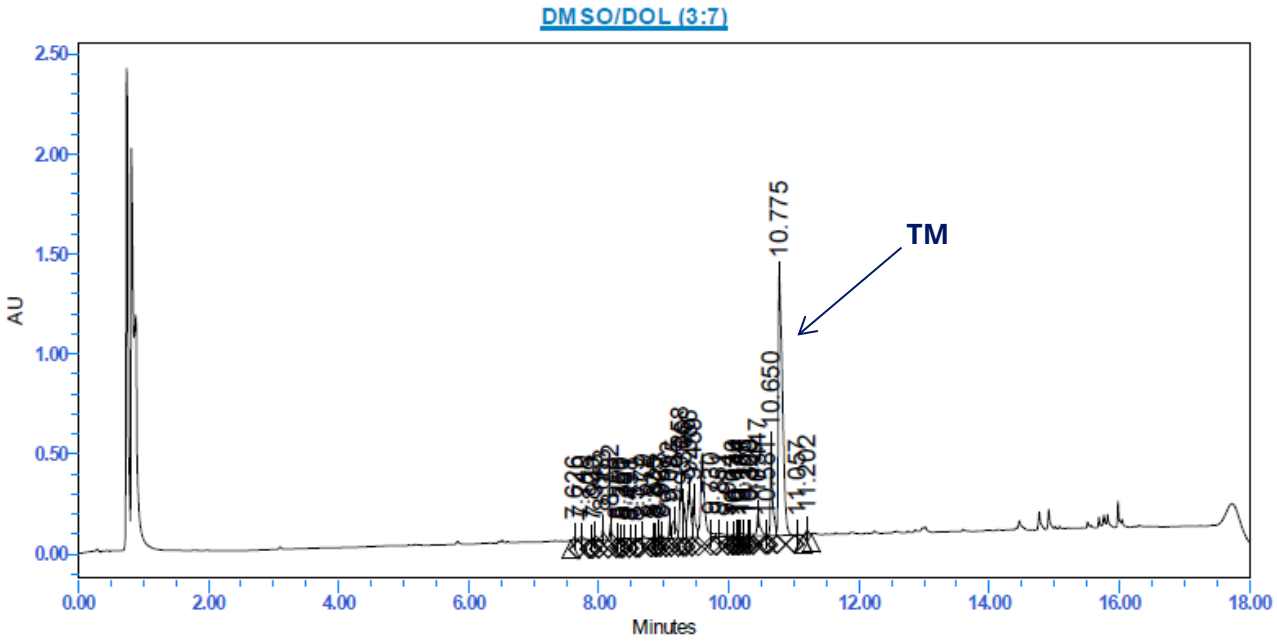
Peak Results

	Name	RT	Area	Height	Amount	% Area
1		8.043	269133	153284		4.36
2		8.197	68748	32309		1.11
3		8.314	173676	78668		2.82
4		8.414	214040	101253		3.47
5		8.627	161715	76447		2.62
6		8.664	59785	36958		0.97
7		8.787	252863	125606		4.10
8		8.993	217413	109254		3.52
9		9.106	44399	26567		0.72
10		9.462	756456	243886		12.26

	Name	RT	Area	Height	Amount	% Area
11		9.855	137881	71695		2.24
12		10.103	558898	287634		9.06
13		10.315	3253694	1602403		52.75

Automated SPPS of Jung-Redemann (H-WFTTLISTIM-NH_2) in DMSO/DOL (3:7), repeated three times

0.15 mmol scale, HPLC method E



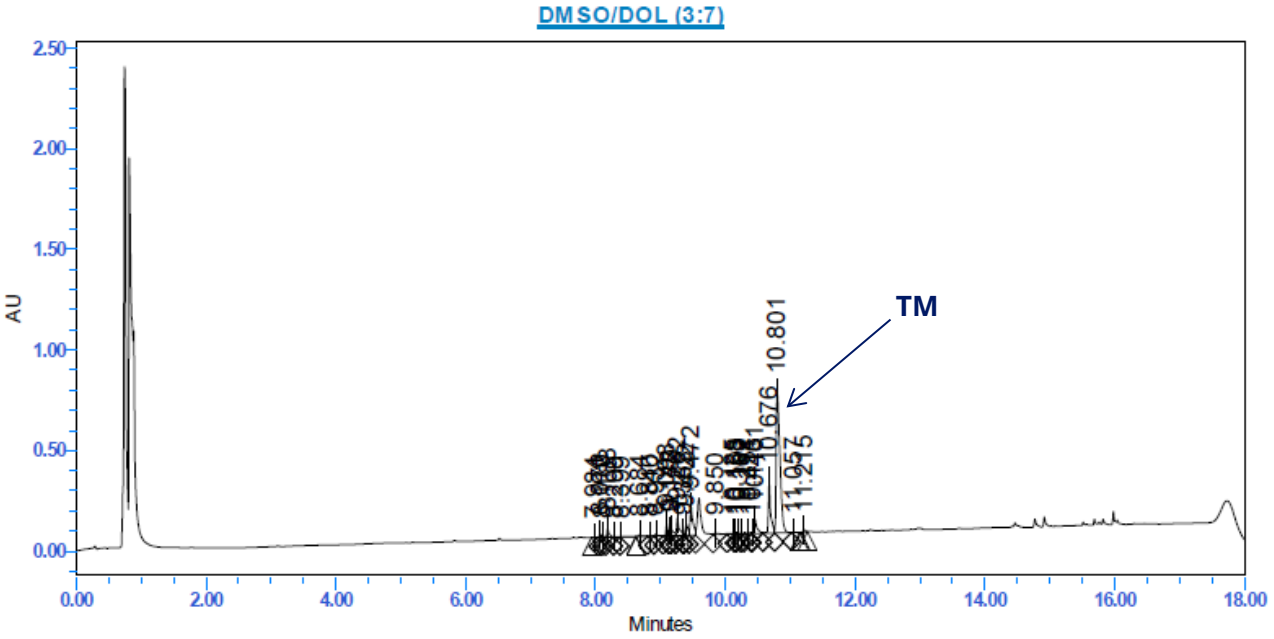
Peak Results

	Name	RT	Area	Height	Amount	% Area
1		7.626	55468	17648		0.48
2		7.740	107664	19391		0.93
3		7.899	14761	7319		0.13
4		7.943	80672	22673		0.69
5		8.063	182151	50975		1.57
6		8.182	200836	76249		1.73
7		8.296	24298	8862		0.21
8		8.350	14042	6184		0.12
9		8.391	23825	5478		0.21
10		8.491	14185	3191		0.12

	Name	RT	Area	Height	Amount	% Area
11		8.579	3335	1502		0.03
12		8.679	57956	17850		0.50
13		8.835	21171	10185		0.18
14		8.875	4639	3524		0.04
15		8.923	39075	22869		0.34
16		8.967	42501	15121		0.37
17		9.092	147447	82753		1.27
18		9.165	381296	90691		3.28
19		9.258	717153	256390		6.18
20		9.306	370067	176653		3.19

	Name	RT	Area	Height	Amount	% Area
21		9.386	839716	233705		7.23
22		9.459	537650	197037		4.63
23		9.730	81741	19648		0.70
24		9.851	148096	18870		1.28
25		9.984	41670	10213		0.36
26		10.070	17262	9400		0.15
27		10.114	29526	12063		0.25
28		10.144	21523	11947		0.19
29		10.187	44933	15570		0.39

30		10.228	32323	10551		0.28
31		10.300	23551	9514		0.20
32		10.326	28981	9044		0.25
33		10.447	391160	113195		3.37
34		10.581	20486	8377		0.18
35		10.650	1084200	447299		9.34
36		10.775	5688146	1297160		48.98
37		11.057	14604	4785		0.13
38		11.202	64787	23571		0.56

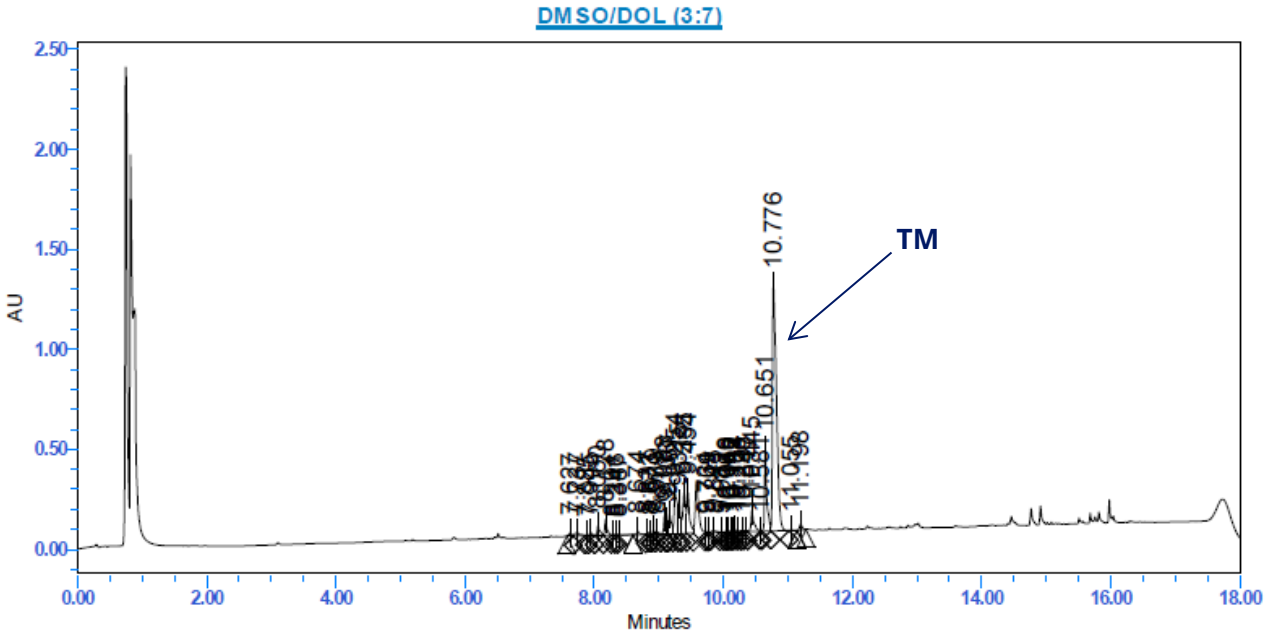


Peak Results

	Name	RT	Area	Height	Amount	% Area
1		7.984	8242	2435		0.16
2		8.072	28832	17098		0.56
3		8.110	19035	7524		0.37
4		8.188	98645	43934		1.93
5		8.299	5580	1620		0.11
6		8.399	5387	1137		0.11
7		8.684	23990	8969		0.47
8		8.840	10410	3914		0.20
9		8.932	28424	11572		0.56
10		9.098	101400	57183		1.99

	Name	RT	Area	Height	Amount	% Area
11		9.142	49810	26521		0.98
12		9.179	80789	26967		1.58
13		9.262	236075	86277		4.62
14		9.353	14768	12457		0.29
15		9.402	153594	54210		3.01
16		9.472	415874	140436		8.14
17		9.850	50699	6854		0.99
18		10.125	21523	4321		0.42
19		10.158	7984	4582		0.16
20		10.193	19999	6023		0.39

	Name	RT	Area	Height	Amount	% Area
21		10.262	11396	4196		0.22
22		10.351	27312	5401		0.53
23		10.415	4232	4144		0.08
24		10.461	204559	69181		4.00
25		10.676	625346	258835		12.24
26		10.801	2817982	704510		55.17
27		11.057	8811	2727		0.17
28		11.215	26987	10785		0.53



Peak Results

	Name	RT	Area	Height	Amount	% Area
1		7.627	54541	16791		0.52
2		7.737	104501	18690		1.00
3		7.885	12127	4866		0.12
4		7.945	48800	15422		0.47
5		8.060	145848	48431		1.39
6		8.178	187249	79735		1.79
7		8.291	10506	4557		0.10
8		8.346	7176	3521		0.07
9		8.387	11196	2507		0.11
10		8.674	48003	18052		0.46

	Name	RT	Area	Height	Amount	% Area
11		8.831	19040	10247		0.18
12		8.873	5852	3426		0.06
13		8.919	41104	22748		0.39
14		8.979	34404	11159		0.33
15		9.088	156006	87572		1.49
16		9.130	113215	65527		1.08
17		9.167	292707	96831		2.79
18		9.254	711528	200028		6.78
19		9.315	334644	150198		3.19
20		9.395	659483	203519		6.29

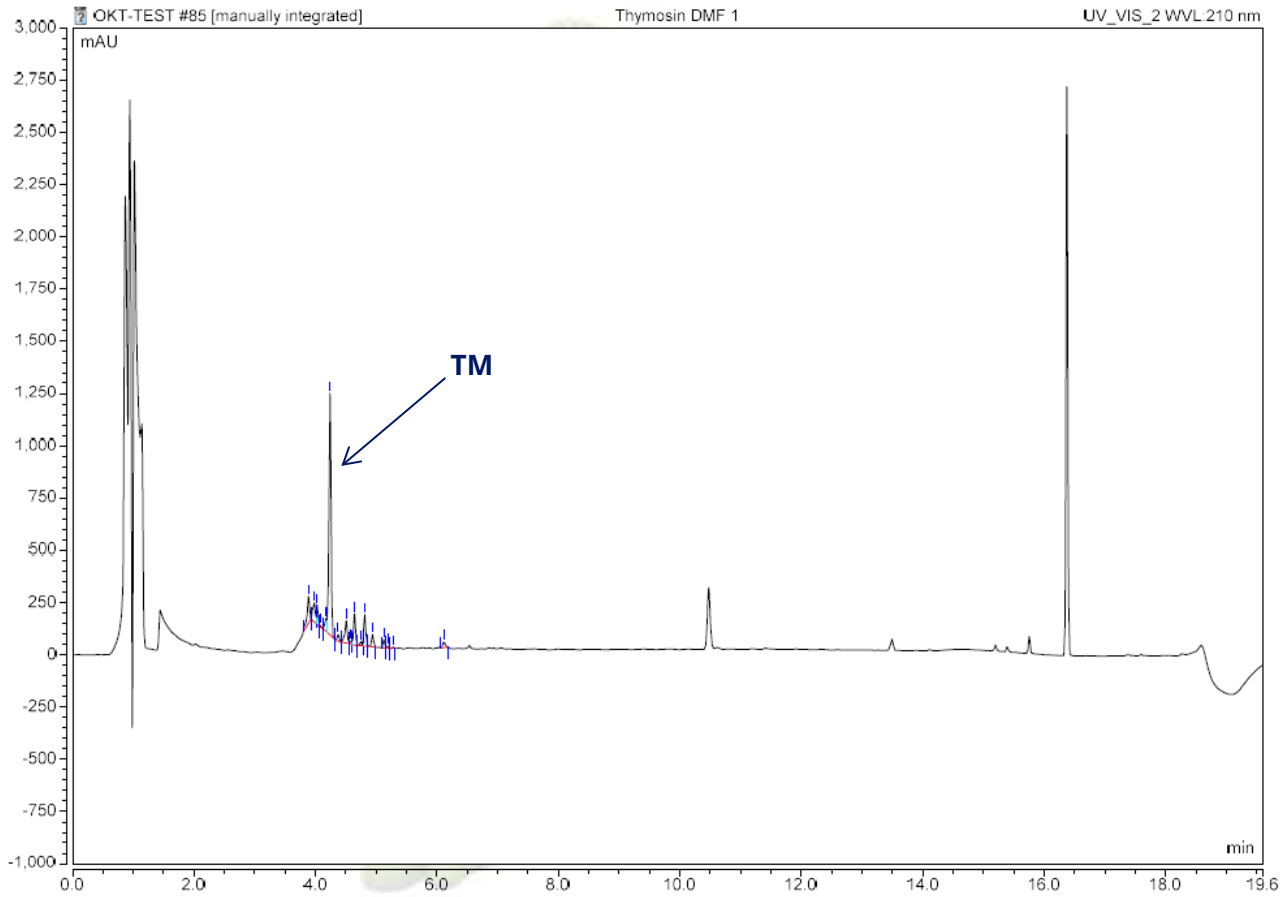
	Name	RT	Area	Height	Amount	% Area
21		9.454	528153	202828		5.04
22		9.734	16704	6776		0.16
23		9.769	11568	5593		0.11
24		9.845	61258	11281		0.58
25		9.982	11933	3624		0.11
26		10.048	8854	3847		0.08
27		10.069	8661	4252		0.08
28		10.112	15535	7156		0.15
29		10.142	11946	7148		0.11
30		10.184	29798	11424		0.28

31		10.223	17018	6204		0.16
32		10.296	13086	5299		0.12
33		10.347	21041	6120		0.20
34		10.445	344435	107221		3.28
35		10.581	10770	5417		0.10
36		10.651	994480	411306		9.48
37		10.776	5286146	1224908		50.40
38		11.055	14916	5096		0.14
39		11.198	84435	29598		0.81

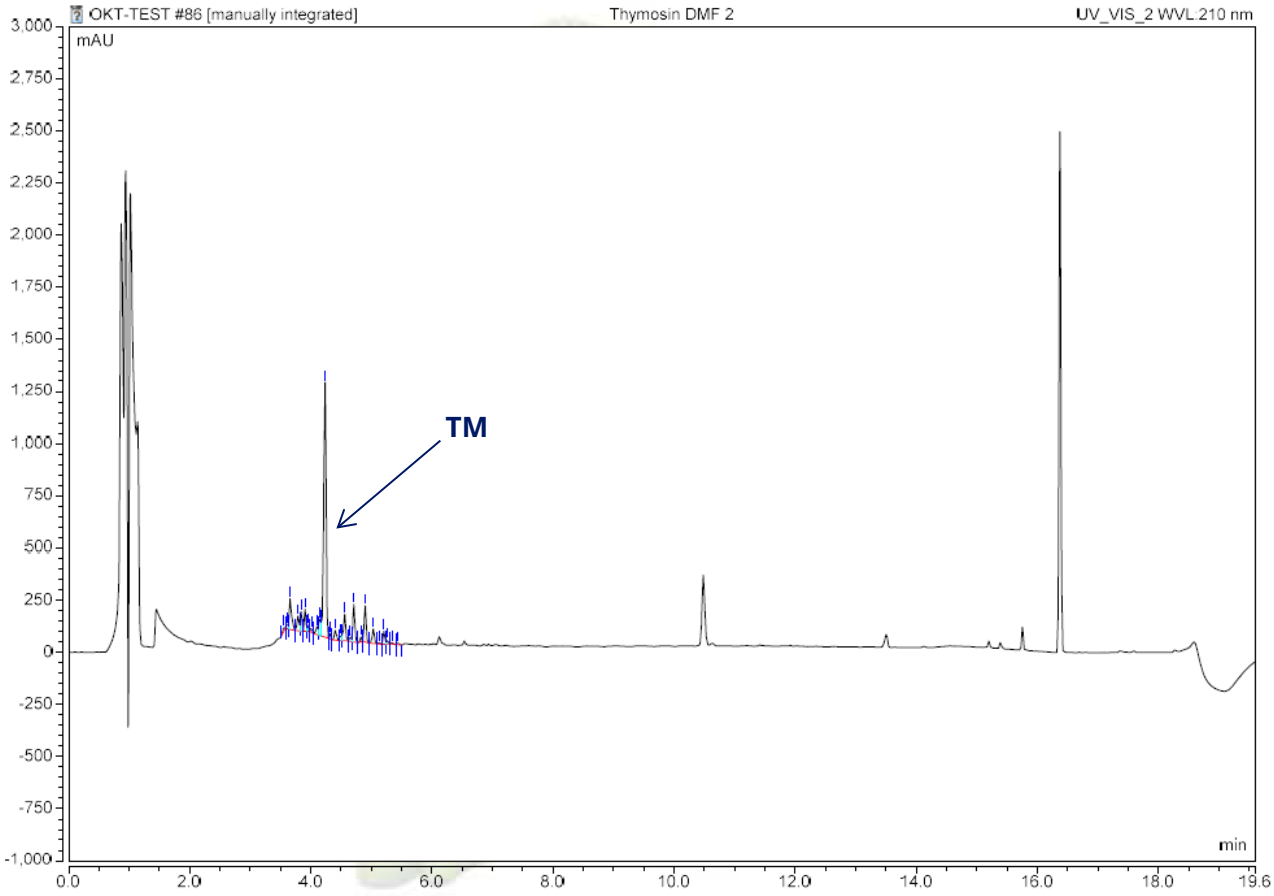
3.5 Automated SPPS of Thymosin α1 (Ac-SDAAVDTSSEITTKDLKEKKEVVEEAEN-NH_2) on 0.15 mmol scale

Automated SPPS of Thymosin α1 (Ac-SDAAVDTSSEITTKDLKEKKEVVEEAEN-NH_2) in DMF, repeated three times

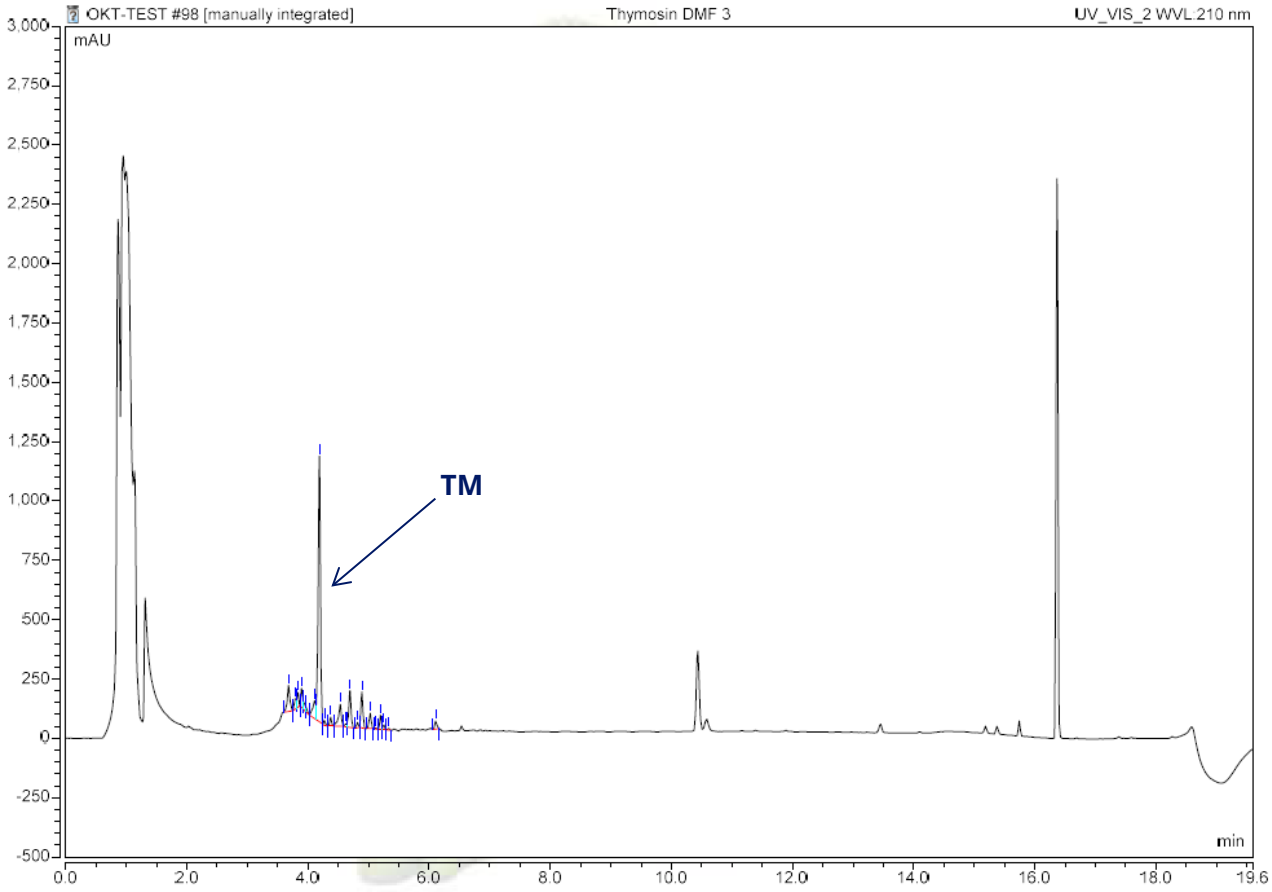
0.15 mmol scale, HPLC method C



Peak No.	Ret.Time	Area	Height	Rel.Area	Peak No.	Ret.Time	Area	Height	Rel.Area
1	3.84	0.7332	25.151	0.85	10	4.64	5.2459	148.233	6.06
2	3.88	5.5793	132.34	6.44	11	4.75	0.4728	16.747	0.55
3	3.98	3.9593	88.874	4.57	12	4.81	5.2939	148.268	6.11
4	4.03	3.0299	89.793	3.5	13	4.94	2.0735	58.518	2.39
5	4.09	0.2537	11.655	0.29	14	5.12	1.3961	42.302	1.61
6	4.24	47.3865	1160.319	54.73	15	5.19	0.3918	13.146	0.45
7	4.37	1.3048	33.896	4.25	16	6.11	1.2661	30.186	1.46
8	4.51	4.7557	106.845	5.49	17	6.53	0.6027	16.384	0.7
9	4.58	0.4675	12.295	0.54					



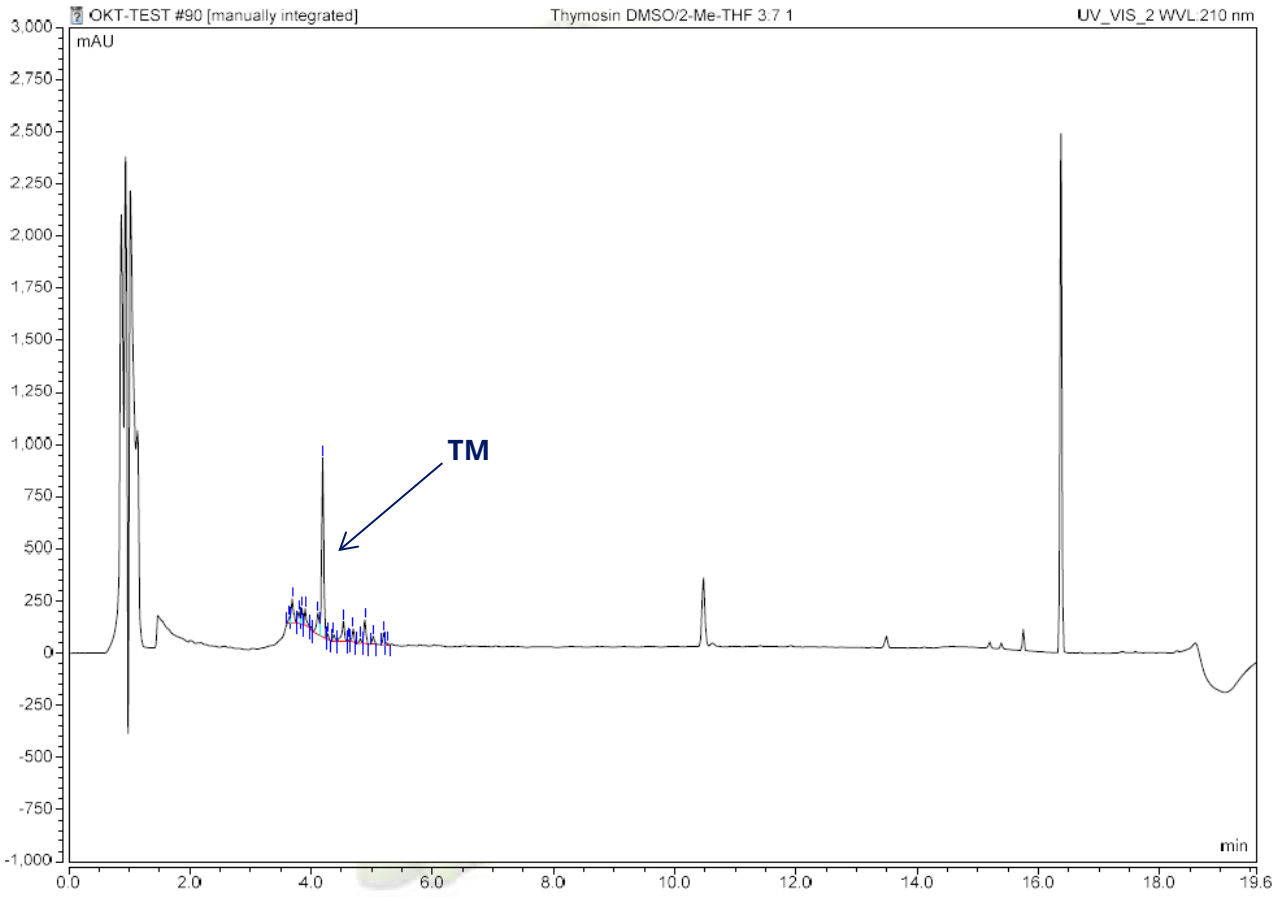
Peak No.	Ret.Time	Area	Height	Rel.Area	Peak No.	Ret.Time	Area	Height	Rel.Area
1	3.56	1.7438	42.575	1.52	13	4.40	1.8519	45.581	1.62
2	3.61	2.0403	45.063	1.78	14	4.49	0.728	23.988	0.64
3	3.66	7.7429	162.458	6.76	15	4.56	5.2233	126.536	4.56
4	3.78	2.7141	69.056	2.37	16	4.65	0.6751	18.072	0.59
5	3.84	2.9126	92.249	2.54	17	4.71	6.6722	181.171	5.83
6	3.90	3.3962	106.67	2.97	18	4.83	1.0109	28.677	0.88
7	3.95	0.8263	31.53	0.72	19	4.90	6.7099	178.624	5.86
8	4.01	0.8525	23.114	0.74	20	5.03	2.6347	70.981	2.3
9	4.10	1.3959	36.629	1.22	21	5.21	1.9976	61.107	1.74
10	4.14	3.44	78.034	3	22	5.26	0.6264	19.64	0.55
11	4.23	56.1403	1220.713	49.03	23	6.12	1.8808	37.287	1.64
12	4.31	0.5514	23.197	0.48	24	6.53	0.7453	20.331	0.65



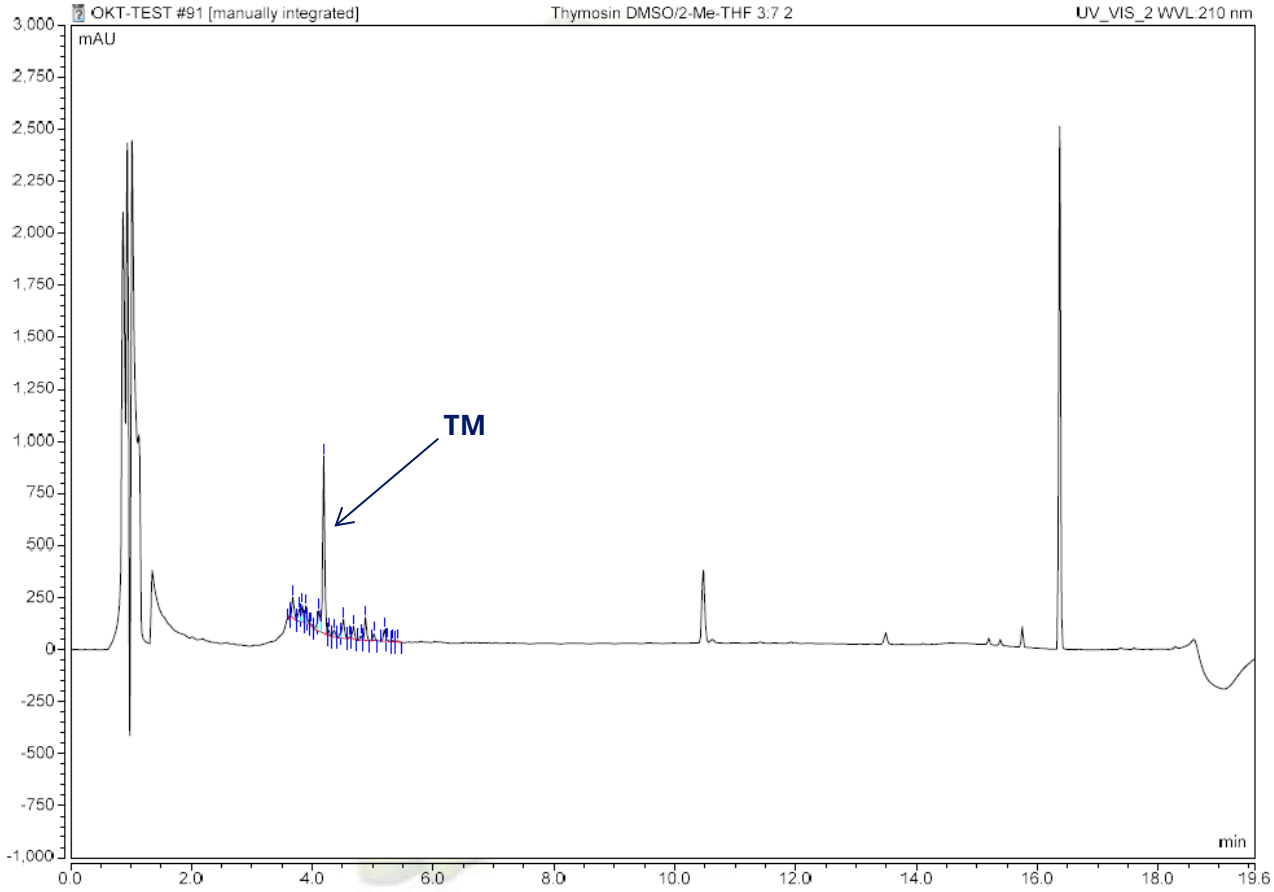
Peak No.	Ret.Time	Area	Height	Rel.Area	Peak No.	Ret.Time	Area	Height	Rel.Area
1	3.57	0.3287	9.565	5.8	11	4.49	0.4463	13.686	0.76
2	3.78	1.5279	41.035	2.6	12	4.55	2.791	69.865	4.74
3	3.83	1.6275	53.543	2.77	13	4.64	0.4063	10.415	0.69
4	3.89	1.5849	54.464	2.69	14	4.70	3.9761	110.304	6.76
5	3.94	0.5737	15.408	1.71	15	4.82	0.4336	13.982	0.74
6	4.09	0.8489	20.908	1.44	16	4.89	3.3707	91.981	5.73
7	4.14	2.1064	45.083	3.58	17	5.03	1.2831	37.906	2.18
8	4.22	31.4377	777.429	53.42	18	5.20	0.9031	30.027	1.53
9	4.31	0.4081	14.221	0.69	19	5.26	0.2437	9.104	0.41
10	4.4	1.0317	24.574	1.75					

Automated SPPS of Thymosin α1 (Ac-SDAAVDTSSEITTKDLKEKKEVVEEAEN-NH_2) in DMSO/2-MeTHF (3:7), repeated two times

0.15 mmol scale, HPLC method C



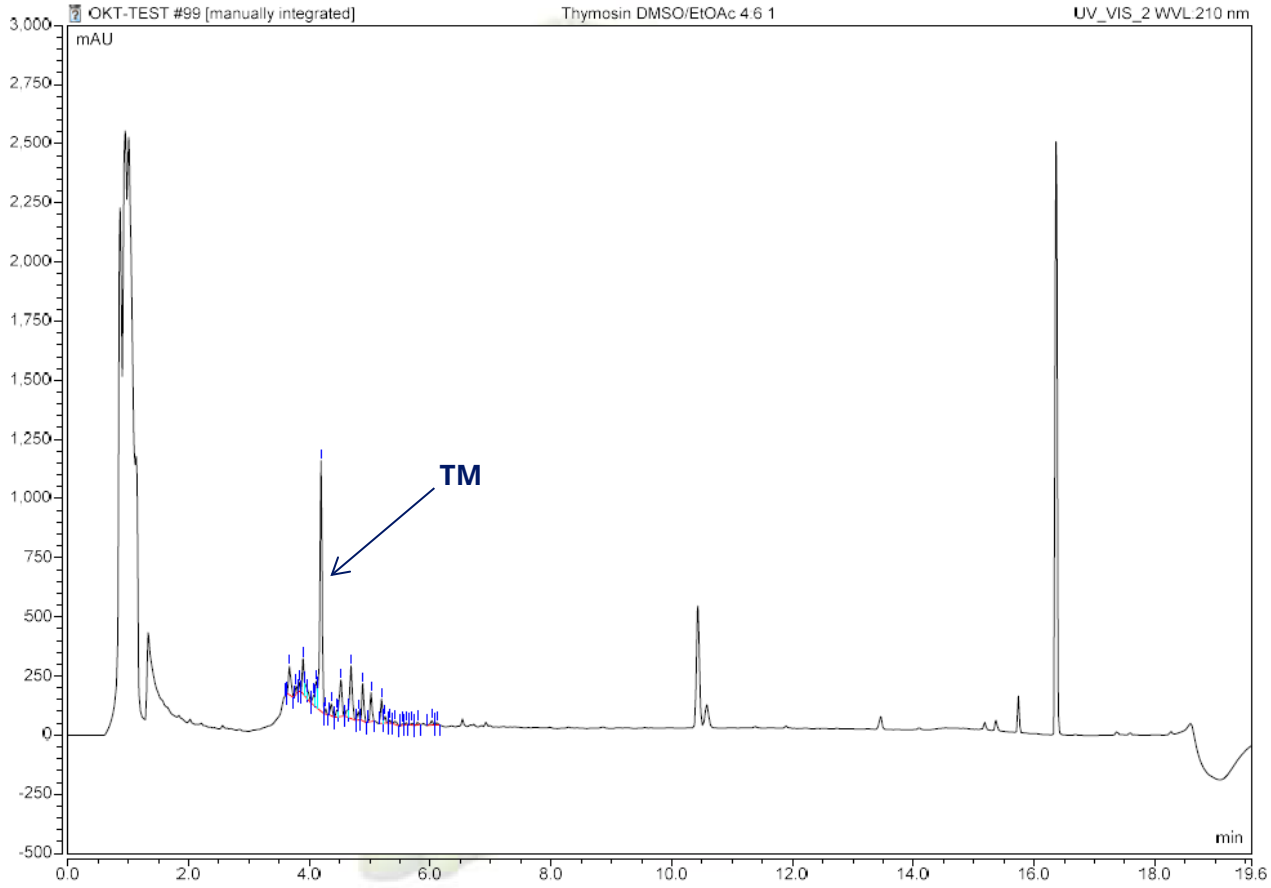
Peak No.	Ret.Time	Area	Height	Rel.Area	Peak No.	Ret.Time	Area	Height	Rel.Area
1	3.61	1.2303	31.87	1.68	11	4.27	0.8164	27.272	1.11
2	3.64	2.1509	56.174	2.93	12	4.37	1.4021	33.26	1.91
3	3.69	5.9887	130.155	8.16	13	4.53	4.6147	93.644	6.29
4	3.79	2.1694	54.7	2.96	14	4.62	0.2193	8.742	0.3
5	3.84	2.0834	72.185	2.84	15	4.69	2.1958	62.526	2.99
6	3.9	2.4845	75.541	3.39	16	4.81	0.6551	21.031	0.89
7	3.99	0.496	13.9	0.68	17	4.89	4.1945	112.566	5.72
8	4.09	2.62	53.0	3.63	18	5.02	1.377	38.702	1.88
9	4.11	2.7002	51.1	3.62	19	5.19	1.6985	53.973	2.31
10	4.19	34.2821	858.734	46.72					



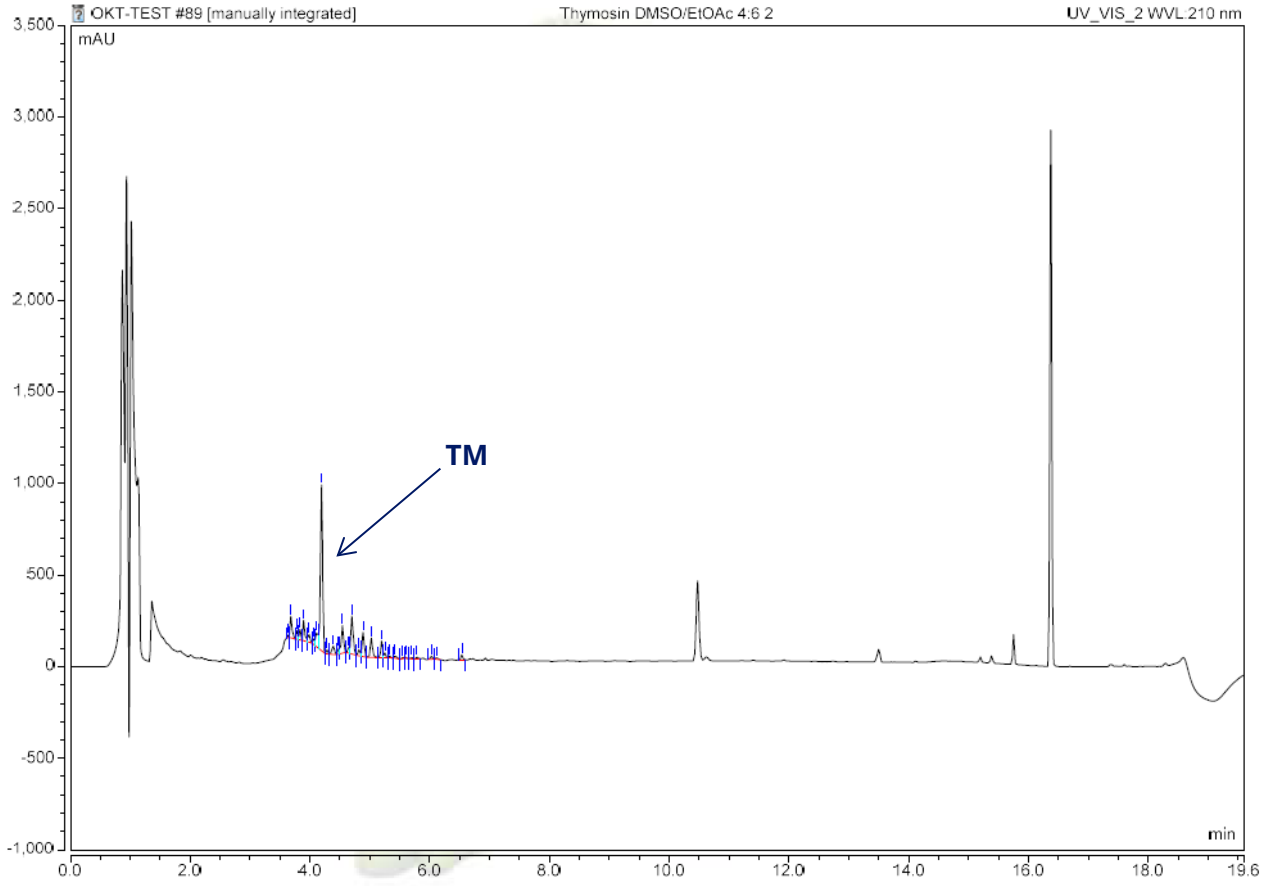
Peak No.	Ret.Time	Area	Height	Rel.Area	Peak No.	Ret.Time	Area	Height	Rel.Area
1	3.58	1.6721	40.303	2.3	12	4.47	0.6432	20.517	0.89
2	3.62	2.1489	55.319	2.96	13	4.51	3.8999	90.158	5.37
3	3.67	5.6283	121.941	7.76	14	4.61	0.197	8.074	0.27
4	3.78	1.7729	48.119	2.44	15	4.68	2.2154	61.512	3.05
5	3.83	2.0272	72.193	2.79	16	4.80	0.701	21.556	0.97
6	3.89	2.3772	74.952	3.28	17	4.88	4.1338	109.921	5.7
7	3.98	0.3307	10.713	0.46	18	5.02	1.3196	37.115	1.82
8	4.11	5.2387	100.617	7.22	19	5.19	1.6865	53.773	2.32
9	4.14	0.8686	28.915	1.2	20	5.33	0.1677	5.516	0.23
10	4.19	33.7401	852.736	46.5	21	5.41	0.3528	6.97	0.49
11	4.36	1.4447	33.768	1.99					

Automated SPPS of Thymosin α1 (Ac-SDAAVDTSSEITTKDLKEKKEVVEEAEN-NH_2) in DMSO/EtOAc (4:6), repeated two times

0.15 mmol scale, HPLC method C



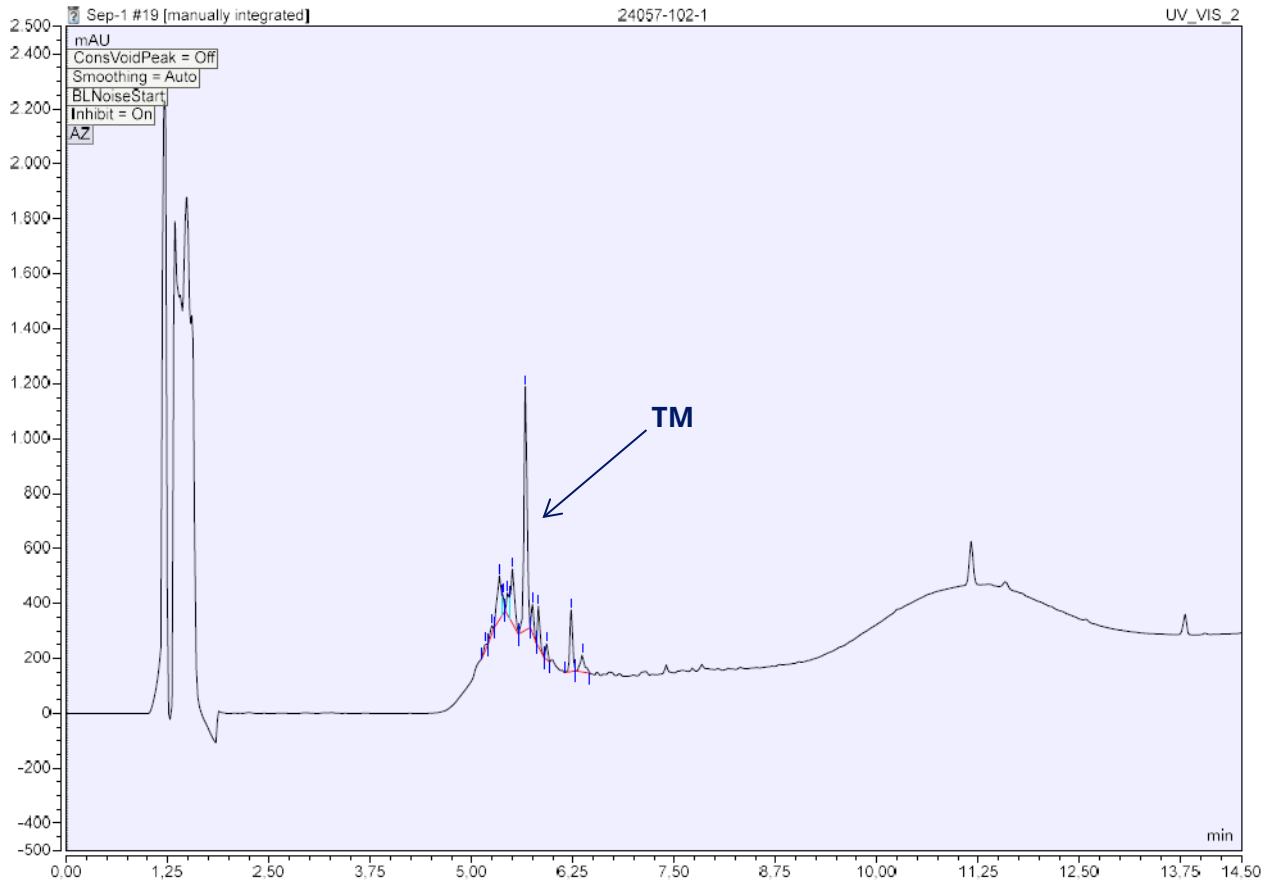
Peak No.	Ret.Time	Area	Height	Rel.Area	Peak No.	Ret.Time	Area	Height	Rel.Area
1	3.62	1.5749	19.076	2.36	12	4.48	0.9011	24.935	1.35
2	3.67	3.7084	89.749	5.55	13	4.55	4.5785	109.832	6.86
3	3.78	1.2974	35.861	1.94	14	4.67	0.9495	24.356	1.42
4	3.83	1.1545	43.442	1.73	15	4.7	6.4913	149.947	9.72
5	3.89	2.5915	83.843	3.88	16	4.82	0.5474	18.587	0.82
6	3.98	0.8721	26.21	1.31	17	4.89	3.4497	94.102	5.17
7	4.07	0.3329	11.065	0.5	18	5.03	2.3694	71.284	3.55
8	4.12	2.1217	47.656	3.18	19	5.19	2.0702	60.799	3.1
9	4.14	0.4827	16.399	0.72	20	5.25	0.6436	18.652	0.96
10	4.20	28.5535	710.22	42.76	21	5.34	0.2914	8.184	0.44
11	4.40	1.2742	31.055	1.91	22	5.42	0.5224	8.255	0.78



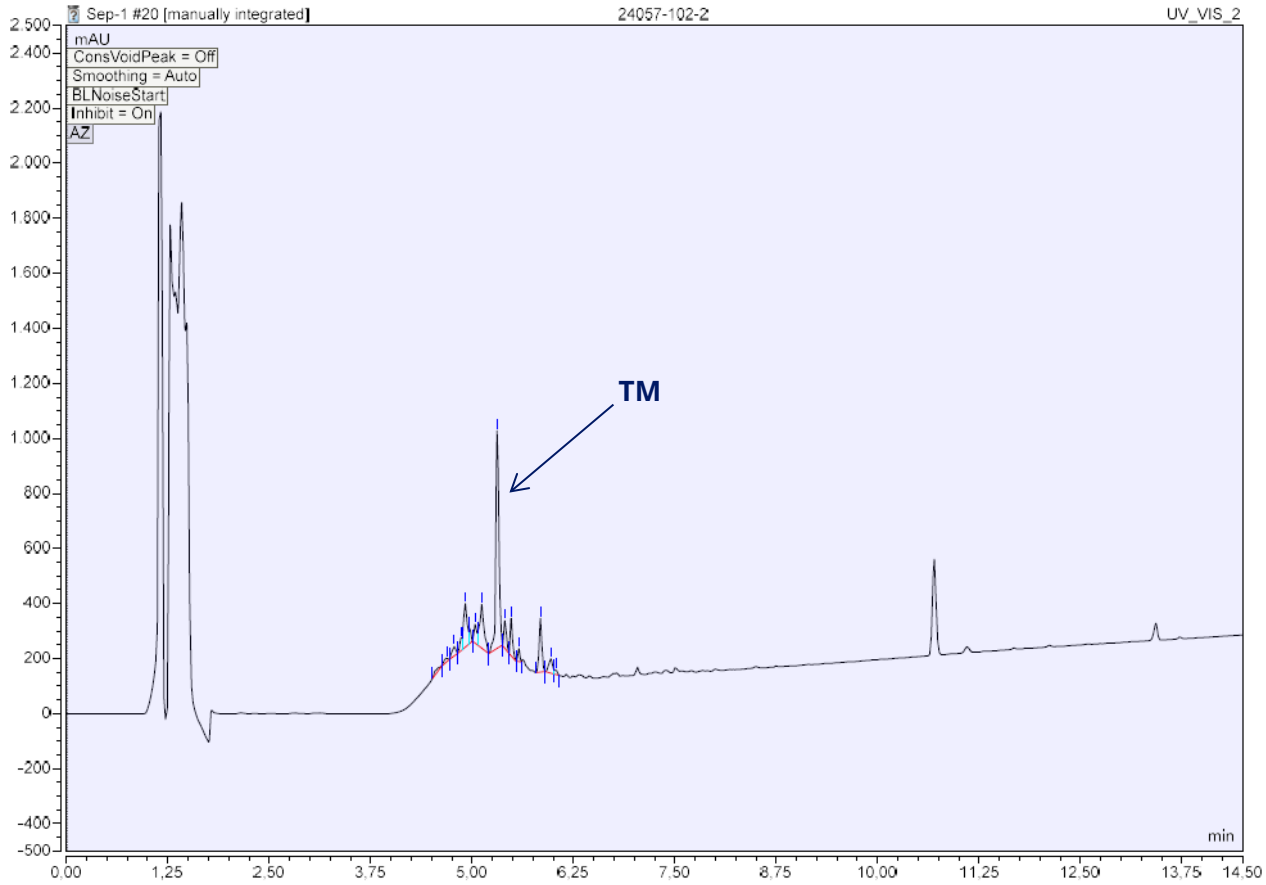
Peak No.	Ret.Time	Area	Height	Rel.Area	Peak No.	Ret.Time	Area	Height	Rel.Area
1	3.63	4.5726	57.659	4.63	12	4.47	1.2027	32.472	1.22
2	3.68	6.7336	144.839	6.82	13	4.54	6.3465	152.579	6.43
3	3.79	1.5988	48.655	1.62	14	4.64	1.0847	28.64	1.1
4	3.83	1.6341	57.768	1.65	15	4.7	9.4512	206.991	9.57
5	3.90	3.9358	111.174	3.98	16	4.82	0.855	28.002	0.87
6	3.98	1.5318	43.29	1.55	17	4.89	4.6709	125.881	4.73
7	4.07	1.1427	34.803	1.16	18	5.02	3.3485	98.182	3.39
8	4.11	4.3727	81.238	4.43	19	5.19	3.1685	91.59	3.21
9	4.19	38.1741	906.625	38.65	20	5.25	0.9147	27.034	0.93
10	4.28	0.6935	23.021	0.7	21	5.33	0.3361	10.572	0.34
11	4.39	2.2304	45.761	2.26	22	5.42	0.7756	13.512	0.79

Automated SPPS of Thymosin α1 (Ac-SDAAVDTSSEITTKDLKEKKEVVEEAEN-NH_2) in DMSO/DOL (3:7), repeated three times

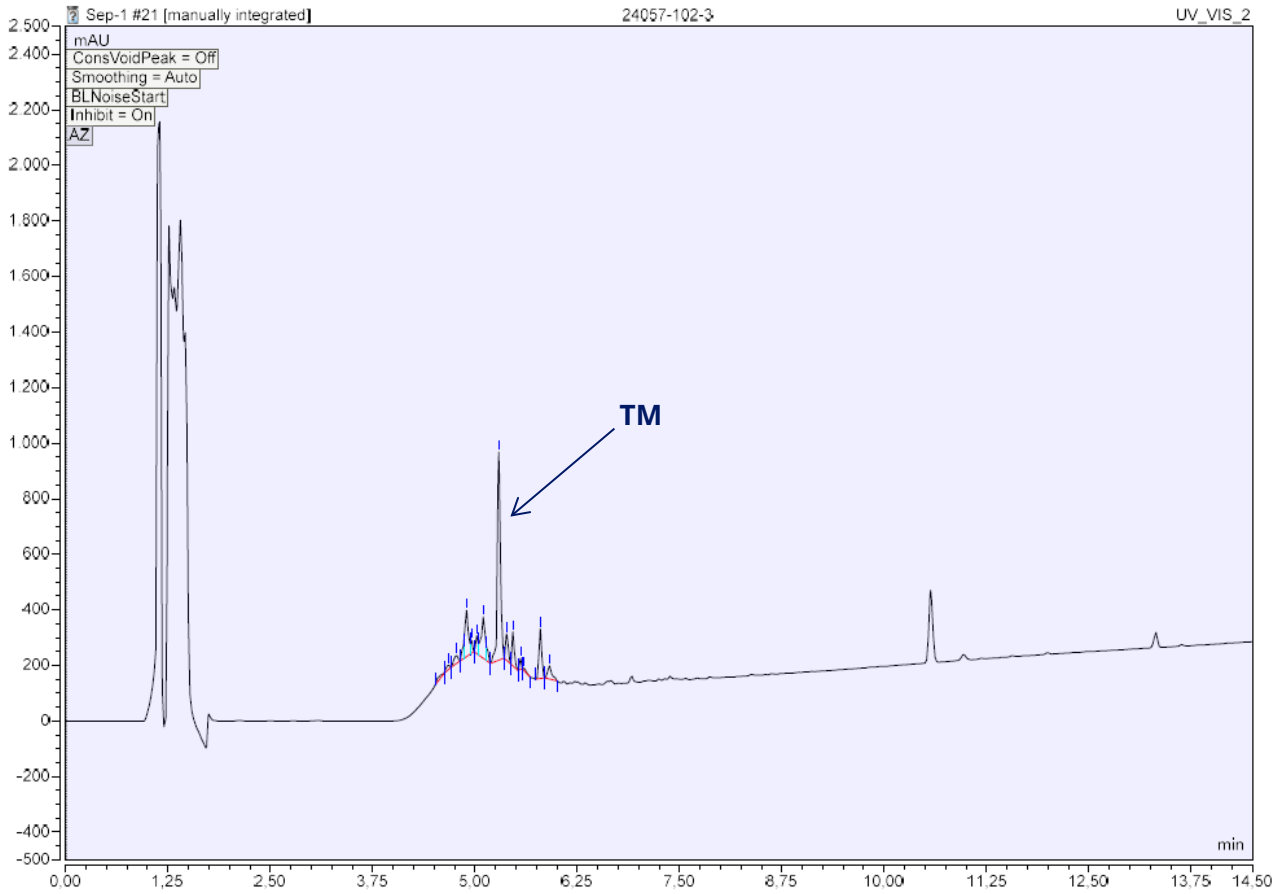
0.15 mmol scale, HPLC method C



Peak No.	Ret.Time	Area	Height	% Area
1	5.17	0.9003	19.973	1
2	5.25	1.2312	28.796	1.37
3	5.34	9.024	159.436	10.04
4	5.38	1.8937	71.831	2.11
5	5.44	3.4848	79.879	3.88
6	5.50	10.3877	198.33	11.56
7	5.66	39.6905	887.599	44.16
8	5.75	3.4996	103.851	3.89
9	5.82	5.0369	149.042	5.6
10	5.93	1.8593	57.903	2.07
11	6.23	8.9186	224.508	9.92
12	6.36	3.949	61.994	4.39



Peak No.	Ret.Time	Area	Height	Rel.Area
1	4.63	0.9592	0	1.17
2	4.69	0.8475	13.214	1.03
3	4.78	1.9482	34.668	2.37
4	4.87	1.5316	45.974	1.86
5	4.92	7.9101	159.726	9.63
6	4.97	1.5355	54.572	1.87
7	5.04	2.5787	68.095	3.14
8	5.12	10.1644	159.545	12.37
9	5.31	33.948	791.066	41.33
10	5.40	3.7952	100.125	4.62
11	5.48	4.4855	135.497	5.46
12	5.58	1.3579	47.55	1.65
13	5.84	7.6573	194.844	9.32
14	5.97	2.7922	52.961	3.4
15	6.03	0.6272	17.509	0.76

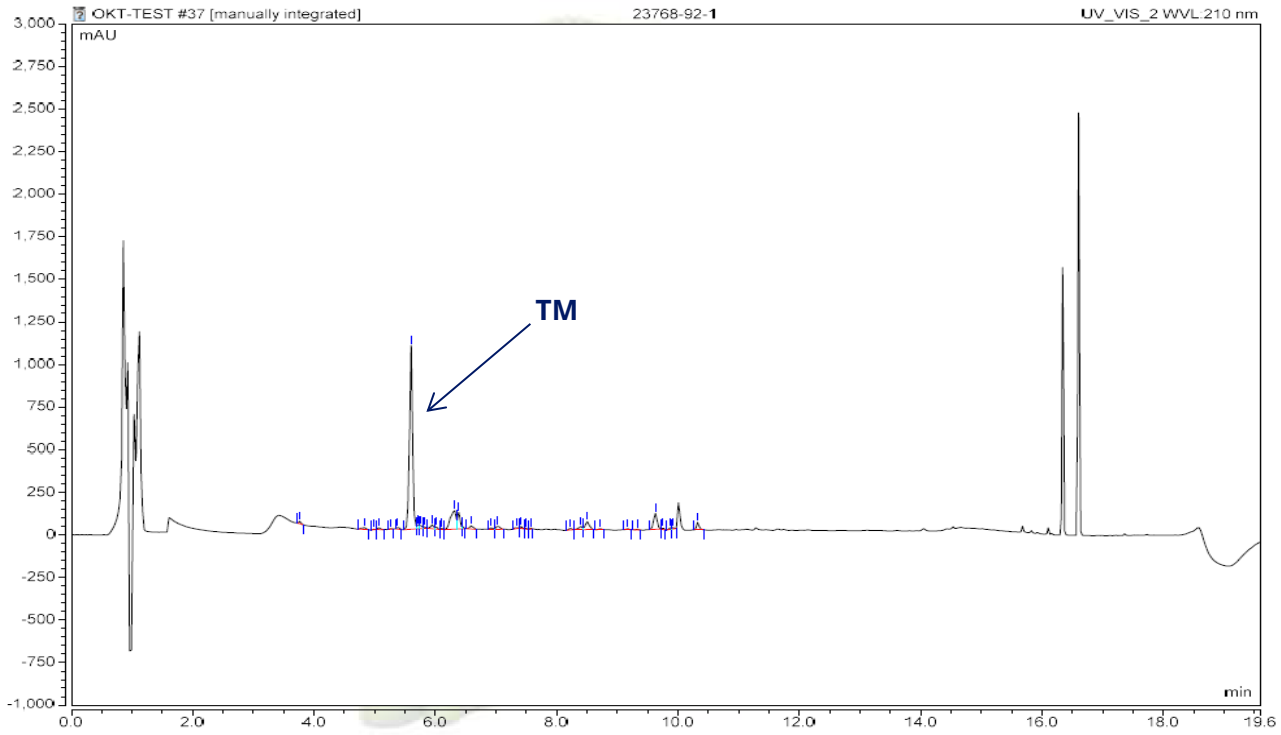


Peak No.	Ret.Time	Area	Height	Rel.Area
1	4.63	0.8304	0	1.09
2	4.67	0.8404	16.996	1.1
3	4.77	1.7718	32.671	2.32
4	4.86	1.22	47.144	1.6
5	4.90	7.8388	166.363	10.28
6	4.96	1.4519	48.09	1.9
7	5.02	2.4755	64.582	3.25
8	5.10	8.244	150.033	10.81
9	5.14	1.1167	46.431	1.46
10	5.29	31.8288	748.999	41.75
11	5.39	3.6393	93.8	4.77
12	5.46	4.003	122.163	5.25
13	5.56	1.025	39.43	1.34
14	5.60	0.3323	9.655	0.44
15	5.79	6.8775	178.318	9.02
16	5.91	2.7391	47.33	3.59

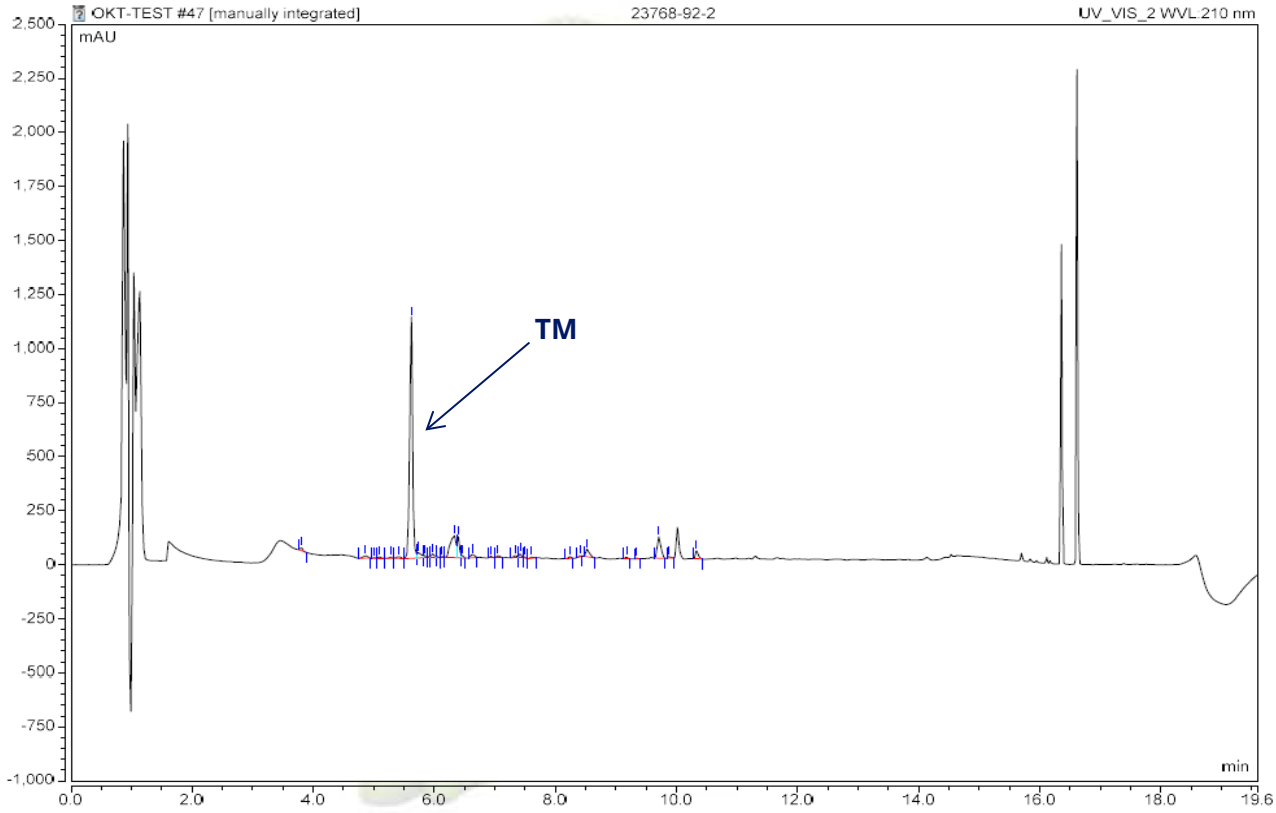
3.6 Automated SPPS of Dasiglucagon amide (H-HSQGTFTSDYSKYLD-Aib-ARAEEFVKWLEST-NH_2) on 0.15 mmol scale

Automated SPPS of Dasiglucagon amide (H-HSQGTFTSDYSKYLD-Aib-ARAEEFVKWLEST-NH_2) in DMF, repeated three times

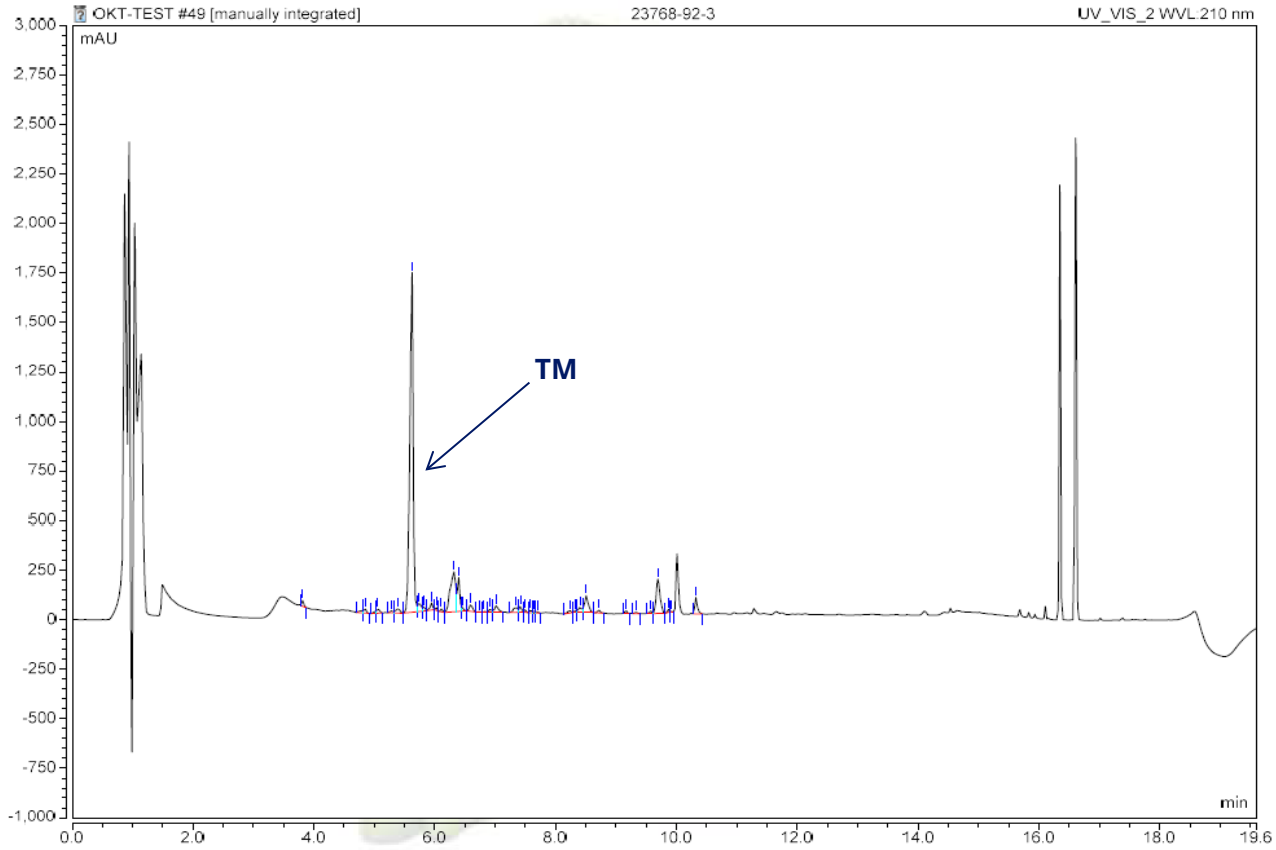
0.15 mmol scale, HPLC method D



Peak No.	Ret.Time	Area	Height	Rel.Area	Peak No.	Ret.Time	Area	Height	Rel.Area
1	3.77	0.6299	17.663	0.57	20	6.44	0.4794	12.583	0.43
2	4.78	0.3805	7.765	0.34	21	6.59	1.3155	18.739	1.19
3	4.83	0.6973	12.41	0.63	22	6.92	0.4443	8.719	0.4
4	4.98	0.1098	3.898	0.1	23	7.03	1.2708	18.895	1.15
5	5.06	0.6885	11.586	0.62	24	7.34	0.689	8.916	0.62
6	5.27	0.2031	5.729	0.18	25	7.41	0.6404	15.368	0.58
7	5.60	64.0796	1079.681	57.8	26	7.49	0.1857	5.695	0.17
8	5.65	0.1033	4.731	0.09	27	7.58	0.2475	6.465	0.22
9	5.67	0.7425	11.459	0.67	28	8.22	0.5209	8.969	0.47
10	5.72	0.8486	22.482	0.77	29	8.40	1.4246	19.004	1.28
11	5.75	0.8506	19.1	0.77	30	8.50	3.6675	49.091	3.31
12	5.81	0.4694	10.095	0.42	31	9.16	0.3354	8.596	0.3
13	5.91	0.066	3.01	0.06	32	9.33	0.174	4.321	0.16
14	5.94	0.692	16.882	0.62	33	9.75	0.3205	10.189	0.29
15	6.01	0.2762	8.343	0.25	34	9.86	0.6823	14.326	0.62
16	6.10	0.2817	8.004	0.25	35	9.92	0.7684	14.562	0.69
17	6.25	2.7688	69.622	2.5	36	10.01	7.5674	159.607	6.83
18	6.31	9.2478	110.909	8.34	37	10.24	0.0599	1.863	0.05
19	6.39	4.5773	97.806	4.13	38	10.32	2.3595	45.264	2.13



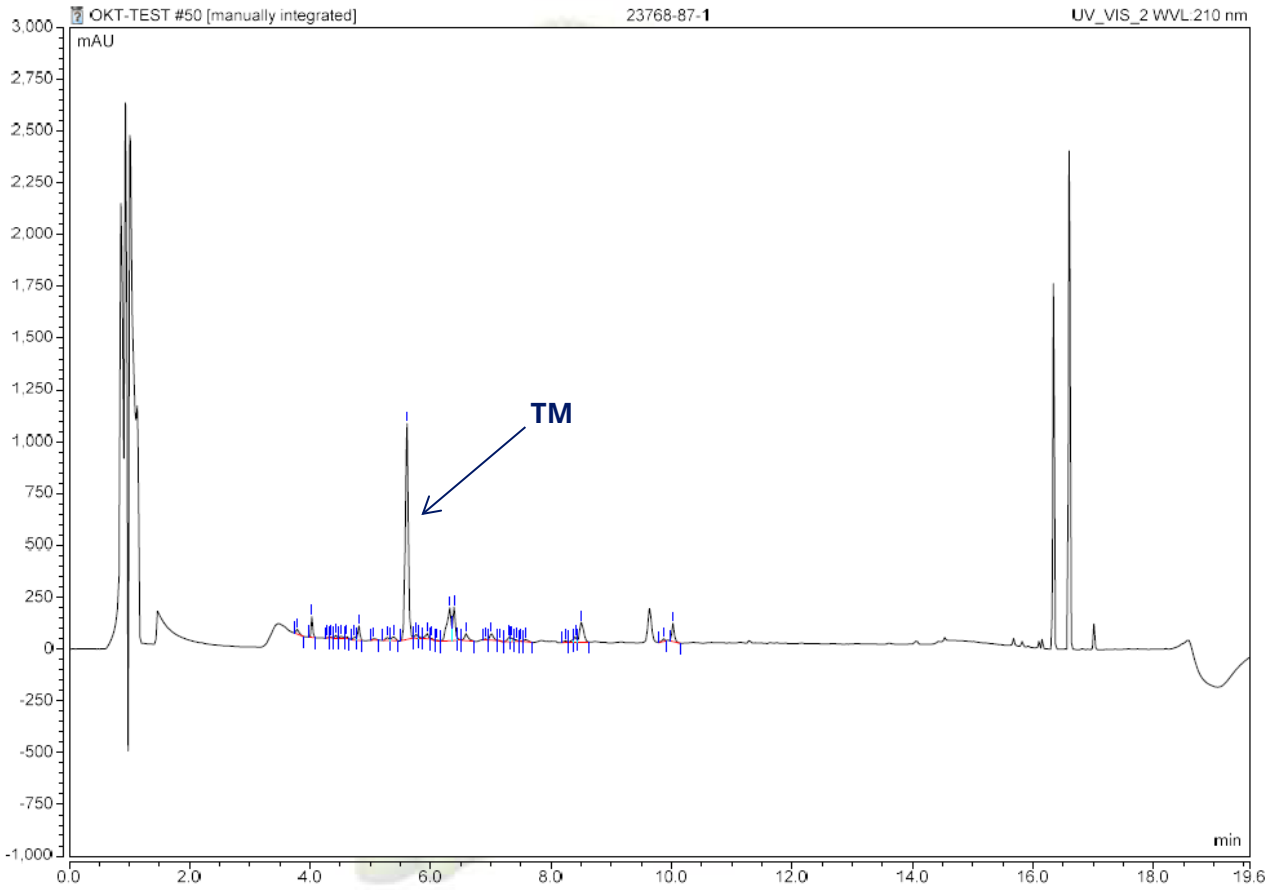
Peak No.	Ret.Time	Area	Height	Rel.Area	Peak No.	Ret.Time	Area	Height	Rel.Area
1	3.80	0.6317	16.563	0.61	19	6.82	0.0541	1.468	0.05
2	4.87	0.9916	11.996	0.97	20	6.93	0.3598	8.068	0.35
3	5.00	0.1288	3.714	0.13	21	7.05	0.6224	9.687	0.61
4	5.09	0.393	7.172	0.38	22	7.33	0.7445	10.718	0.72
5	5.28	0.2047	5.115	0.2	23	7.42	1.0711	22.066	1.04
6	5.42	0.5252	7.803	0.51	24	7.49	0.3033	8.018	0.3
7	5.62	62.0545	1116.87	60.39	25	7.61	0.3592	7.839	0.35
8	5.74	1.8706	24.658	1.82	26	7.64	0.2307	5.862	0.22
9	5.83	0.5277	11.462	0.51	27	7.72	0.2323	4.073	0.23
10	5.92	0.16	5.527	0.16	28	8.24	0.4874	9.156	0.47
11	5.97	0.9247	16.955	0.9	29	8.42	1.0777	14.056	1.05
12	6.04	0.3601	8.611	0.35	30	8.52	3.32	41.366	3.23
13	6.13	0.1612	5.267	0.16	31	9.17	0.2772	7.047	0.27
14	6.27	2.2123	63.827	2.15	32	9.34	0.0617	3.37	0.06
15	6.29	2.9474	82.516	2.87	33	9.58	0.2079	5.164	0.2
16	6.33	5.151	95.482	5.01	34	9.88	0.4554	8.843	0.44
17	6.39	3.5587	88.167	3.46	35	10.02	6.9174	142.911	6.73
18	6.63	1.185	14.713	1.15	36	10.33	1.9851	37.998	1.93



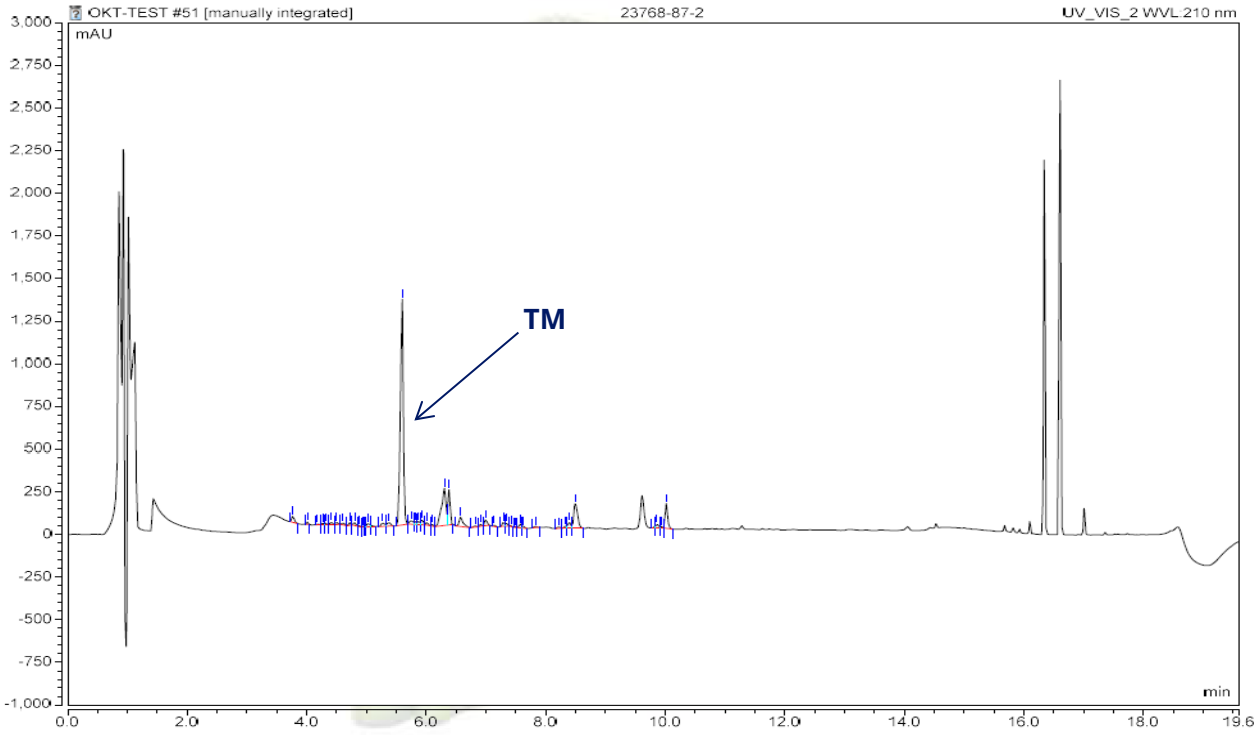
Peak No.	Ret.Time	Area	Height	Rel.Area	Peak No.	Ret.Time	Area	Height	Rel.Area
1	3.81	1.1437	28.635	0.59	18	7.02	2.2542	31.428	1.17
2	4.8	0.5992	10.792	0.31	19	7.35	2.1641	25.251	1.12
3	4.85	1.1856	21.097	0.62	20	7.42	1.2192	30.529	0.63
4	5.03	0.4541	10.504	0.24	21	7.49	0.4124	11.887	0.21
5	5.06	1.1586	21.757	0.6	22	7.58	0.5214	12.46	0.27
6	5.28	0.676	9.528	0.35	23	7.64	0.3313	8.016	0.17
7	5.39	1.6064	20.836	0.83	24	7.70	0.3096	6.841	0.16
8	5.62	110.4583	1714.722	57.32	25	8.24	0.7084	10.692	0.37
9	5.73	3.1553	40.519	1.64	26	8.36	0.573	10.881	0.3
10	5.82	0.9097	21.433	0.47	27	8.40	1.5875	25.968	0.82
11	5.95	1.7788	35.355	0.92	28	8.50	6.0992	85.671	3.17
12	6.02	0.4733	14.419	0.25	29	8.71	0.6942	14.183	0.36
13	6.11	0.5857	15.159	0.3	30	9.16	0.585	15.204	0.3
14	6.32	21.4234	201.612	11.12	31	9.32	0.3012	8.315	0.16
15	6.39	8.4976	171.311	4.41	32	9.57	0.5181	10.762	0.27
16	6.60	2.1512	35.29	1.12	33	10.01	13.4517	297.258	6.98
17	6.92	0.7337	15.301	0.38	34	10.32	3.9707	82.853	2.06

Automated SPPS of Dasiglucagon amide (H-HSQGTFTSDYSKYLD-Aib-ARAEEFVKWLEST-NH_2) in DMSO/2-Me-THF (3:7), repeated two times

0.15 mmol scale, HPLC method D



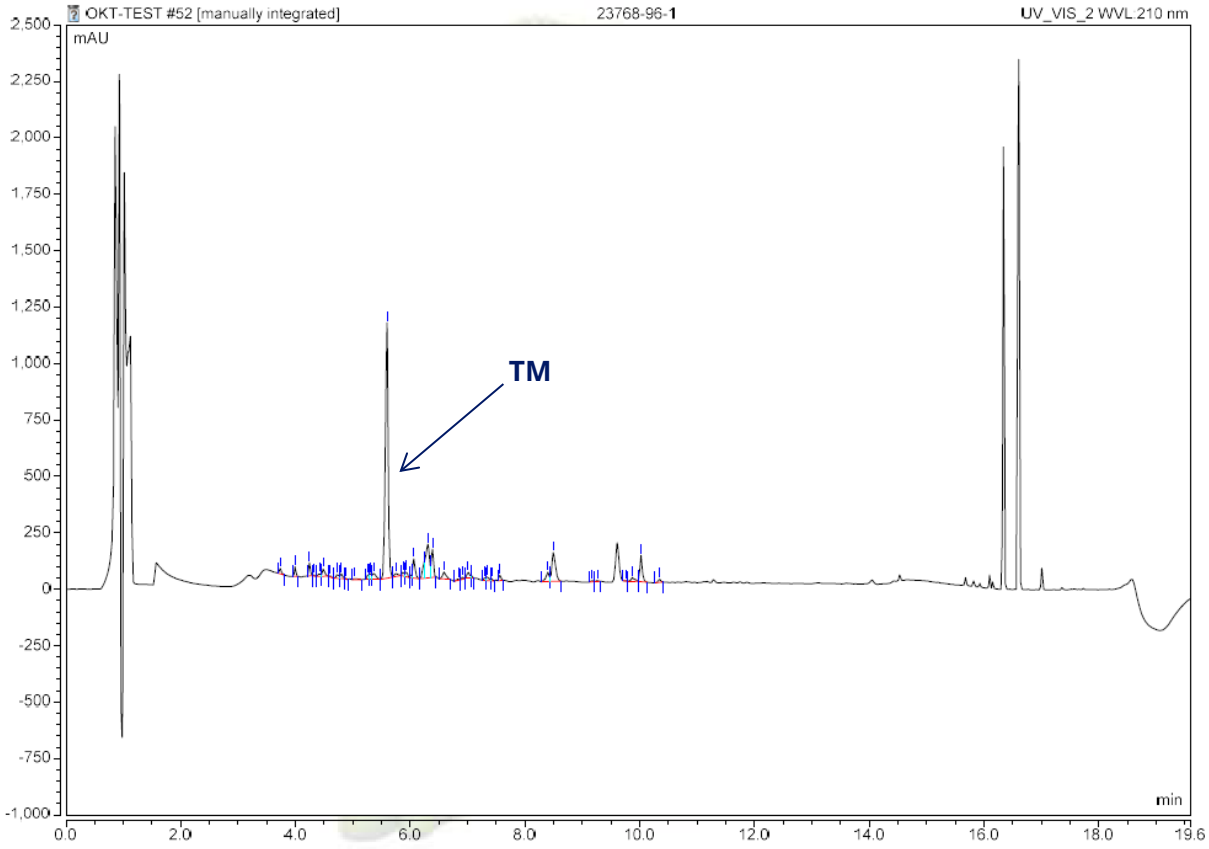
Peak No.	Ret.Time	Area	Height	Rel.Area	Peak No.	Ret.Time	Area	Height	Rel.Area
1	3.79	1.2233	21.945	1.06	15	5.94	1.4083	25.147	1.23
2	4.03	2.8544	101.51	2.48	16	6.32	12.528	155.574	10.9
3	4.28	0.1745	6.034	0.15	17	6.39	6.7032	154.186	5.83
4	4.34	0.2362	6.241	0.21	18	6.59	2.2721	34.223	1.98
5	4.43	0.5397	12.624	0.47	19	7.01	2.0462	33.105	1.78
6	4.51	0.4365	8.701	0.38	20	7.31	2.1097	24.169	1.84
7	4.61	0.2409	8.637	0.21	21	7.42	0.3839	9.321	0.33
8	4.74	0.7929	15.848	0.69	22	7.58	0.403	9.339	0.35
9	4.81	2.4419	69.32	2.12	23	8.37	0.385	9.064	0.34
10	5.05	0.5358	8.621	0.47	24	8.40	1.3755	25.426	1.2
11	5.28	0.9565	14.194	0.83	25	8.50	6.9527	93.87	6.05
12	5.39	1.3093	17.722	1.14	26	9.87	1.1604	19.639	1.01
13	5.61	58.5396	1040.887	50.94	27	9.95	0.8284	13.594	0.72
14	5.76	1.3618	17.995	1.18	28	10.02	4.7201	94.957	4.11



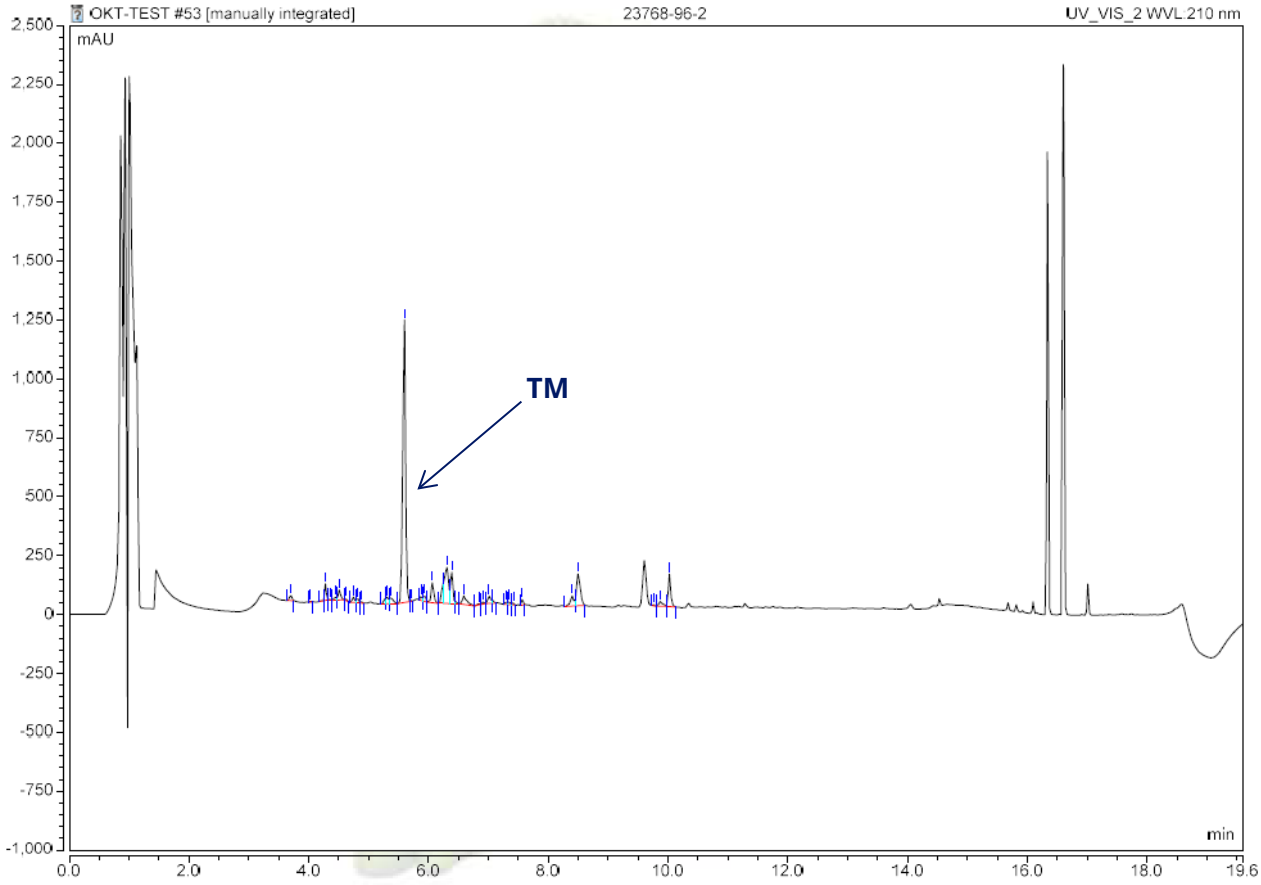
Peak No.	Ret.Time	Area	Height	Rel.Area	Peak No.	Ret.Time	Area	Height	Rel.Area
1	3.77	1.9098	37.894	1.24	21	6.30	18.4764	217.27	11.97
2	4.01	0.4269	15.534	0.28	22	6.38	8.7959	206.262	5.7
3	4.27	0.2644	7.035	0.17	23	6.57	3.8349	54.735	2.48
4	4.32	0.3158	8.888	0.2	24	6.82	0.4462	8.505	0.29
5	4.41	0.7728	16.526	0.5	25	6.90	0.4237	12.509	0.27
6	4.49	0.6126	14.122	0.4	26	6.99	2.0344	36.393	1.32
7	4.60	0.6843	11.02	0.44	27	7.13	0.3693	6.84	0.24
8	4.72	0.9075	20.455	0.59	28	7.29	1.5551	29.801	1.01
9	4.81	1.0808	20.963	0.7	29	7.34	1.2836	25.893	0.83
10	4.87	0.2067	6.297	0.13	30	7.42	0.4121	10.525	0.27
11	5.02	1.5319	19.328	0.99	31	7.49	0.1219	3.714	0.08
12	5.27	1.3052	20.863	0.85	32	7.57	0.9992	21.243	0.65
13	5.37	1.5307	19.493	0.99	33	7.62	0.4757	10.71	0.31
14	5.45	1.6937	23.62	1.1	34	8.33	0.5982	13.233	0.39
15	5.51	0.6233	17.293	0.4	35	8.39	2.0874	37.978	1.35
16	5.57	0.8914	18.536	0.58	36	8.49	10.7627	144.563	6.97
17	5.58	1.2549	29.078	0.81	37	9.83	0.2617	8.965	0.17
18	5.60	75.6322	1323.577	48.99	38	9.86	1.2228	24.301	0.79
19	6.01	0.6545	17.28	0.42	39	9.93	0.6852	12.641	0.44
20	6.09	0.4269	11.898	0.28	40	10.02	6.7997	149.024	4.4

Automated SPPS of Dasiglucagon amide (H-HSQGTFTSDYSKYLD-Aib-ARAEEFVKWLEST-NH_2) in DMSO/EtOAc (4:6), repeated two times

0.15 mmol scale, HPLC method D



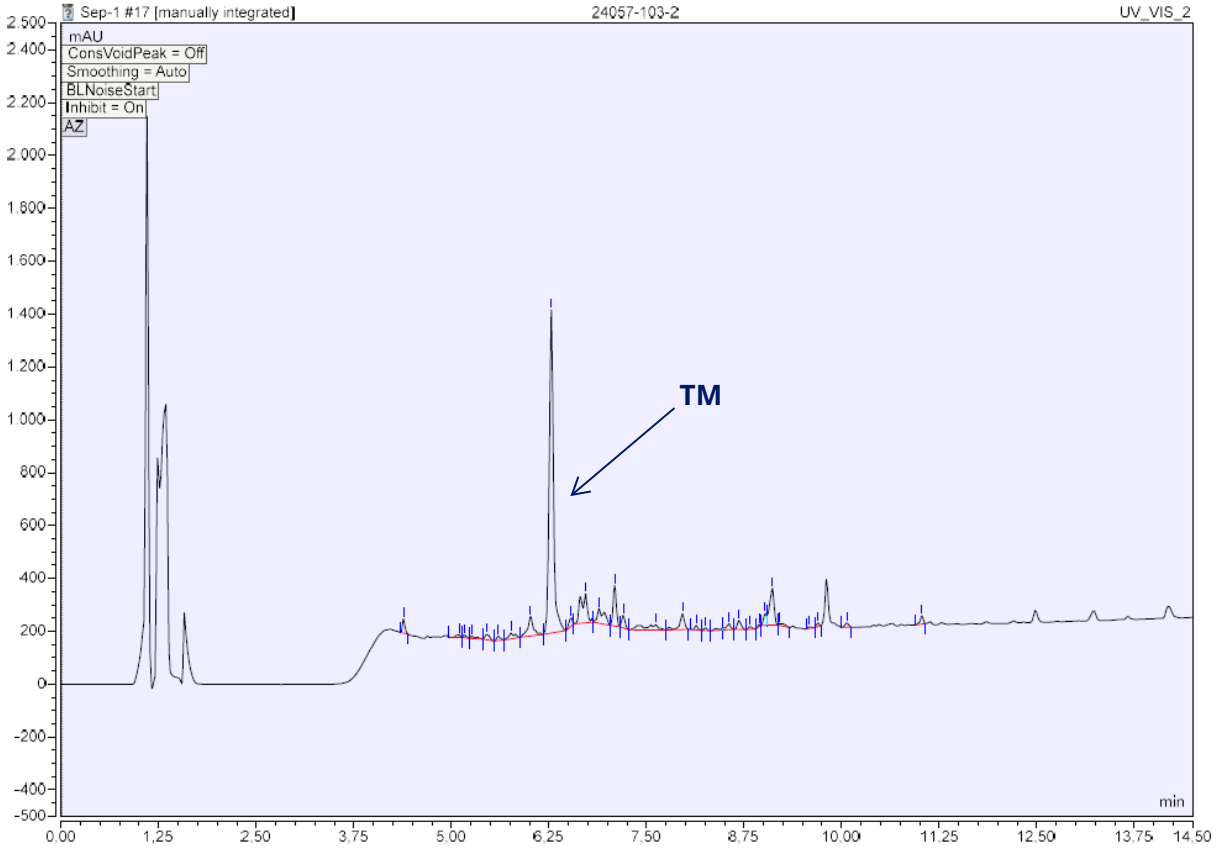
Peak No.	Ret.Time	Area	Height	Rel.Area	Peak No.	Ret.Time	Area	Height	Rel.Area
1	3.73	1.0556	23.875	0.81	16	6.39	5.1541	123.227	3.98
2	3.99	1.1434	43.389	0.88	17	6.59	2.8965	33.608	2.23
3	4.24	1.6497	57.175	1.27	18	6.84	0.5047	9.155	0.39
4	4.41	0.5788	12.49	0.45	19	6.91	1.0806	16.422	0.83
5	4.49	1.6046	34.077	1.24	20	7.01	2.4404	38.129	1.88
6	4.73	0.913	19.961	0.7	21	7.07	1.0099	21.467	0.78
7	4.80	1.0361	21.451	0.8	22	7.15	0.6983	10.133	0.54
8	5.30	2.0328	25.033	1.57	23	7.30	0.5314	12.267	0.41
9	5.36	1.829	26.161	1.41	24	7.34	0.8326	18.025	0.64
10	5.39	1.016	15.154	0.78	25	7.42	0.4356	11.344	0.34
11	5.41	0.7166	17.594	0.55	26	7.56	0.8976	23.21	0.69
12	5.52	1.0513	23.741	0.81	27	8.39	2.7289	43.238	2.11
13	5.53	4.3222	85.613	3.33	28	8.49	9.1636	127.176	7.07
14	5.56	14.1372	148.035	10.91	29	10.02	5.2475	115.739	4.05
15	5.59	62.8994	1131.763	48.53					



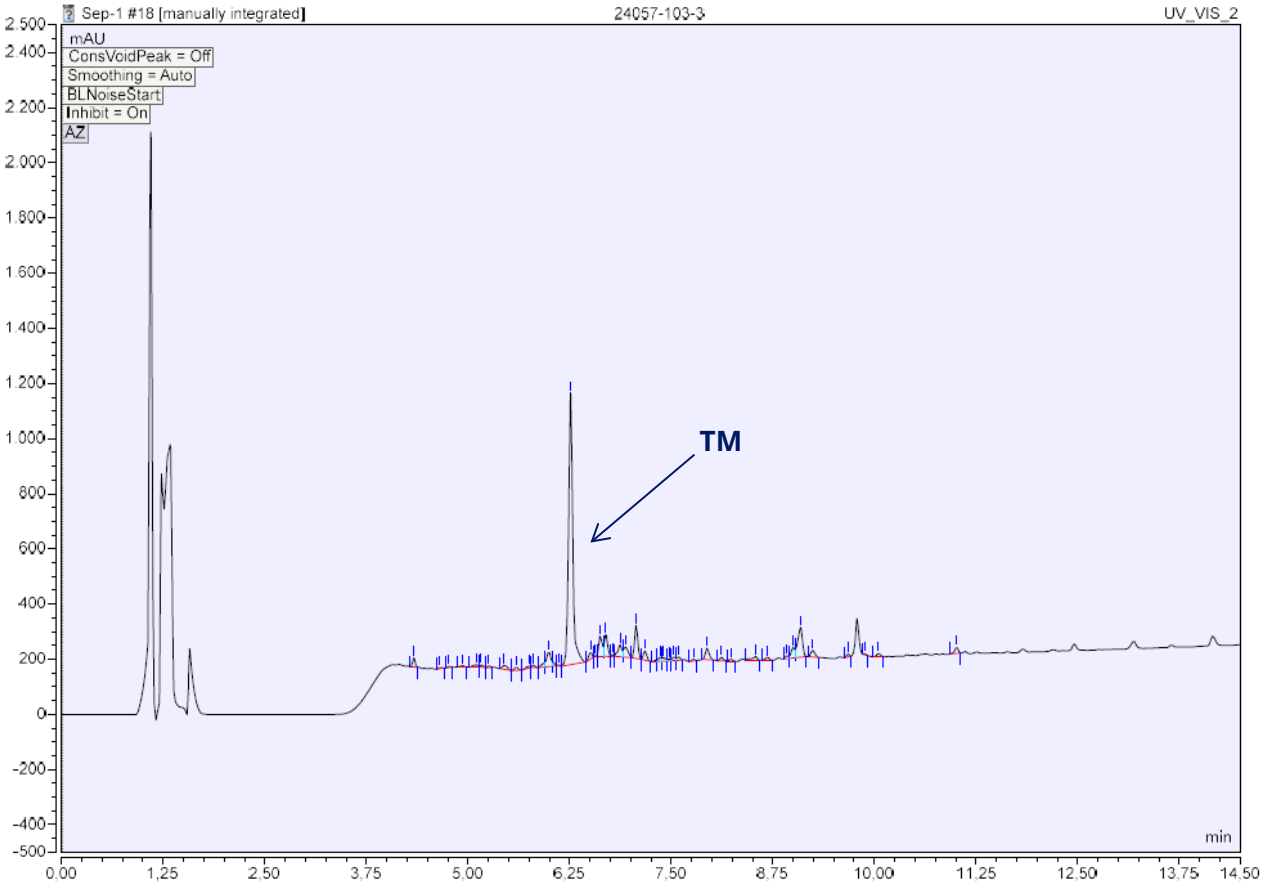
Peak No.	Ret.Time	Area	Height	Rel.Area	Peak No.	Ret.Time	Area	Height	Rel.Area
1	4.28	2.087	70.677	1.56	17	6.39	6.2625	132.01	4.7
2	4.36	0.0959	3.37	0.07	18	6.59	3.1189	35.617	2.34
3	4.44	0.7122	15.028	0.53	19	6.84	0.2381	4.525	0.18
4	4.51	1.9914	43.499	1.49	20	6.91	0.3029	8.936	0.23
5	4.63	0.2865	11.055	0.21	21	7.01	1.5581	29.537	1.17
6	4.75	0.9253	21.512	0.69	22	7.07	0.4214	11.769	0.32
7	4.82	0.8533	16.158	0.64	23	7.29	0.4641	11.654	0.35
8	4.88	0.1716	5.275	0.13	24	7.34	0.6786	14.705	0.51
9	5.31	2.1567	28.037	1.62	25	7.42	0.3526	9.737	0.26
10	5.37	1.7968	24.307	1.35	26	7.56	0.831	22.017	0.62
12	5.47	0.0836	3.79	0.06	27	8.40	2.8812	44.824	2.16
13	5.51	0.7335	18.163	0.55	28	8.50	10.0598	136.521	7.54
14	5.54	1.0587	25.349	0.79	29	9.87	1.4898	19.384	1.12
15	5.57	4.1519	85.195	3.11	30	10.02	6.26	137.318	4.69
11	5.60	65.2866	1197.633	48.95	31	10.34	0.9234	19.254	0.69
16	6.31	15.1441	152.023	11.35					

Automated SPPS of Dasiglucagon amide (H-HSQGTFTSDYSKYLD-Aib-ARAEEFVKWLEST-NH_2) in DMSO/DOL (3:7), repeated two times

0.15 mmol scale, HPLC method D



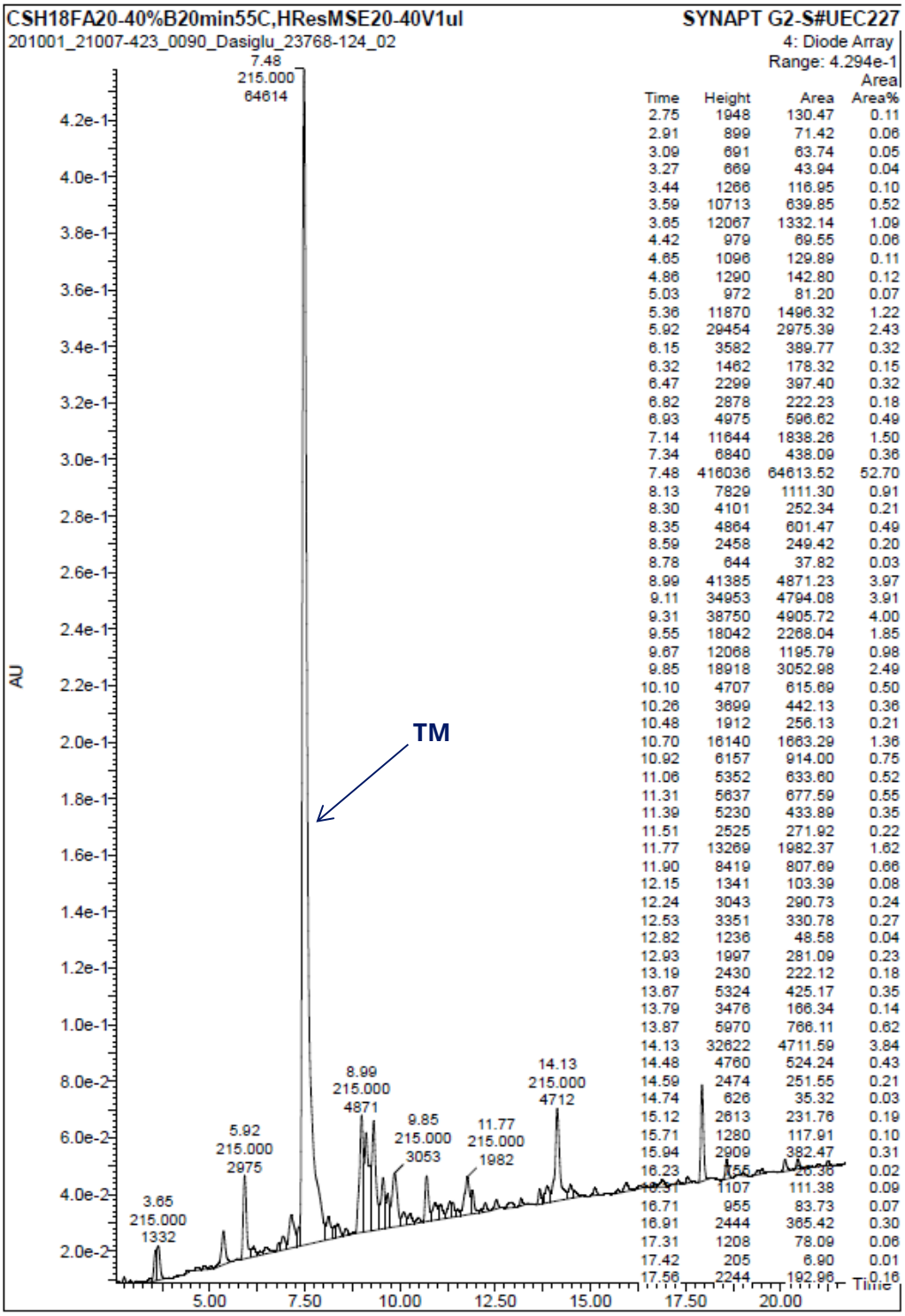
Peak No.	Ret.Time	Area	Height	Rel.Area	Peak No.	Ret.Time	Area	Height	Rel.Area
1	4.39	1.8475	54.177	1.24	16	7.96	4.1823	60.839	2.81
2	5.1	0.5569	9.773	0.37	17	8.14	0.7096	17.25	0.48
3	5.16	0.4368	9.853	0.29	18	8.26	0.4531	10.454	0.3
4	5.26	0.7407	9.398	0.5	19	8.48	0.7606	7.587	0.51
5	5.45	1.4185	21.046	0.95	20	8.56	1.4921	23.876	1
6	5.60	0.8796	17.445	0.59	21	8.68	1.9244	34.695	1.29
7	5.77	1.9669	24.394	1.32	22	8.83	0.5345	10.876	0.36
8	6.01	6.1823	74.511	4.16	23	8.94	0.2373	7.764	0.16
9	6.28	77.0703	1222.529	51.86	24	9.02	2.2235	44.469	1.5
10	6.53	1.6464	33.156	1.11	25	9.11	9.0685	139.189	6.1
11	6.72	10.766	110.472	7.24	26	9.21	0.8191	7.125	0.55
12	6.90	5.5408	55.679	3.73	27	9.59	0.256	5.315	0.17
13	7.09	7.0995	153.334	4.78	28	9.69	0.5677	16.023	0.38
14	7.20	2.3447	48.684	1.58	29	10.07	0.8396	17.736	0.56
15	7.62	4.5804	20.622	3.08	30	11.03	1.4687	31.45	0.99


Sep-1 #18 [manually integrated]
24057-103-3
UV_VIS_2
mAU
ConsVoidPeak = Off
Smoothing = Auto
BLNoiseStart
Inhibit = On
AZ
TM
min
2.500
2.400
2.200
2.000
1.800
1.600
1.400
1.200
1.000
800
600
400
200
0
-200
-400
-500
0.00
1.25
2.50
3.75
5.00
6.25
7.50
8.75
10.00
11.25
12.50
13.75
14.50

Peak No.	Ret.Time	Area	Height	Rel.Area	Peak No.	Ret.Time	Area	Height	Rel.Area
1	4.33	1.0611	33.376	0.97	24	7.07	5.4634	119.743	4.98
2	4.65	0.2169	4.501	0.2	25	7.18	1.642	34.212	1.5
3	4.76	0.1809	5.233	0.16	26	7.31	0.1863	5.525	0.17
4	4.94	0.2648	5.631	0.24	27	7.36	0.6018	14.557	0.55
5	5.10	0.4737	7.495	0.43	28	7.41	0.6625	13.035	0.6
6	5.16	0.3651	7.656	0.33	29	7.48	0.3348	6.709	0.31
7	5.25	0.2446	7.133	0.22	30	7.53	0.6054	13.124	0.55
8	5.44	1.0491	14.621	0.96	31	7.59	0.5273	13.563	0.48
9	5.59	0.5839	12.579	0.53	32	7.77	0.3183	7.28	0.29
10	5.75	0.3927	7.178	0.36	33	7.94	2.1681	42.565	1.98
11	5.80	0.3269	8.674	0.3	34	8.12	0.5824	13.447	0.53
12	5.95	0.7482	16.623	0.68	35	8.23	0.3128	7.29	0.29
13	5.99	3.0464	54.645	2.78	36	8.54	0.8886	13.053	0.81
14	6.04	0.4473	15.439	0.41	37	8.69	0.5699	10.093	0.52
15	6.12	0.1153	4.091	0.11	38	8.92	0.3956	10.039	0.36
16	6.26	58.8635	985.925	53.64	39	9.00	2.1186	38.774	1.93
17	6.51	1.1527	24.123	1.05	40	9.09	7.1592	108.223	6.52
18	6.57	0.0694	3.978	0.06	41	9.24	1.3757	24.245	1.25
19	6.63	3.4396	72.76	3.13	42	9.67	0.3974	11.611	0.36
20	6.69	3.7756	79.801	3.44	43	9.88	0.1466	4.897	0.13
21	6.78	0.1228	5.9	0.11	44	10.05	0.5862	12.467	0.53
22	6.87	2.3267	43.795	2.12	45	11.01	1.0725	23.794	0.98
23	6.94	2.3538	37.732	2.14					

3.7 Automated SPPS of Dasiglucagon amide (H-HSQGTFTSDYSKYLD-Aib-ARAEEFVKWLEST-NH_2) on 5 mmol scale

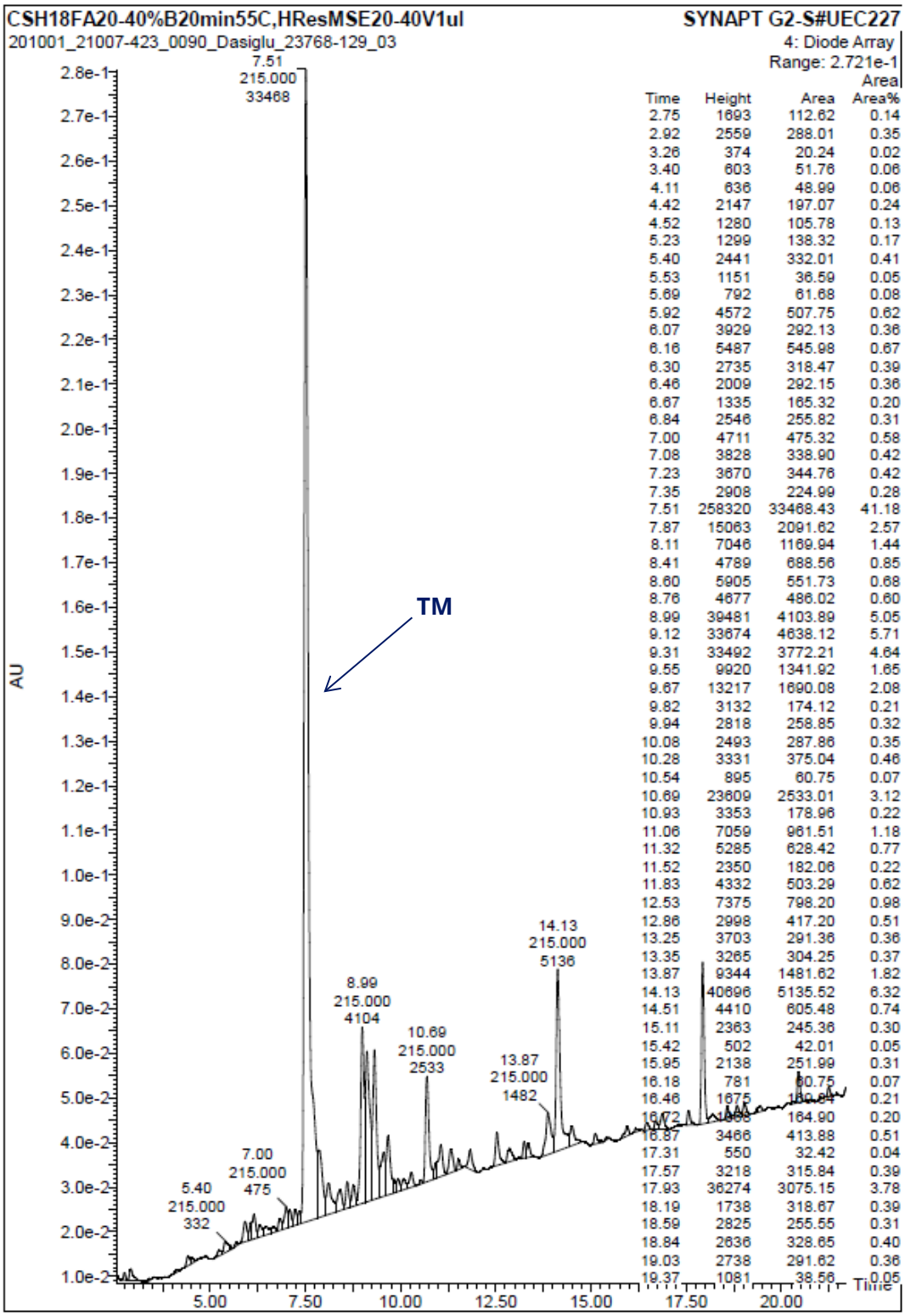
Automated SPPS of Dasiglucagon amide (H-HSQGTFTSDYSKYLD-Aib-ARAEEFVKWLEST-NH_2) in DMF 5 mmol scale, HPLC method F



Time	Height	Area	Area%
2.75	1948	130.47	0.11
2.91	899	71.42	0.06
3.09	691	63.74	0.05
3.27	669	43.94	0.04
3.44	1266	116.95	0.10
3.59	10713	639.85	0.52
3.65	12067	1332.14	1.09
4.42	979	69.55	0.06
4.65	1096	129.89	0.11
4.86	1290	142.80	0.12
5.03	972	81.20	0.07
5.36	11870	1496.32	1.22
5.92	29454	2975.39	2.43
6.15	3582	389.77	0.32
6.32	1462	178.32	0.15
6.47	2299	397.40	0.32
6.82	2878	222.23	0.18
6.93	4975	596.62	0.49
7.14	11644	1838.26	1.50
7.34	6840	438.09	0.36
7.48	416036	64613.52	52.70
8.13	7829	1111.30	0.91
8.30	4101	252.34	0.21
8.35	4864	601.47	0.49
8.59	2458	249.42	0.20
8.78	644	37.82	0.03
8.99	41385	4871.23	3.97
9.11	34953	4794.08	3.91
9.31	38750	4905.72	4.00
9.55	18042	2268.04	1.85
9.67	12068	1195.79	0.98
9.85	18918	3052.98	2.49
10.10	4707	615.69	0.50
10.26	3699	442.13	0.36
10.48	1912	256.13	0.21
10.70	16140	1663.29	1.36
10.92	6157	914.00	0.75
11.06	5352	633.60	0.52
11.31	5637	677.59	0.55
11.39	5230	433.89	0.35
11.51	2525	271.92	0.22
11.77	13269	1982.37	1.62
11.90	8419	807.69	0.66
12.15	1341	103.39	0.08
12.24	3043	290.73	0.24
12.53	3351	330.78	0.27
12.82	1236	48.58	0.04
12.93	1997	281.09	0.23
13.19	2430	222.12	0.18
13.67	5324	425.17	0.35
13.79	3476	166.34	0.14
13.87	5970	766.11	0.62
14.13	32622	4711.59	3.84
14.48	4760	524.24	0.43
14.59	2474	251.55	0.21
14.74	626	35.32	0.03
15.12	2613	231.76	0.19
15.71	1280	117.91	0.10
15.94	2909	382.47	0.31
16.23	456	26.36	0.02
16.51	1107	111.38	0.09
16.71	955	83.73	0.07
16.91	2444	365.42	0.30
17.31	1208	78.09	0.06
17.42	205	6.90	0.01
17.56	2244	192.96	0.16

Automated SPPS of Dasiglucagon amide (H-HSQGTFTSDYSKYLD-Aib-ARAEEFVKWLEST-NH_2) in DMSO/2-Me-THF (3:7)

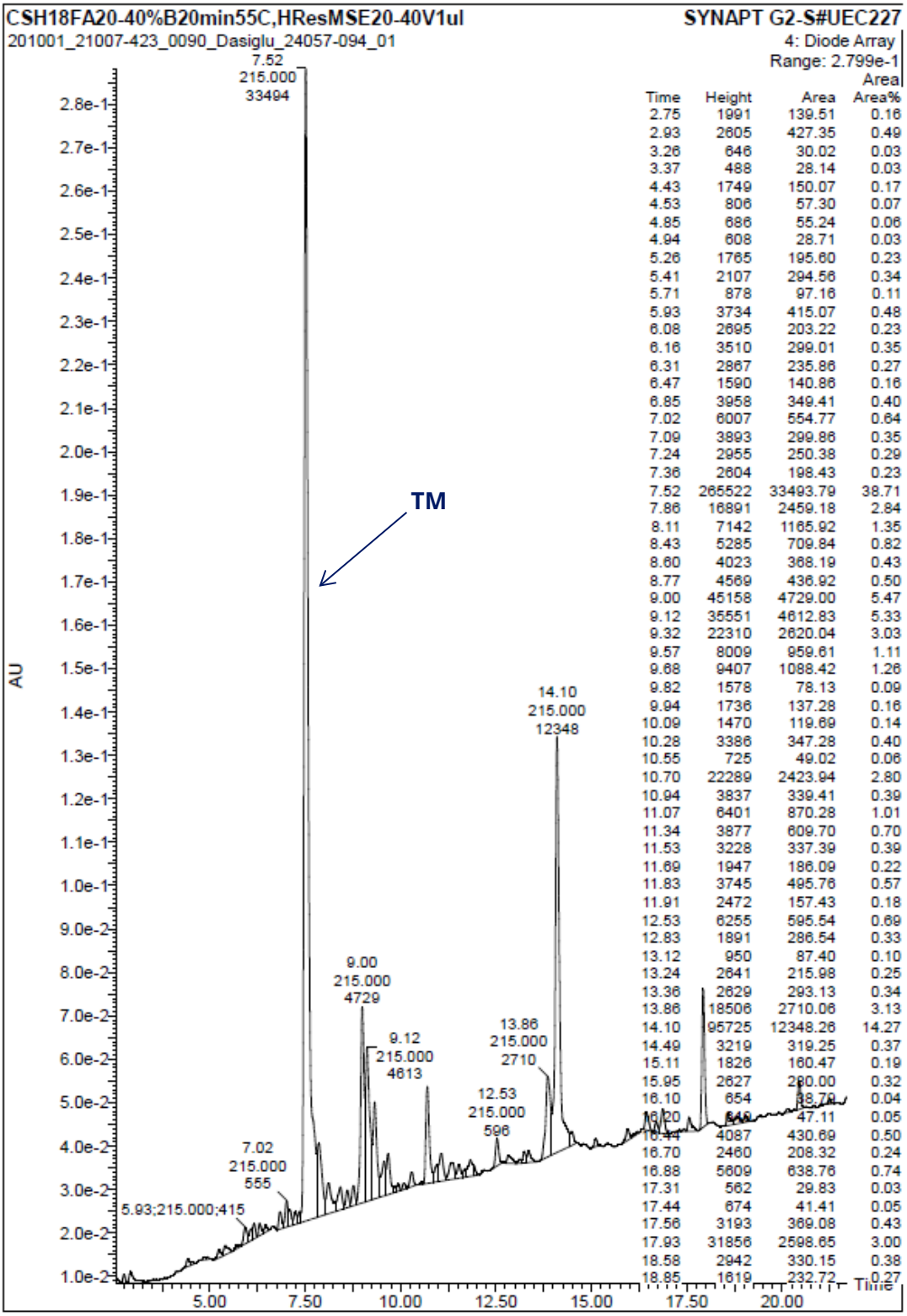
5 mmol scale, HPLC method F



Time	Height	Area	Area%
2.75	1693	112.62	0.14
2.92	2559	288.01	0.35
3.26	374	20.24	0.02
3.40	603	51.76	0.06
4.11	636	48.99	0.06
4.42	2147	197.07	0.24
4.52	1280	105.78	0.13
5.23	1299	138.32	0.17
5.40	2441	332.01	0.41
5.53	1151	36.59	0.05
5.69	792	61.68	0.08
5.92	4572	507.75	0.62
6.07	3929	292.13	0.36
6.16	5487	545.98	0.67
6.30	2735	318.47	0.39
6.46	2009	292.15	0.36
6.67	1335	165.32	0.20
6.84	2546	255.82	0.31
7.00	4711	475.32	0.58
7.08	3828	338.90	0.42
7.23	3670	344.76	0.42
7.35	2908	224.99	0.28
7.51	258320	33468.43	41.18
7.87	15063	2091.62	2.57
8.11	7046	1169.94	1.44
8.41	4789	688.56	0.85
8.60	5905	551.73	0.68
8.76	4677	486.02	0.60
8.99	39481	4103.89	5.05
9.12	33674	4638.12	5.71
9.31	33492	3772.21	4.64
9.55	9920	1341.92	1.65
9.67	13217	1690.08	2.08
9.82	3132	174.12	0.21
9.94	2818	258.85	0.32
10.08	2493	287.86	0.35
10.28	3331	375.04	0.46
10.54	895	60.75	0.07
10.69	23609	2533.01	3.12
10.93	3353	178.96	0.22
11.06	7059	961.51	1.18
11.32	5285	628.42	0.77
11.52	2350	182.06	0.22
11.83	4332	503.29	0.62
12.53	7375	798.20	0.98
12.86	2998	417.20	0.51
13.25	3703	291.36	0.36
13.35	3265	304.25	0.37
13.87	9344	1481.62	1.82
14.13	40696	5135.52	6.32
14.51	4410	605.48	0.74
15.11	2363	245.36	0.30
15.42	502	42.01	0.05
15.95	2138	251.99	0.31
16.18	781	60.75	0.07
16.46	1675	180.64	0.21
16.72	[illegible]	164.90	0.20
16.87	3466	413.88	0.51
17.31	550	32.42	0.04
17.57	3218	315.84	0.39
17.93	36274	3075.15	3.78
18.19	1738	318.67	0.39
18.59	2825	255.55	0.31
18.84	2636	328.65	0.40
19.03	2738	291.62	0.36
19.37	1081	38.56	0.05

Automated SPPS of Dasiglucagon amide (H-HSQGTFTSDYSKYLD-Aib-ARAEEFVKWLEST-NH_2) in DMSO/DOL (3:7)

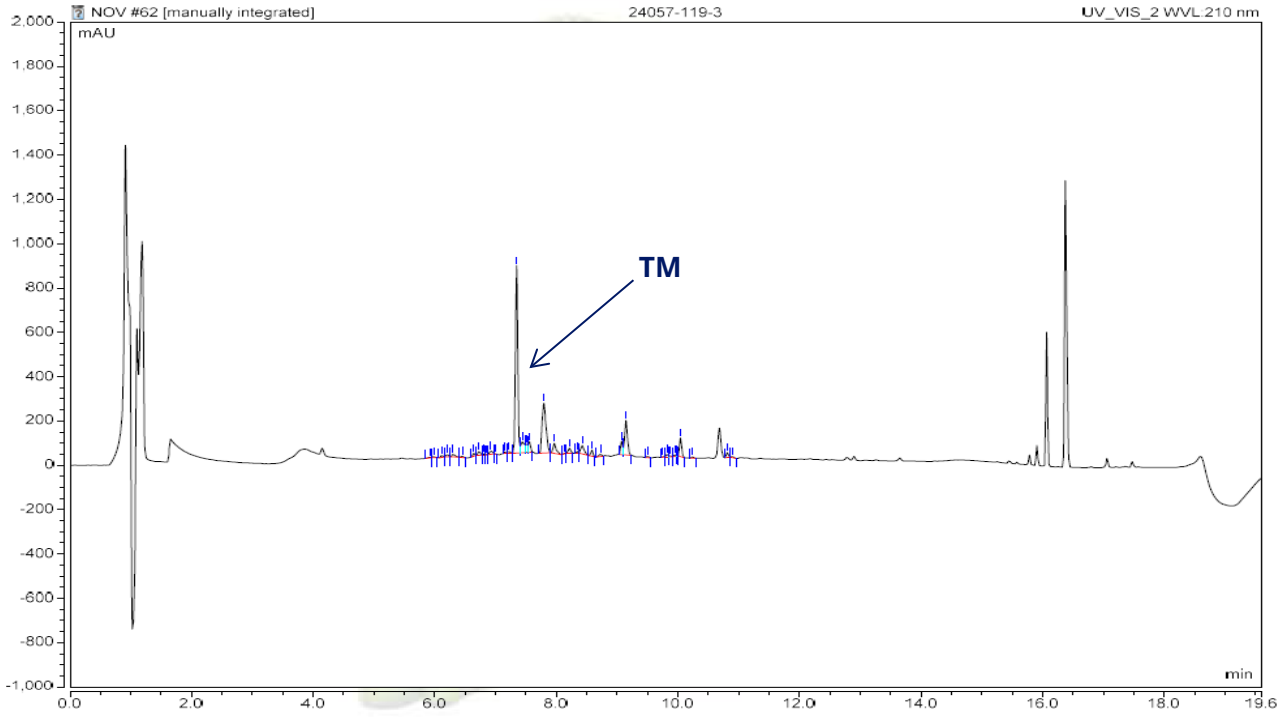
5 mmol scale, HPLC method F



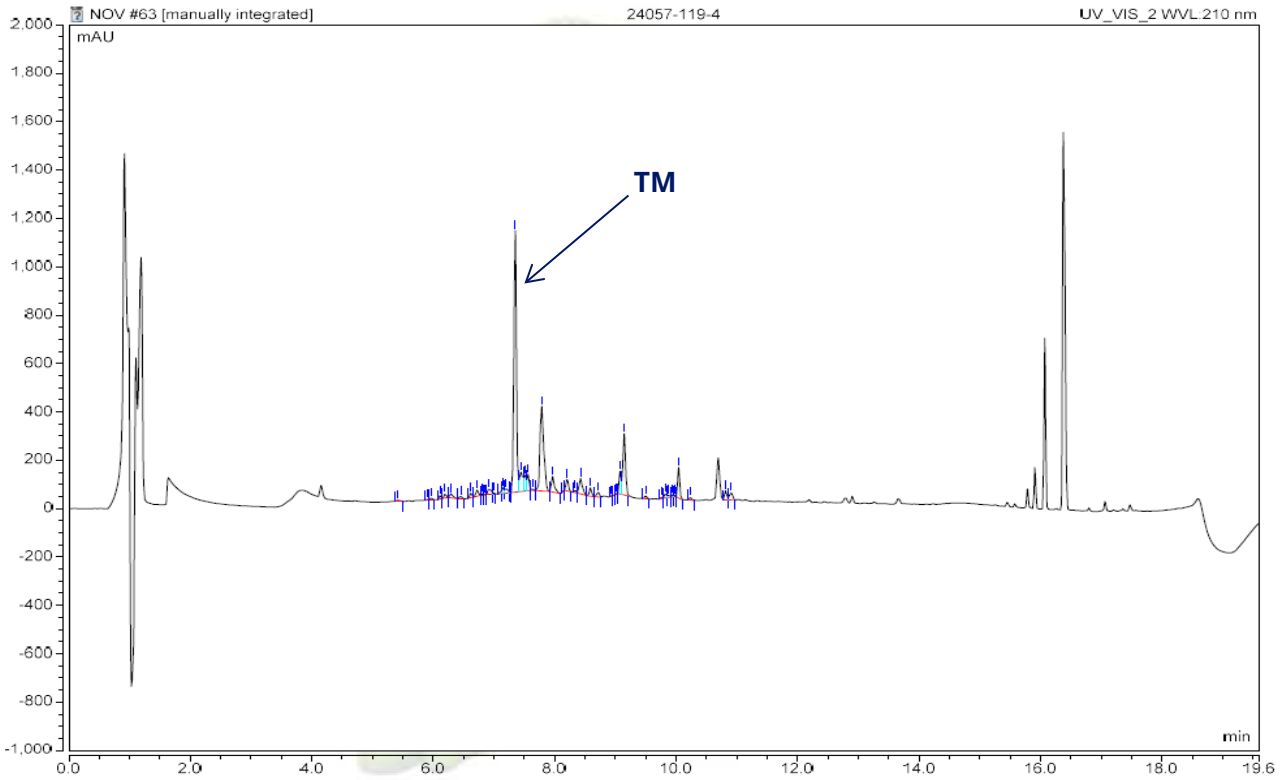
3.8 Optimised automated SPPS of Dasiglucagon amide (Ac-HSQGTFTSDYSKYLD-Aib-ARAEEFVKWLEST-NH_2) on 0.45 mmol scale

Automated SPPS of Dasiglucagon amide (H-HSQGTFTSDYSKYLD-Aib-ARAEEFVKWLEST-NH_2) in DMSO/DOL (3:7), repeated two times

0.45 mmol scale, HPLC method D, no optimisation



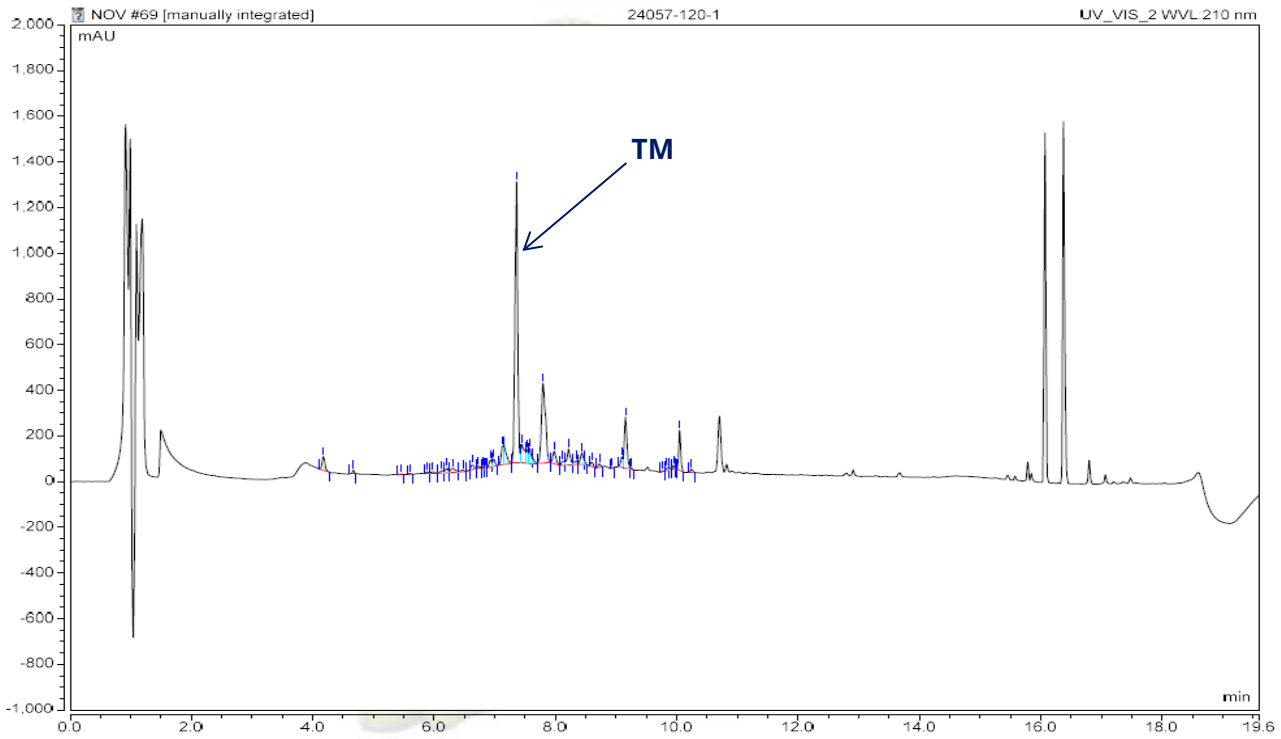
Peak No.	Ret.Time	Area	Height	Rel.Area	Peak No.	Ret.Time	Area	Height	Rel.Area
1	5.92	0.1536	2.644	0.17	20	7.97	2.5756	45.227	2.81
2	5.98	0.1883	4.647	0.21	21	8.13	0.0957	2.662	0.10
3	6.12	0.3829	9.688	0.42	22	8.21	1.1643	23.800	1.27
4	6.20	0.5024	12.581	0.55	23	8.34	0.2431	8.151	0.27
5	6.30	0.7464	11.943	0.81	24	8.43	2.4679	38.599	2.69
6	6.45	0.3107	6.203	0.34	25	8.58	1.1238	27.079	1.23
7	6.64	0.5496	13.002	0.60	26	8.73	0.5581	11.461	0.61
8	6.73	0.8112	17.713	0.88	27	9.07	2.5117	59.388	2.74
9	6.80	0.1354	5.518	0.15	28	9.14	8.0434	155.188	8.77
10	6.85	0.0596	2.239	0.07	29	9.50	0.2925	6.876	0.32
11	6.92	0.8303	15.211	0.91	30	9.75	0.1073	3.835	0.12
12	7.00	0.0519	2.399	0.06	31	9.82	0.4962	12.583	0.54
13	7.15	0.1765	6.642	0.19	32	9.87	0.2164	4.724	0.24
14	7.21	0.3942	8.066	0.43	33	9.95	0.2334	6.481	0.25
15	7.35	37.4133	844.927	40.79	34	9.98	0.0809	4.029	0.09
16	7.44	3.3912	49.842	3.70	35	10.04	3.2347	84.312	3.53
17	7.51	1.6695	38.414	1.82	36	10.24	0.2630	6.686	0.29
18	7.56	1.9261	48.080	2.10	37	10.81	0.8222	22.733	0.90
19	7.80	17.2288	226.184	18.78	38	10.89	0.2646	5.401	0.29



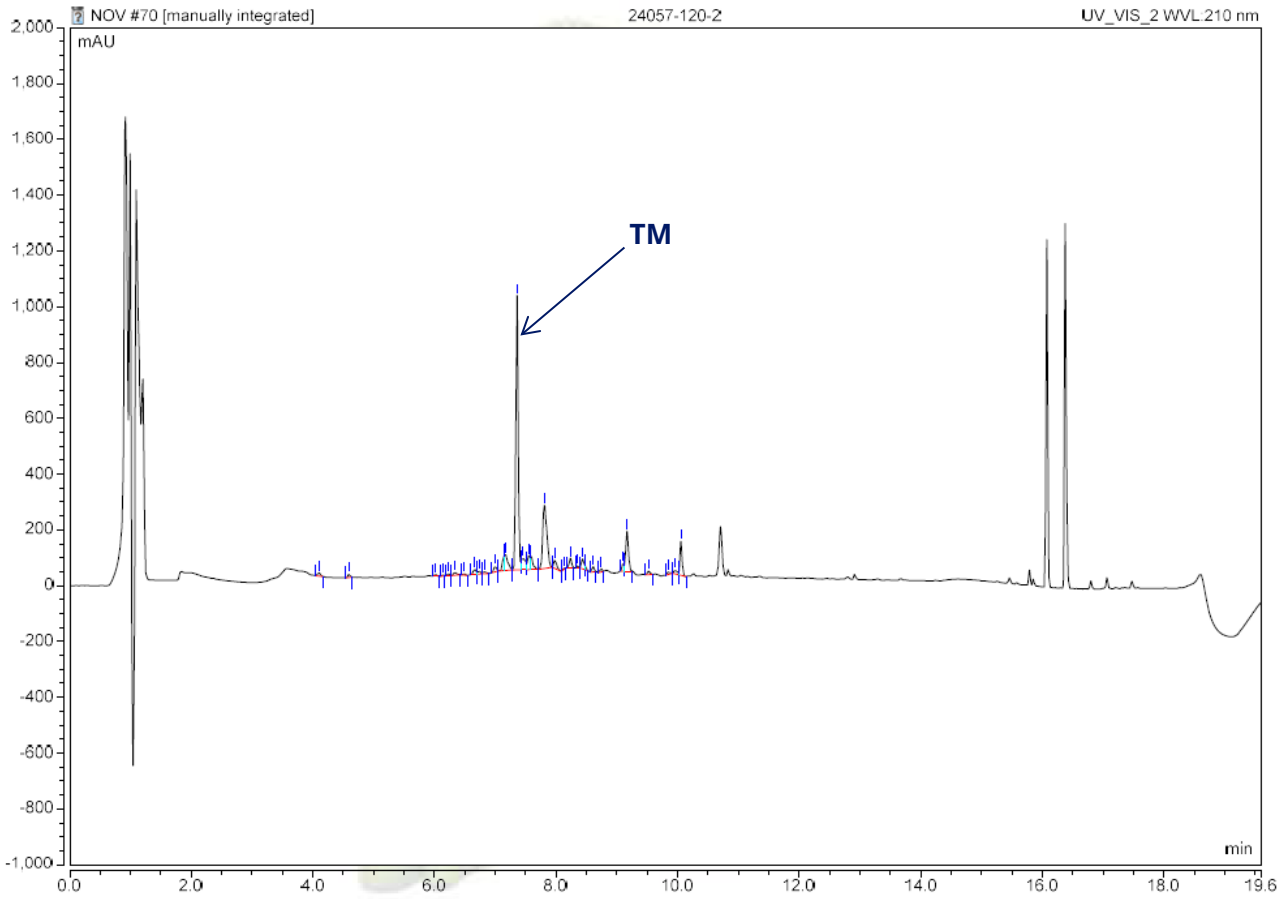
Peak No.	Ret.Time	Area	Height	Rel.Area	Peak No.	Ret.Time	Area	Height	Rel.Area
1	5.413	0.2996	4.906	0.21	22	7.967	3.5731	64.395	2.55
2	5.907	0.0820	2.591	0.06	23	8.163	0.0692	0.000	0.05
3	5.963	0.2825	7.517	0.20	24	8.203	2.1787	48.298	1.55
4	6.113	0.5188	14.539	0.37	25	8.333	0.3519	10.770	0.25
5	6.193	0.6936	17.293	0.49	26	8.427	4.0109	65.126	2.86
6	6.297	0.9926	16.375	0.71	27	8.590	1.5344	35.430	1.09
7	6.457	0.5692	10.052	0.41	28	8.717	1.0296	20.017	0.73
8	6.620	0.8021	19.882	0.57	29	8.980	0.0572	1.847	0.12
9	6.717	1.2629	26.527	0.90	30	9.077	3.7755	95.130	2.69
10	6.797	0.2056	7.841	0.15	31	9.143	12.6204	253.027	9.00
11	6.853	0.0521	2.043	0.04	32	9.497	0.5193	12.304	0.37
12	6.917	1.5677	23.922	1.12	33	9.753	0.2280	7.100	0.16
13	6.997	0.1906	6.648	0.14	34	9.823	0.7270	18.854	0.52
14	7.147	0.9793	24.291	0.90	35	9.873	0.3750	8.548	0.27
15	7.193	1.0616	18.497	0.82	36	9.940	0.3527	11.040	0.25
16	7.350	53.2824	1081.822	38.00	37	9.980	0.1852	6.961	0.13
17	7.447	4.8377	79.576	3.45	38	10.040	4.7949	126.505	3.42
18	7.510	3.0977	65.511	2.21	39	10.230	0.4277	10.890	0.31
19	7.560	2.6455	68.150	1.89	40	10.813	1.4664	37.596	1.05
20	7.630	0.2533	6.197	0.18	41	10.900	1.8036	29.885	1.29
21	7.787	25.9769	349.900	18.53					

Automated SPPS of Dasiglucagon amide (H-HSQGTFTSDYSKYLD-Aib-ARAEEFVKWLEST-NH_2) in DMSO/DOL (3:7), repeated two times

0.45 mmol scale, HPLC method D, with optimisation



Peak No.	Ret.Time	Area	Height	Rel.Area	Peak No.	Ret.Time	Area	Height	Rel.Area
1	4.173	3.1875	60.530	2.12	21	7.627	0.7411	22.903	0.49
2	4.667	0.5632	17.301	0.38	22	7.797	27.5012	351.463	18.33
3	5.460	0.2170	3.792	0.14	23	7.980	3.1425	55.159	2.09
4	5.600	0.1687	3.998	0.11	24	8.117	1.0533	22.299	0.70
5	5.977	0.4952	7.564	0.33	25	8.217	4.1538	70.721	2.77
6	6.127	0.6346	11.975	0.42	26	8.340	0.9607	17.805	0.64
7	6.207	1.1249	22.313	0.75	27	8.437	2.5612	50.929	1.71
8	6.310	1.5949	18.893	1.06	28	8.470	0.5165	18.407	0.34
9	6.580	0.0891	3.009	0.06	29	8.600	1.0204	21.063	0.68
10	6.627	1.1869	23.522	0.79	30	8.727	0.7257	16.946	0.48
11	6.720	0.5929	10.706	0.40	31	8.933	0.1544	5.810	0.10
12	6.853	0.0773	2.590	0.05	32	9.090	1.7508	45.003	1.17
13	6.927	1.3391	31.039	0.89	33	9.763	0.1341	4.521	0.09
14	6.983	1.5450	32.946	1.03	34	9.833	0.5124	12.837	0.34
15	7.127	3.0802	83.165	2.05	35	9.877	0.2702	8.197	0.18
16	7.153	4.5612	80.998	3.04	36	9.947	0.4982	15.998	0.33
17	7.360	65.2279	1233.487	43.48	37	9.970	0.1645	6.163	0.11
18	7.450	5.4892	82.675	3.66	38	10.047	6.6397	176.838	4.43
19	7.527	2.3959	61.565	1.60	39	10.240	0.6826	16.329	0.46
20	7.577	3.2516	65.728	2.17					

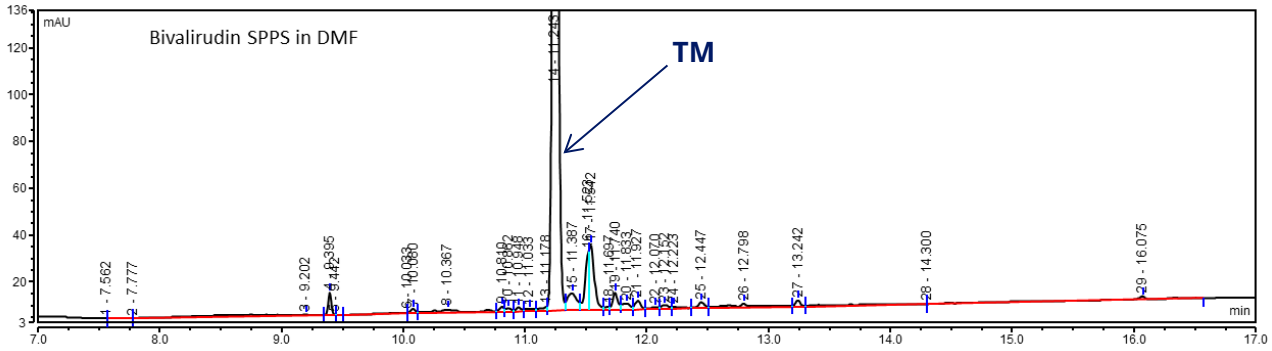


Peak No.	Ret.Time	Area	Height	Rel.Area	Peak No.	Ret.Time	Area	Height	Rel.Area
1	4.097	0.6402	11.533	0.68	16	7.547	2.2382	49.426	2.39
2	4.593	0.4817	13.744	0.52	17	7.587	2.4991	48.394	2.67
3	6.017	0.3761	6.672	0.40	18	7.807	18.4787	226.556	19.76
4	6.130	0.1775	4.424	0.19	19	7.980	1.7250	31.374	1.84
5	6.230	0.3489	8.555	0.37	20	8.127	0.2020	6.323	0.22
6	6.333	0.6643	10.595	0.71	21	8.233	1.6300	35.413	1.74
7	6.490	0.2455	4.954	0.26	22	8.353	0.2548	8.497	0.27
8	6.657	0.9570	19.075	1.02	23	8.437	1.6941	35.928	1.81
9	6.733	0.5317	10.492	0.57	24	8.470	0.3711	14.885	0.40
10	6.817	0.3988	7.389	0.43	25	8.610	0.8015	18.690	0.86
11	6.997	1.0203	20.598	1.09	26	8.743	0.5042	11.042	0.54
12	7.140	1.8806	53.890	2.01	27	9.103	1.0547	28.334	1.13
13	7.167	3.5720	57.552	3.82	28	9.523	0.6274	13.064	0.67
14	7.360	45.5448	984.356	48.70	29	9.843	0.5696	9.886	0.61
15	7.453	3.2100	42.879	3.43	30	9.957	0.8299	15.125	0.89

3.9 Automated SPPS of Bivalirudin (H-fPRPGGGGNGDFEEIPEEYL-OH) on 7.5 mmol scale

Automated SPPS of Bivalirudin (H-fPRPGGGGNGDFEEIPEEYL-OH) in DMF

7.5 mmol scale, HPLC method A

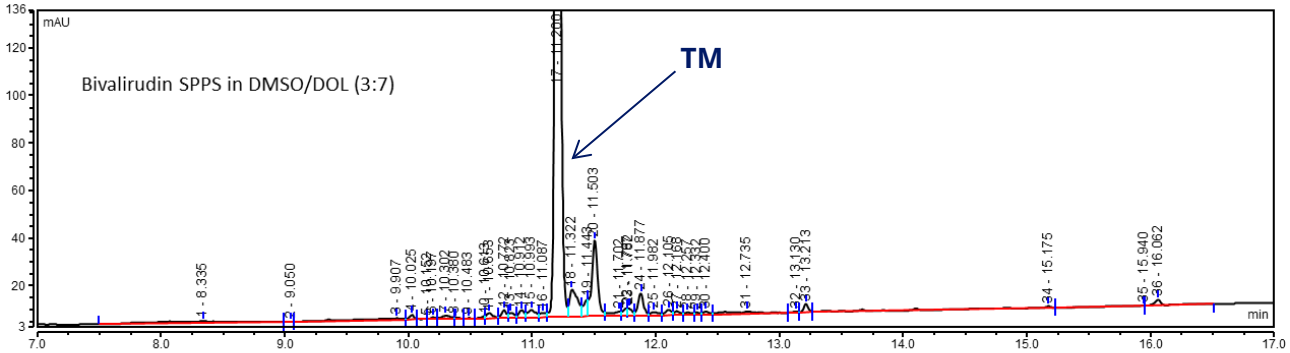


Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
4	9.395	Ac-[Glu^{17}-Leu^{20}]-OH	1.10
7	10.080	des[Ile^{15}-Pro^{16}]	0.38
14	11.243	Bivalirudin	73.82
15	11.387	des[Gly-Gly]	2.40
16	11.523	Ac-[Gly^{7}-Leu^{20}]-OH	2.93

Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
17	11.542	Ac-[Pro^{4}-Leu^{20}]-OH	5.10
19	11.740	Ac-[Asn^{9}-Leu^{20}]-OH	1.30
20	11.833	des[Arg-Pro]	0.75
21	11.927	des[Arg^{3}]	0.75
25	12.447	[Asp^{11}-Leu^{20}]-OH	0.57
other peaks			10.9

Automated SPPS of Bivalirudin (H-fPRPGGGGNGDFEEIPEEYL-OH) in DMSO/DOL (3:7)

7.5 mmol scale, HPLC method A

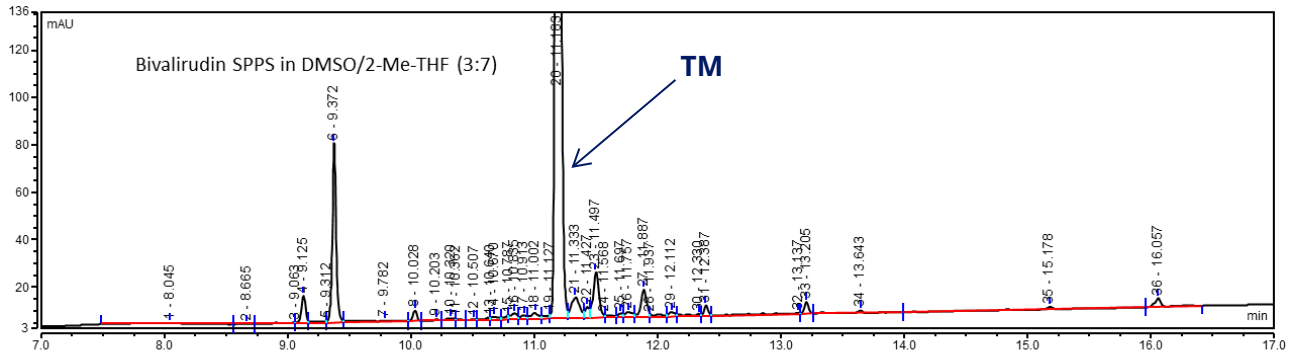


Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
4	10.025	des[Ile^{15}-Pro^{16}]	0.31
17	11.200	Bivalirudin	72.75
18	11.322	des[Gly-Gly]	3.11
19	11.443	des[Gly]	1.01
20	11.503	Ac-[Pro^{4}-Leu^{20}]-OH	6.23

Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
22	11.767	Ac-[Asn^{9}-Leu^{20}]-OH	0.38
23	11.782	des[Arg-Pro]	0.49
24	11.877	des[Arg^{3}]	1.73
30	12.400	[Asp^{11}-Leu^{20}]-OH	0.26
other peaks			13.73

Automated SPPS of Bivalirudin (H-fPRPGGGGNGDFEEIPEEYL-OH) in DMSO/2-Me-THF (3:7)

7.5 mmol scale, HPLC method A

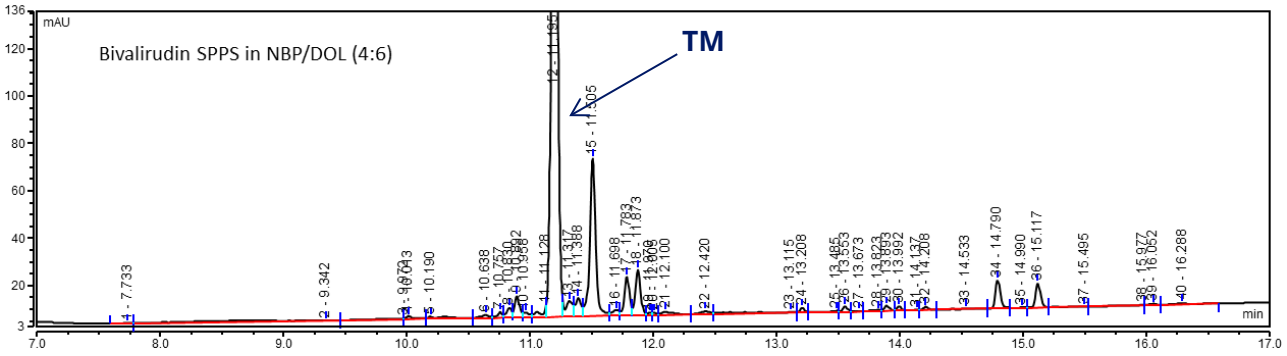


Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
4	9.125	Ac-[Glu^{18}-Leu^{20}]-OH	1.35
6	9.372	Ac-[Glu^{17}-Leu^{20}]-OH	8.33
8	10.028	des[Ile^{15}-Pro^{16}]	0.52
20	11.183	Bivalirudin	70.66
21	11.333	des[Gly-Gly]	2.30
22	11.427	des[Gly]	0.58

Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
23	11.497	Ac-[Pro^{4}-Leu^{20}]-OH	3.96
25	11.697	Ac-[Asn^{9}-Leu^{20}]-OH	0.52
26	11.757	des[Arg-Pro]	0.52
27	11.887	des[Arg^{3}]	1.96
31	12.387	[Asp^{11}-Leu^{20}]-OH	0.58
other peaks			8.72

Automated SPPS of Bivalirudin (H-fPRPGGGGNGDFEEIPEEYL-OH) in NBP/DOL (4:6)

7.5 mmol scale, HPLC method A

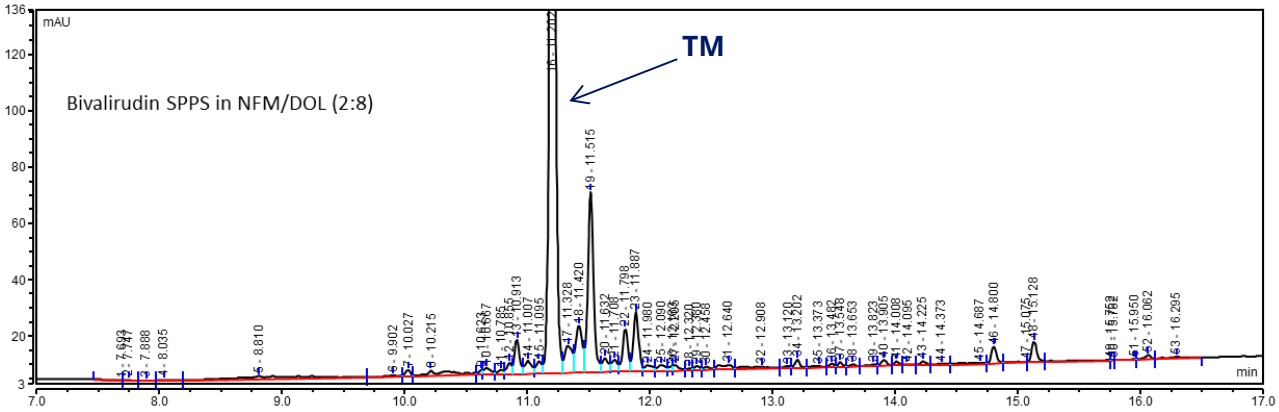


Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
4	10.013	des[Ile^{15}-Pro^{16}]	0.34
12	11.195	Bivalirudin	62.69
13	11.317	des[Gly-Gly]	1.57
14	11.388	des[Gly]	1.35
15	11.505	Ac-[Pro^{4}-Leu^{20}]-OH	12.75

Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
16	11.698	Ac-[Asn^{9}-Leu^{20}]-OH	0.55
17	11.783	des[Arg-Pro]	2.55
18	11.873	des[Arg^{3}]	3.27
22	12.420	[Asp^{11}-Leu^{20}]-OH	0.45
other peaks			14.48

Automated SPPS of Bivalirudin (H-fPRPGGGGNGDFEEIPEEYL-OH) in NFM/DOL (2:8)

7.5 mmol scale, HPLC method A



Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
7	10.027	des[Ile^{15}-Pro^{16}]	0.27
16	11.202	Bivalirudin	58.58
17	11.328	des[Gly-Gly]	2.07
18	11.420	des[Gly]	3.40
19	11.515	Ac-[Pro^{4}-Leu^{20}]-OH	11.47

Peak No.	Ret. Time (min)	Peak Name	Rel. Area %
21	11.708	Ac-[Asn^{9}-Leu^{20}]-OH	0.76
22	11.798	des[Arg-Pro]	2.29
23	11.887	des[Arg^{3}]	3.46
29	12.380	[Asp^{11}-Leu^{20}]-OH	0.24
other peaks			17.36

4. References

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2. C. Reichardt, *Chem. Rev.*, 1994, **94**, 2319-2358.
3. S. Eissler, M. Kley, D. Bachle, G. Loidl, T. Meier and D. Samson, *J. Pept. Sci.*, 2017, **23**, 757-762.