In-situ Fmoc Removal – A Sustainable Solid-Phase Peptide Synthesis Approach

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General Information

All reagents and solvents were purchased from commercial suppliers and used without further purification. Fmoc amino acids, Fmoc-Rink Amide AM resin (loading 0.64 mmol/g) were purchased from Iris Biotech. OxymaPure and DIC were gifted from Luxembourg Biotech. and piperidine (PIP)/ 4-methylpiperidine (4-MP) were supplied by Sigma-Aldrich. Organic solvents dimethylformamide (DMF) and HPLC quality acetonitrile (CH_3CN) were purchased from Merck. Milli-Q water was used for RP-HPLC analyses. Analytical HPLC was performed on an Agilent 1100 system using a Phenomenex Aeris^{TM}C18 (3.6µm, 4.6 × 150 mm) column, with flow rate of 1.0 mL/min and UV detection at 220 nm. Chemstation software was used for data processing. Buffer A: 0.1% TFA in H_2O; buffer B: 0.1% TFA in CH_3CN. LC-MS was performed on Thermo Fisher Scientific UltiMate 3000 UHPLC-ISQTM EC single quadrupole mass spectrometer in positive ion mode using a Phenomenex Aeris^{TM} C18 (3.6 µm, 4.6 × 150 mm) column. Buffer A: 0.1% formic acid in H_2O; buffer B: 0.1% formic acid in CH_3CN.

HPLC method: 5-60% B into A in 15 min.

Experimental

SPPS

All peptides were assembled manually in plastic syringes fitted with a porous polyethylene disc, as Fmoc/*t*Bu methodology using DIC/OxymaPure as coupling agents. Fmoc removal was carried as in situ i.e., PIP or 4-MP was added to peptidyl resin without filtering of coupling cocktail after coupling. In some experiment Fmoc removal was carried out by treatment with a solution of 20% PIP or 4-MP in DMF (v/v).

SPPS of tripeptide H-Gly-Phe-Leu-NH_2 (H-GFL-NH_2) as using in situ Fmoc removal

Tripeptide H-Gly-Phe-Leu-NH_2 was synthesized using Fmoc-Rink -amide AMPS resin (loading 0.64 mmol/g) The coupling was performed using a ratio Fmoc-AA-OH/DIC/Oxyma [1:1:1, 3.0 or 1.5 eq.) in DMF at rt for 1 h as 1 min pre-activation. Fmoc removal was performed as *in situ* with PIP at different time and temperature (7 min, rt/45 ºC, 20 min, rt). Tripeptide was cleaved from

the resin by treating with TFA/TIS/H_2O (95:2.5:2.5) for 1 h at rt and precipitated with chilled diethyl ether. HPLC analysis method for coupling quantification was 5-60 % B into A.

SPPS of tripeptidyl resin (H-GFL-Rink amide AMPS) for coupling of Fmoc-Gly-OH and Fmoc-Tyr(*t*Bu)-OH unit

The tripeptidyl resin (H-GFL-resin) for coupling of Gly and Tyr units, was synthesized as standard protocol (Coupling → Washing → Deprotection → Washing) on Rink-amide-AMPS (loading 0.64 mmol/g). The coupling of each residue was performed using a ratio Fmoc-AA-OH/DIC/Oxyma [1:1:1, 3.0 eq.] in DMF at rt for 1 h as 1 min pre-activation. Fmoc removal was performed by using 20% PIP/DMF (v/v) for 1x1 min + 1x7 min at rt.

Coupling of Fmoc-Gly-OH and Fmoc-Tyr(*t*Bu)-OH, on tripeptidyl resin (H-GFL-Rink amide AMPS) and in situ Fmoc removal

Sixty-minute couplings were done by *in situ* activation or pre-activating 0.6 M solutions of coupling cocktail [Fmoc-AA-OH: OxymaPure: DIC (1:1:1, 3.0 eq.) in 0.5 mL DMF] for 1 min, prior to addition to the tripeptidyl resin and in case of 1.5 eq. of reagent the concentration of coupling cocktail was 0.3 M at rt/45 ºC. Fmoc removal step was carried out by treatment with neat PIP or 4-MP i.e 0.12 mL PIP or 4-MP was added to the coupling cocktail after coupling without filtering coupling reaction mixture and washing of peptidyl resin (*in situ* Fmoc removal) for 1x7 or 10 or 20 min at rt/45 ºC. After Fmoc removal resin was washed with DMF or 1% OxymaPure or both.

SPPS of Leu-Enkephalin pentapeptide (H-Tyr-Gly-Gly-Phe-Leu-NH_2)

Previous standard SPPS protocol for comparison

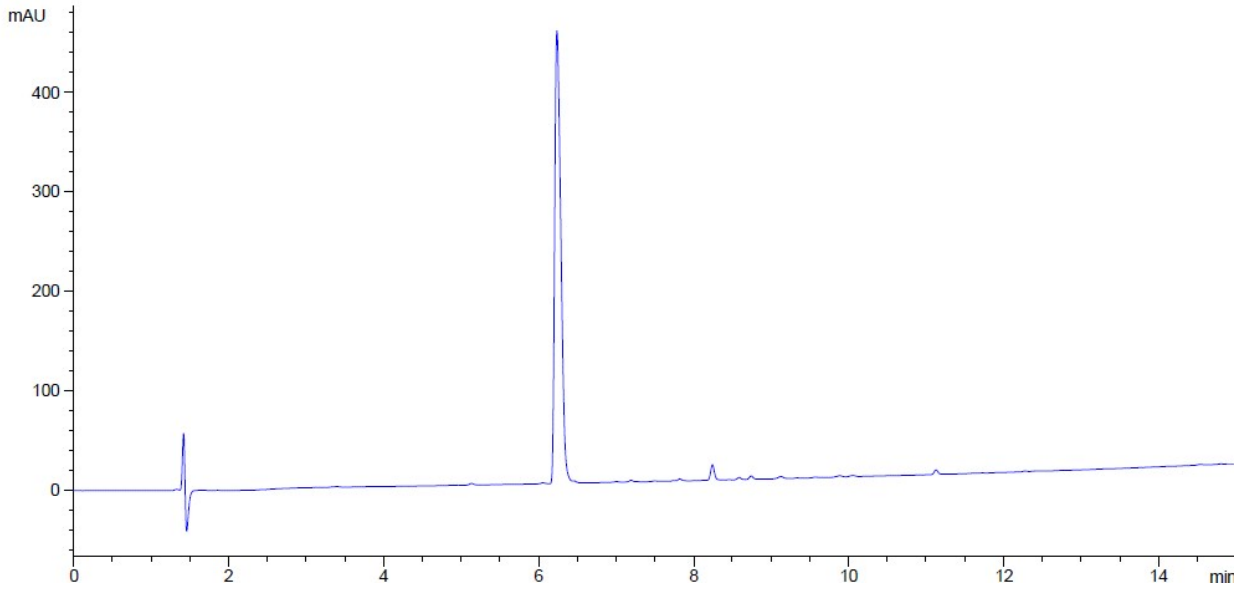
SPPS was carried out manually in plastic syringe fitted with polyethylene porous using Fmoc/*t*Bu methodology. Fmoc-Rink-Amide AM resin (0.64 mmol/g) was washed with DMF (2×5 min). Deprotection of the Fmoc group was achieved by treatment of the resin with 20% PIP/DMF (1×1 min and 1×7 min) followed by washing with DMF. The protected Fmoc-amino acids (1.5 eq.) and OxymaPure (1.5 eq.) in 0.5 mL DMF (0.6M) preactivated 1 min with DIC (1.5eq.) prior to added on resin for 1h at rt. This was repeated until the pentapeptides were achieved. Washes after couplings with DMF (2x3 mL) and after deprotections (3x3mL) were performed. Peptide was

cleaved from resin by treating with TFA/TIS/H_2O (95:2.5:2.5) for 1 h at rt., precipitated with chilled diethyl ether, centrifuged to afford desired peptide, and analyzed by HPLC and LCMS.

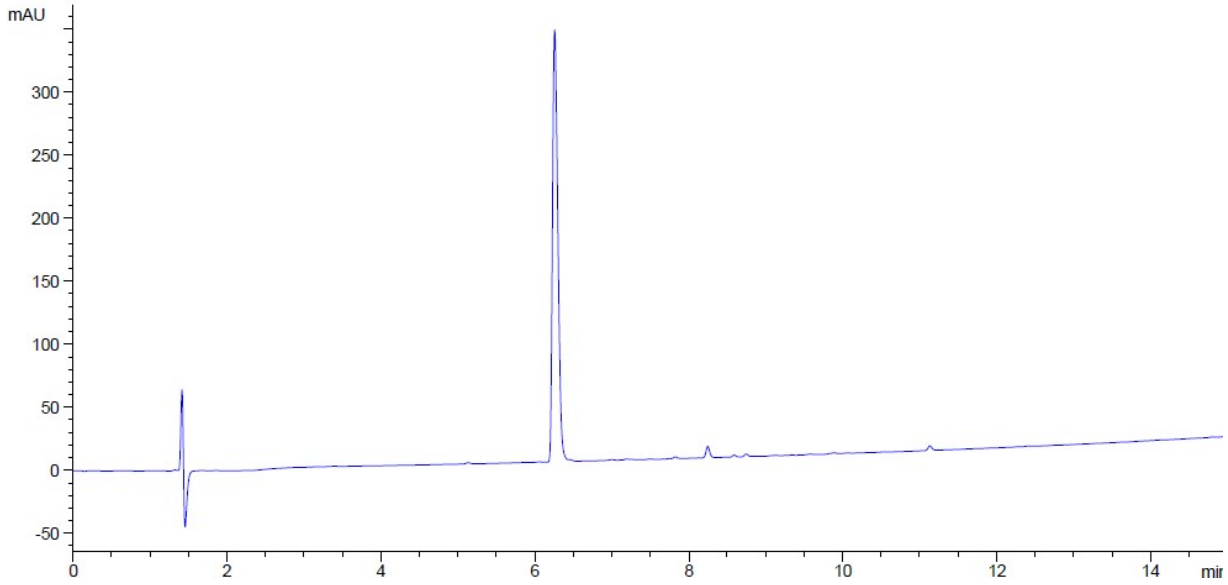
New adopted SPPS protocol (In situ Fmoc removal)

SPPS was carried out manually in plastic syringe fitted with polyethylene porous using Fmoc/*t*Bu methodology. Fmoc-Rink-Amide AM resin (0.64 mmol/g) was washed with DMF (2×1 mL for 5 min). Deprotection of the Fmoc group was achieved by treatment of the resin with 20% 4-MP/DMF (1×7 min) followed by washing with 1% OxymaPure/DMF (2 x 1 mL). The protected Fmoc-amino acids (1.5 eq.) and OxymaPure (1.5 eq.) in 0.5 mL DMF (0.3M) preactivated 1 min with DIC (1.5 eq.) prior to added on resin as a coupling system, 1 h at rt. After 1 h of coupling 0.12 mL neat 4-MP was added to coupling reaction mixture without removing of coupling reaction mixture and washing for 1x7 min at rt. After 7 min reaction mixture was filtered and peptidyl resin was washed with 1% OxymaPure/DMF (2x1 mL). Coupling and deprotection was repeated until the peptide was assembled onto the resin. All the peptides were dried and cleaved from resin by treating with TFA/TIS/H_2O (95:2.5:2.5) for 1 h at rt. The cleavage mixture was, precipitated with chilled diethyl ether, centrifuged to afford desired peptide, and confirmed by HPLC and LCMS analysis.

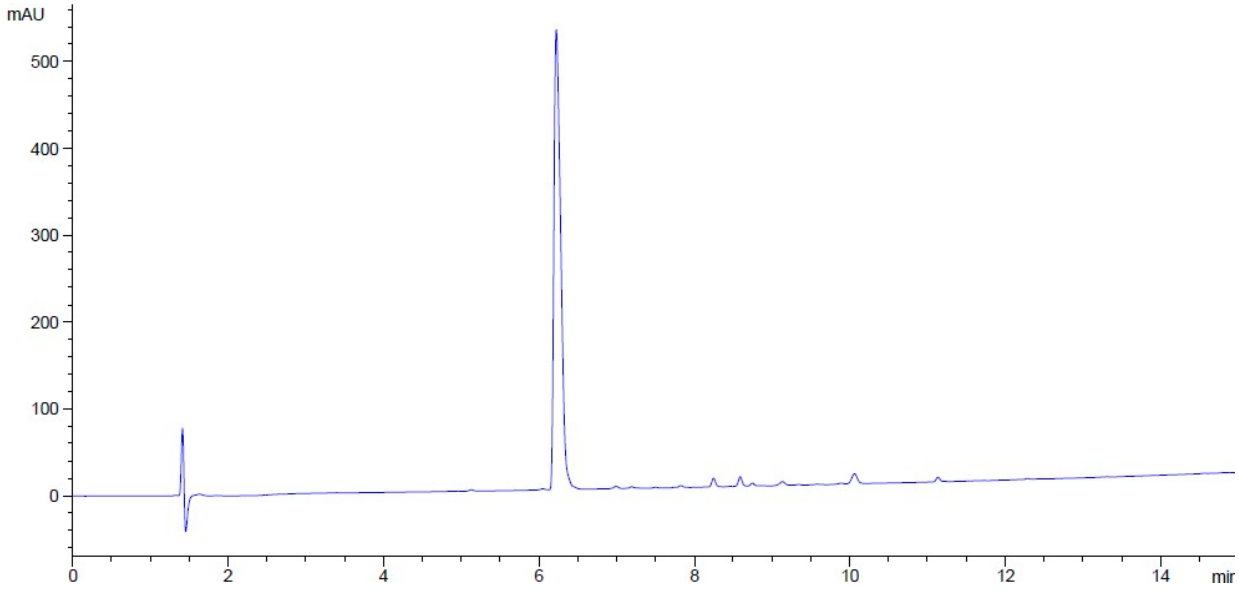
HPLC -S1. Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 3.0 eq], 1 min pre-activation, 1h, rt in DMF, *in situ* Fmoc removal 1x7min, rt.



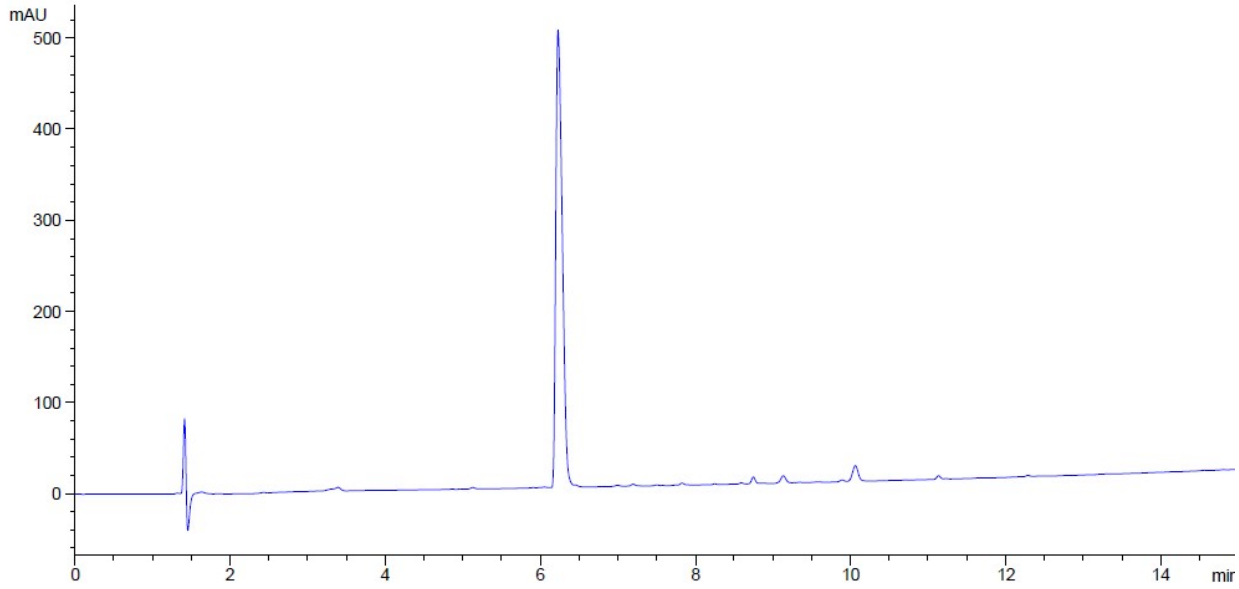
HPLC -S2. Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 3.0 eq.], 1 min pre-activation, 1h, rt in DMF, *in situ* Fmoc removal 1x7 min, 45 ºC.



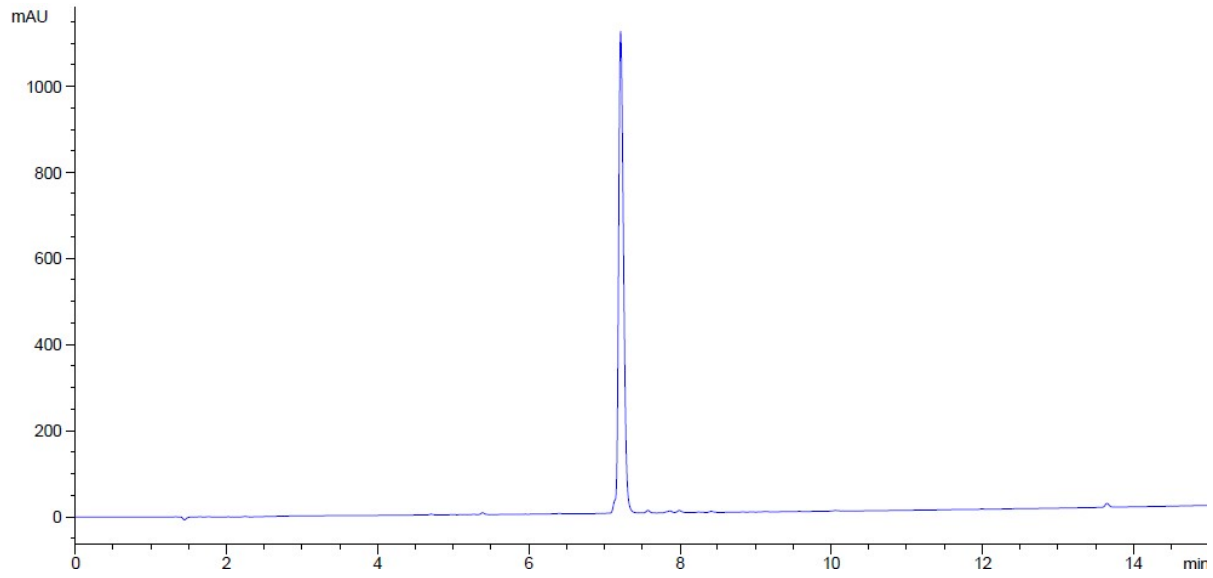
HPLC -S3. Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 3.0 eq.], 1 min pre-activation, 1h, rt in DMF, *in situ* Fmoc removal 1x20 min, rt.



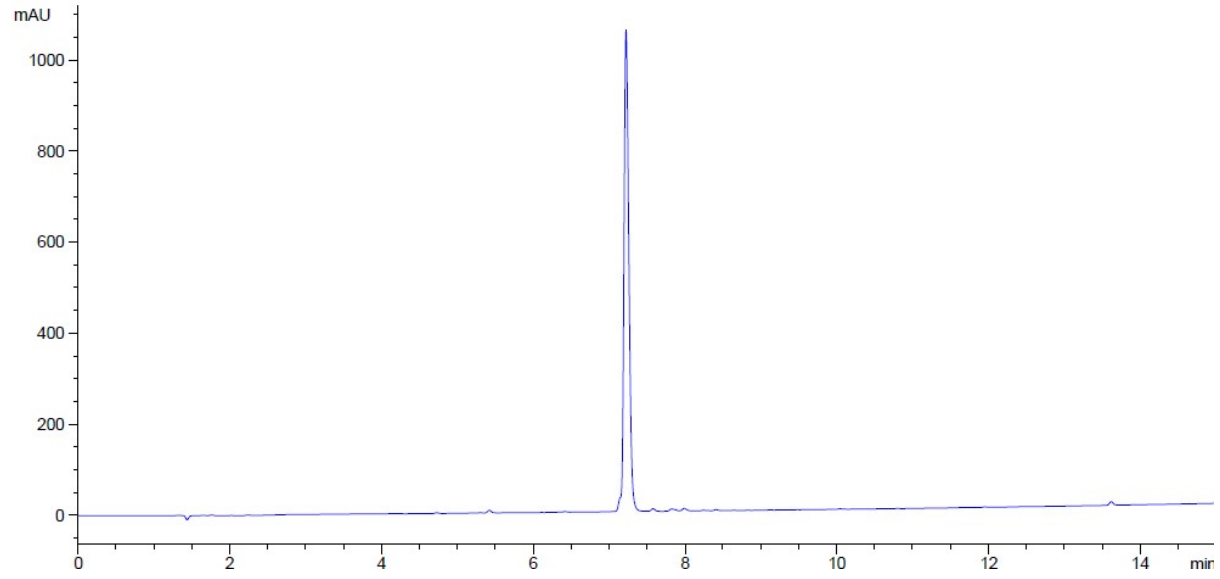
HPLC -S4. Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], 1 min pre-activation, 1h, rt in DMF, *in situ* Fmoc removal 1x20 min, rt.



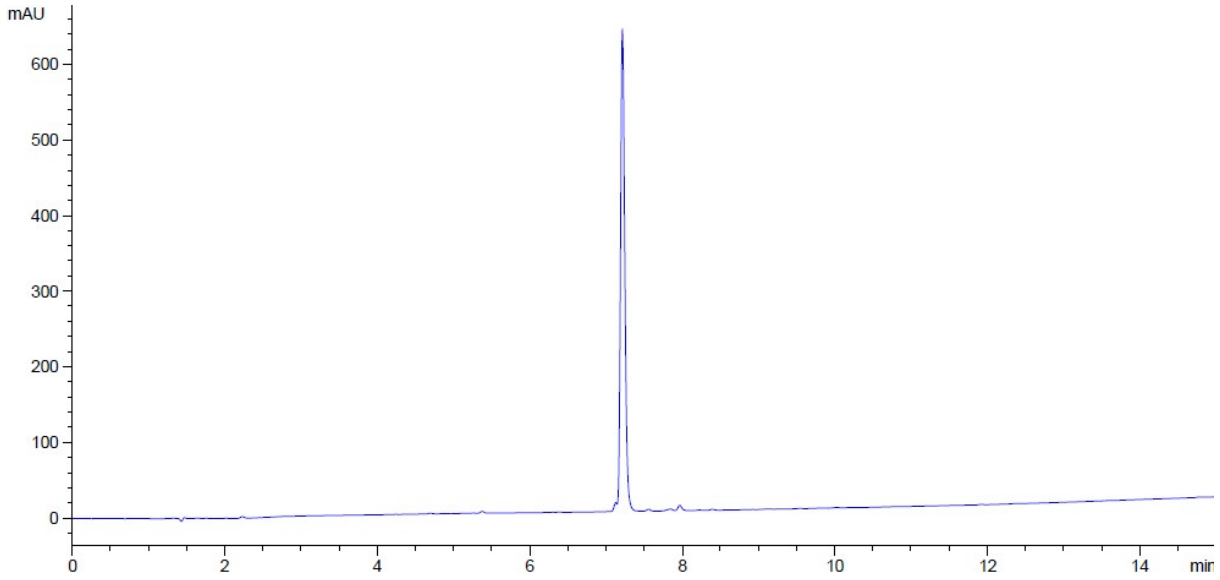
HPLC -S5. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 3.0 eq.], 1 min pre-activation, 1h, rt in DMF, *in situ* Fmoc removal 1x7 min, rt. Washing after Fmoc removal with DMF (3x3 mL).



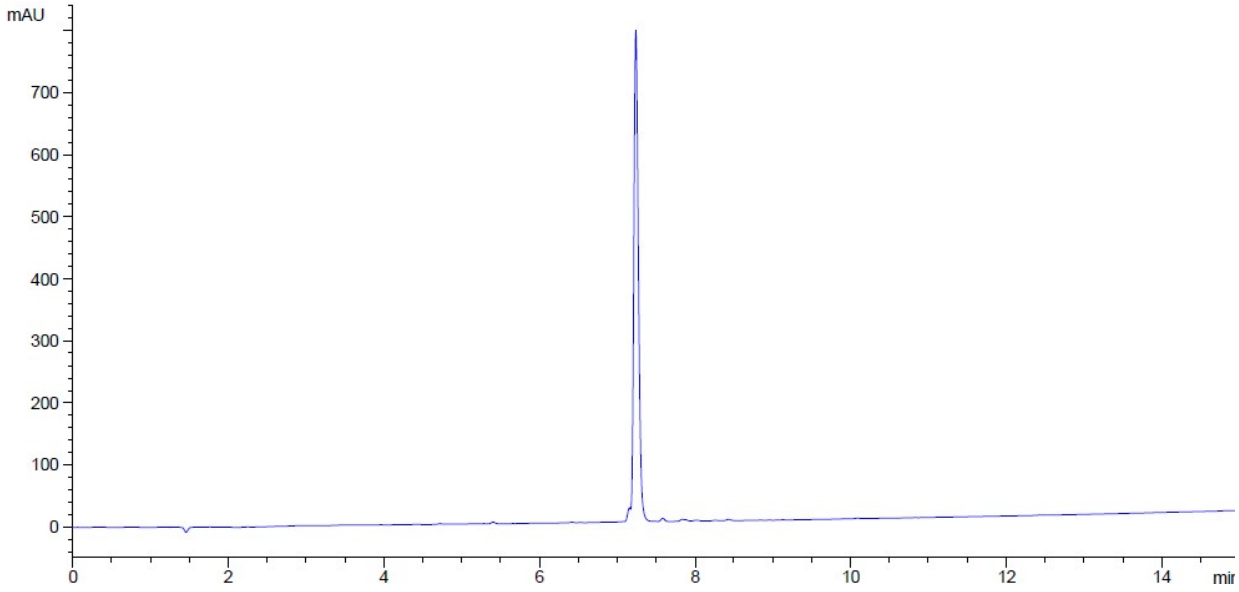
HPLC -S6. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 3.0 eq.], 1 min pre-activation, 1h, rt in DMF, *in situ* Fmoc removal 1x7 min, 45 ºC. Washing after Fmoc removal with DMF (3x3 mL).



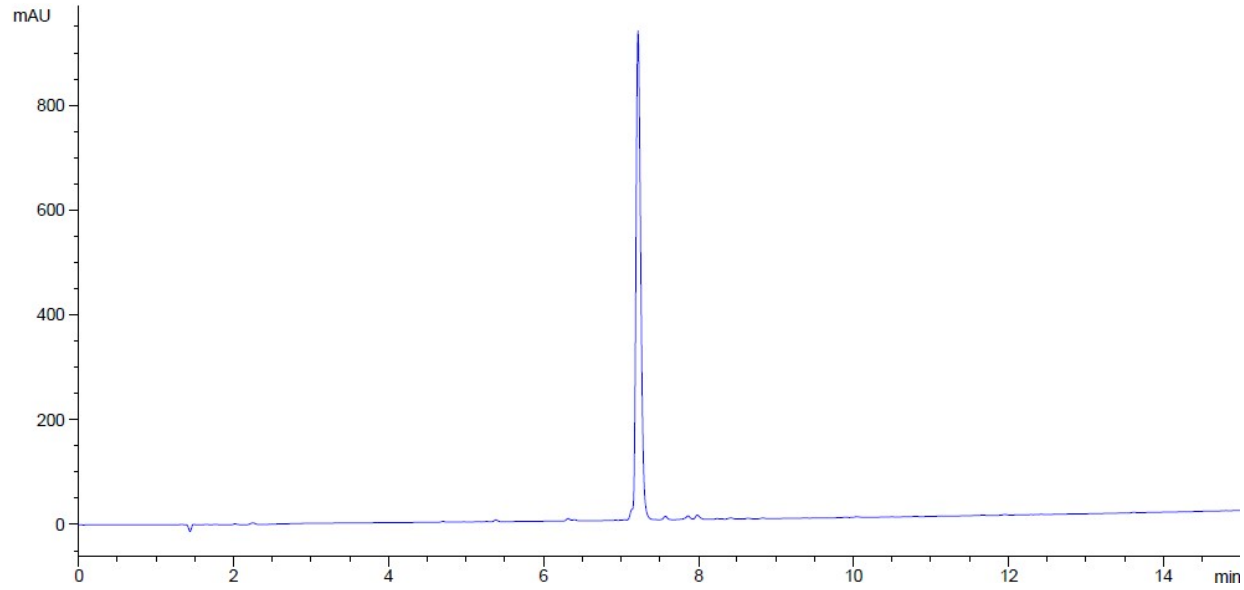
HPLC -S7. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 3.0 eq.], 1 min pre-activation, 1h, rt in DMF, *in situ* Fmoc removal 1x20 min, rt. Washing after Fmoc removal with DMF (3x3 mL).



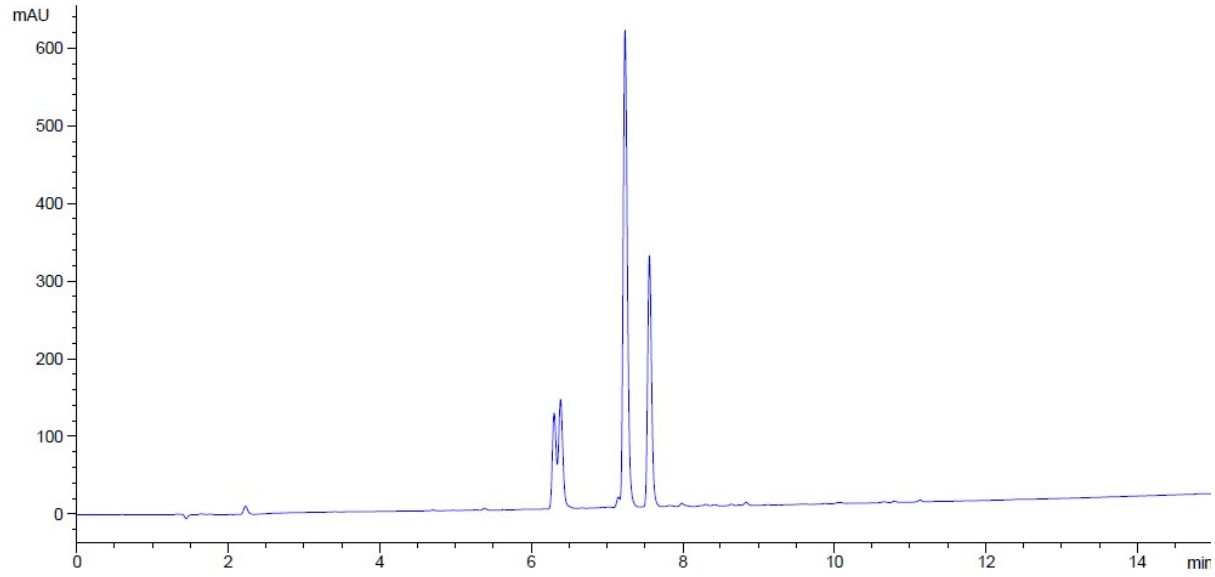
HPLC -S8. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], 1 min pre-activation, 1h, rt in DMF, *in situ* Fmoc removal 1x7 min, rt. Washing after Fmoc removal with DMF (3x3 mL).



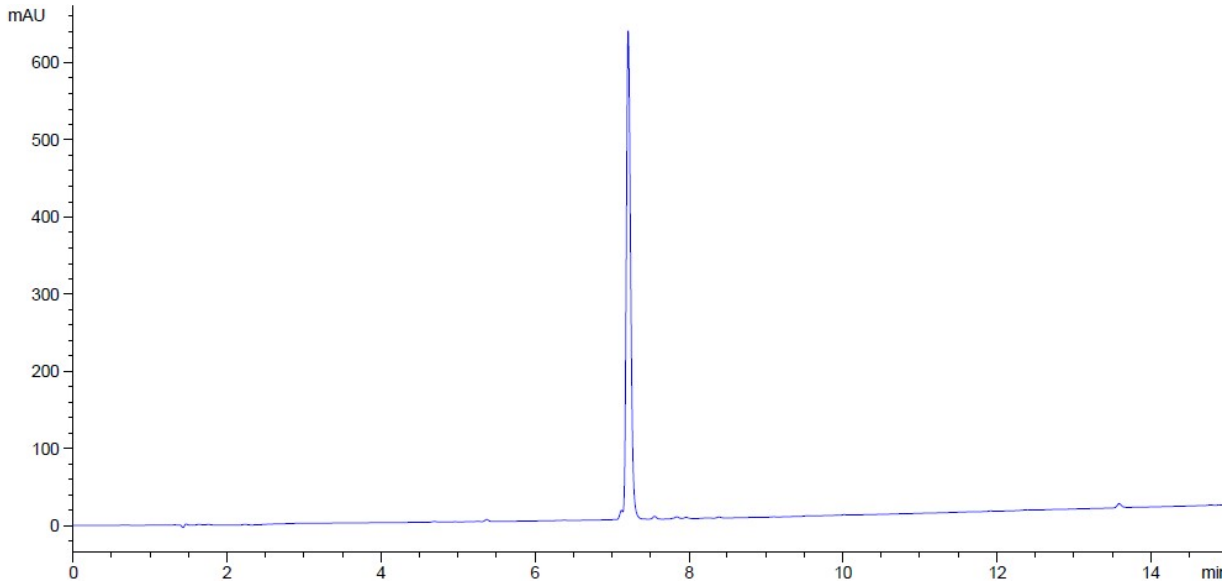
HPLC -S9. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], 1 min pre-activation, 1h, rt in DMF, *in situ* Fmoc removal 1x7 min, 45 ºC. Washing after Fmoc removal with DMF (3x3 mL).



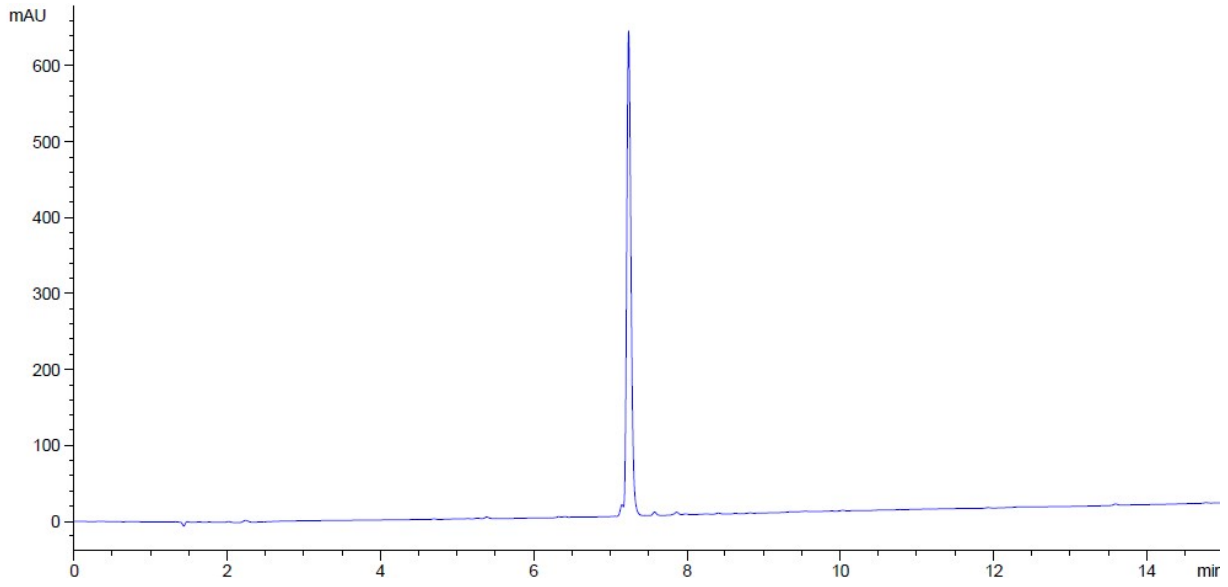
HPLC -S10. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Before coupling added 0.1 mL of 20% PIP/DMF to peptidyl resin, mix well then added coupling cocktail. Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 3.0 eq.], 1 min pre-activation, 1h, rt in DMF, *in situ* Fmoc removal 1x7 min, rt. Washing after Fmoc removal with DMF (3x3 mL).



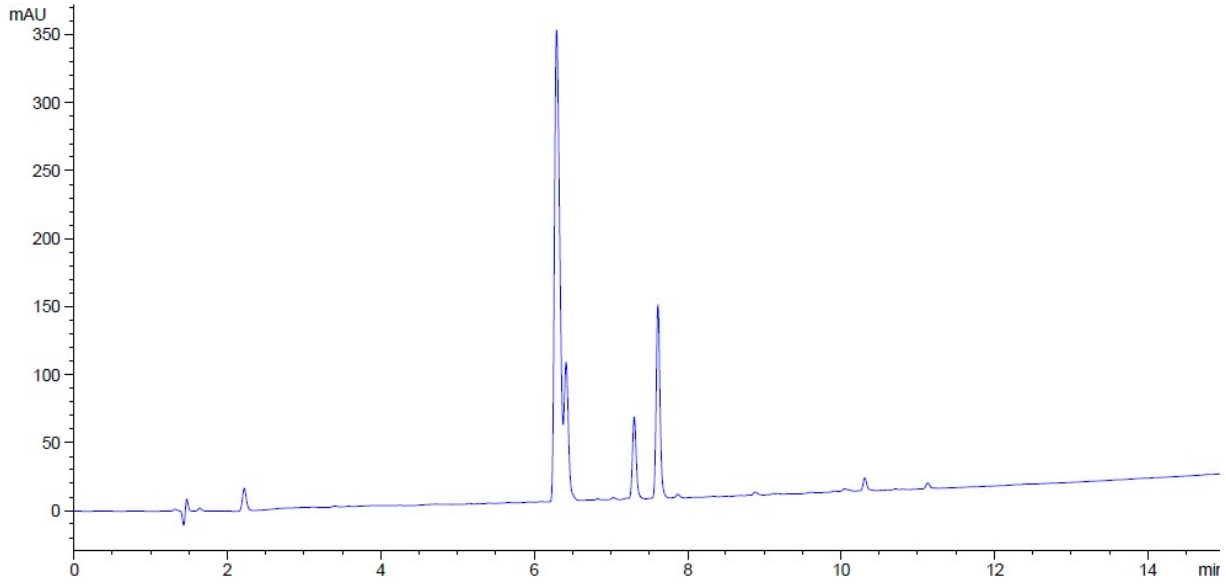
HPLC -S11. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Before coupling added 0.1 mL of 5% PIP/DMF to peptidyl resin, mix well then added coupling cocktail. Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 3.0 eq.], 1 min pre-activation, 1h, rt in DMF, *in situ* Fmoc removal 1x7min, rt. Washing after Fmoc removal with DMF (3x3 mL).



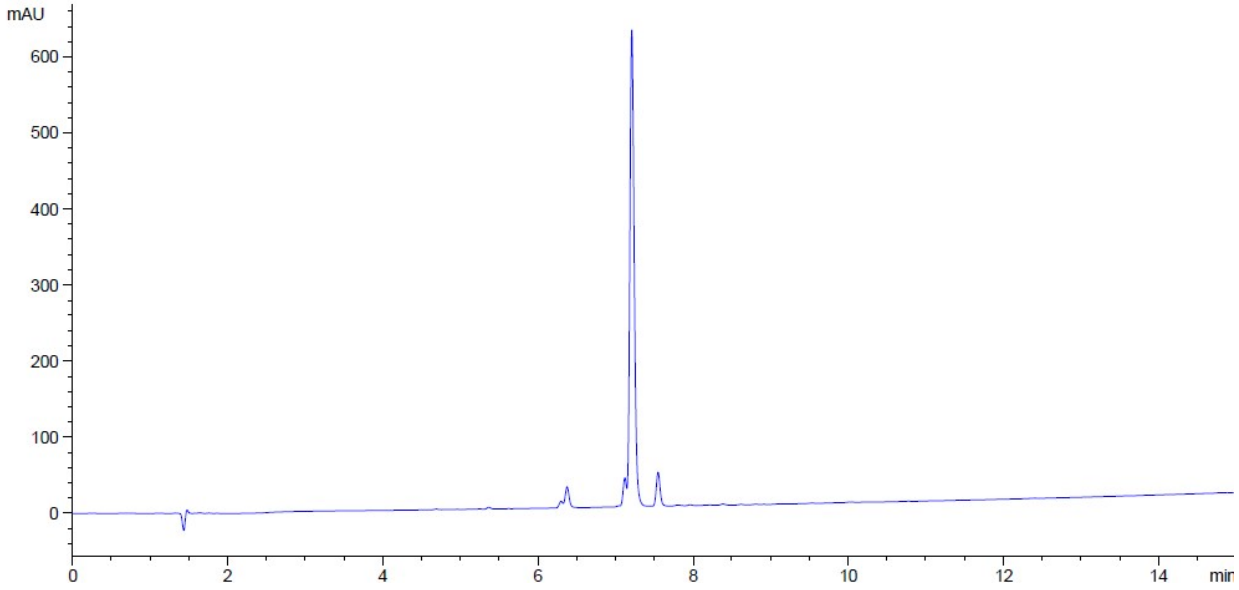
HPLC -S12. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Before coupling added 0.1 mL of 1% PIP/DMF to peptidyl resin, mix well then added coupling cocktail. Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 3.0 eq.], 1 min pre-activation, 1h, rt in DMF, *in situ* Fmoc removal 1x7min, rt.



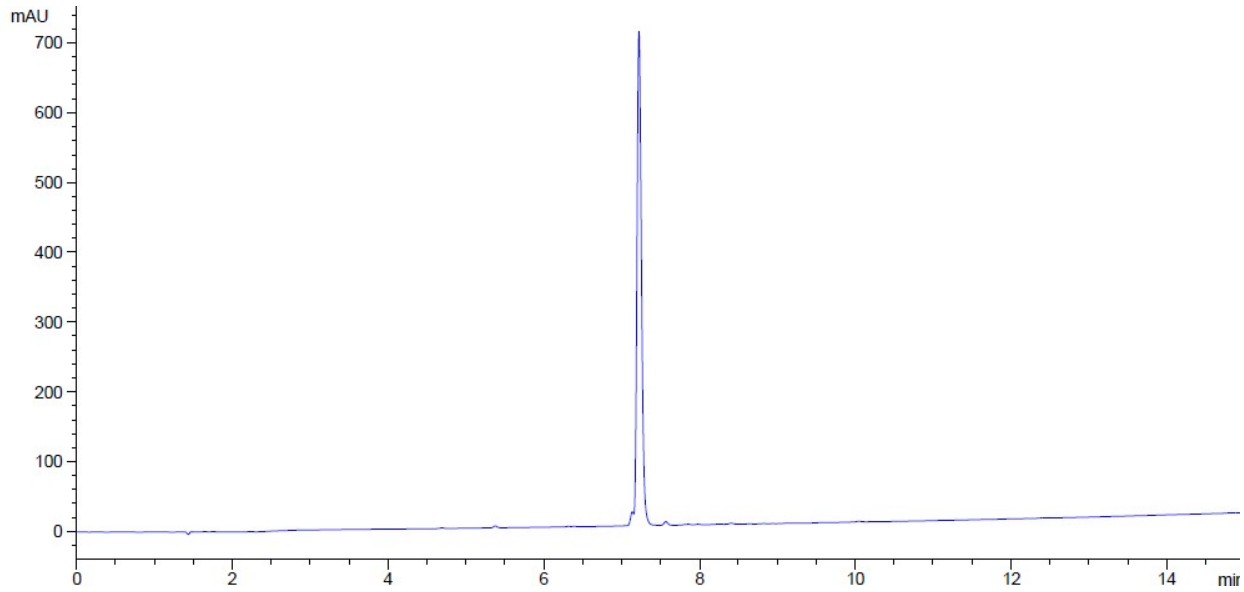
HPLC -S13. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Before coupling added 0.1 mL of 20% PIP/DMF to peptidyl resin, mix well then added coupling cocktail. Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], 1 min pre-activation, 1h, rt in DMF, *in situ* Fmoc removal 1x7min, rt.



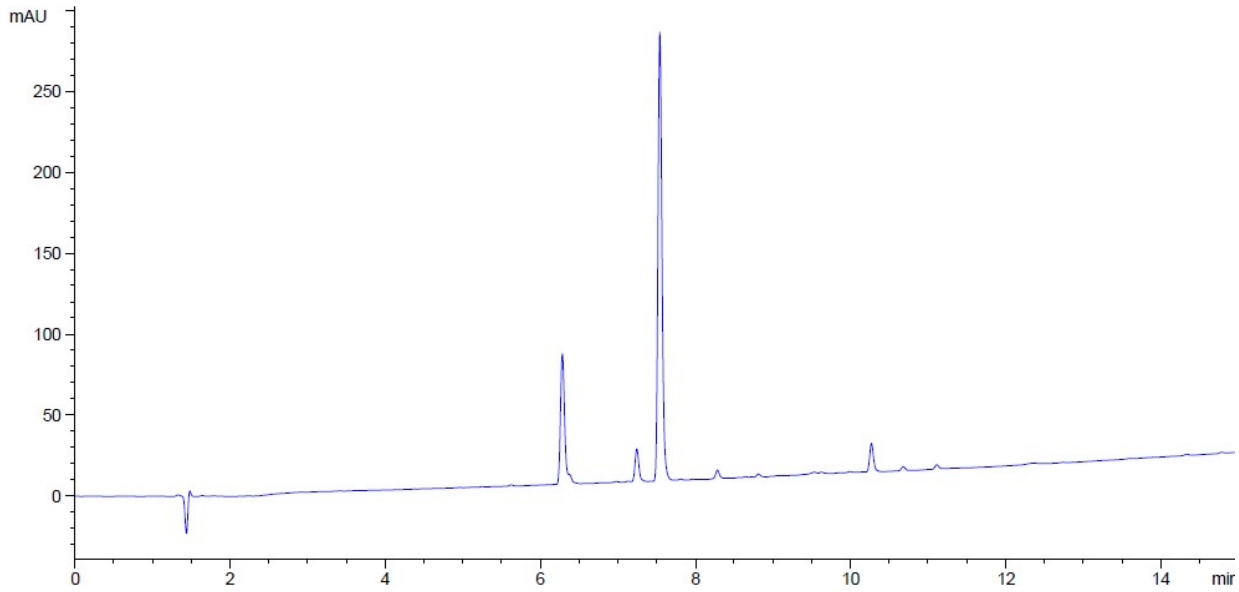
HPLC -S14. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Before coupling added 0.1 mL of 5% PIP/DMF to peptidyl resin, mix well then added coupling cocktail. Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], 1 min pre-activation, 1h, rt in DMF, *in situ* Fmoc removal 1x7min, rt.



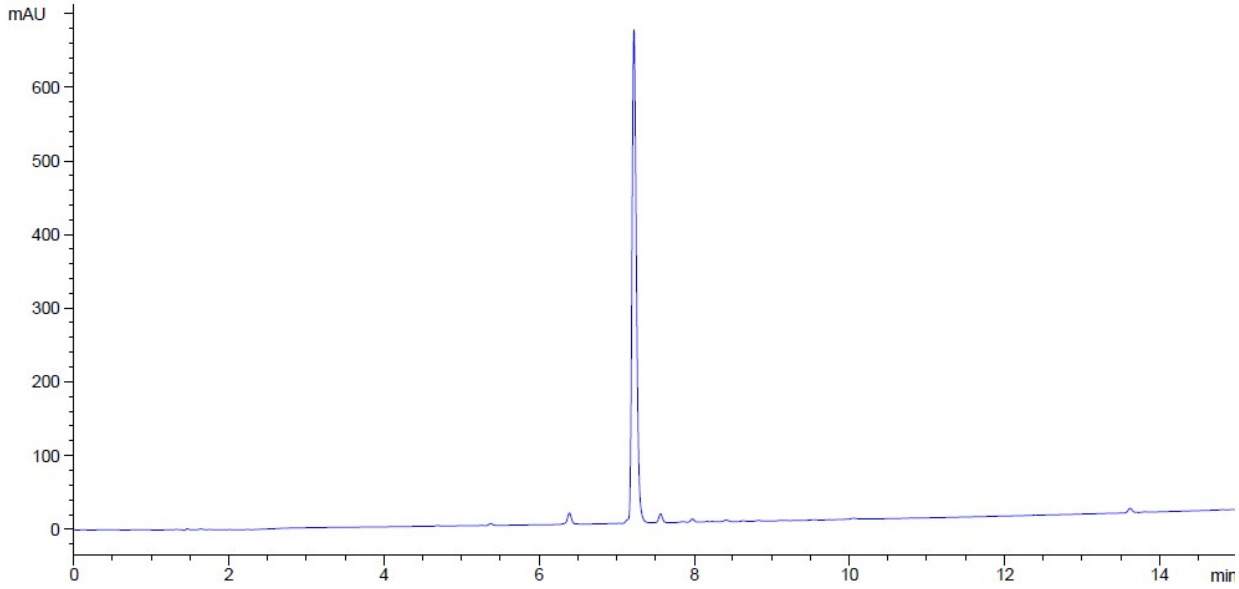
HPLC -S15. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Before coupling added 0.1 mL of 1% PIP/DMF to peptidyl resin, mix well then added coupling cocktail. Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], 1 min pre-activation, 1h, rt in DMF, *in situ* Fmoc removal 1x7min, rt.



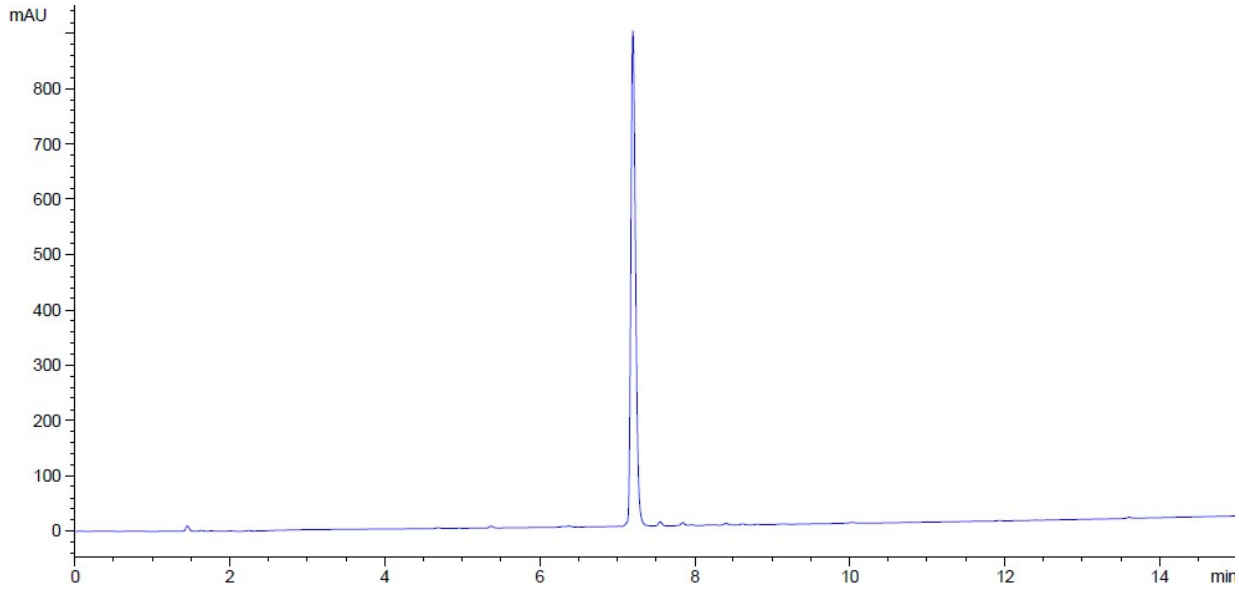
HPLC -S16. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Before coupling added 0.1 mL of 20% PIP/DMF to peptidyl resin, mix well then added coupling cocktail. Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], 1 min pre-activation, 1h, 45 ºC in DMF, *in situ* Fmoc removal 1x7min, 45 ºC.



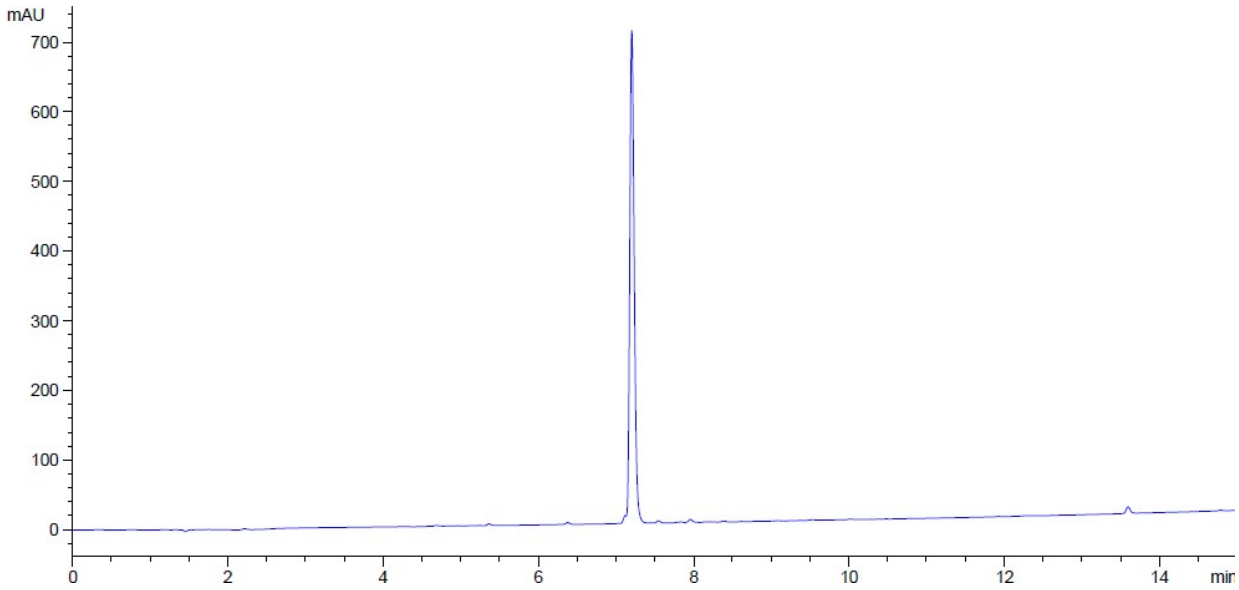
HPLC -S17. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Before coupling added 0.1 mL of 5% PIP/DMF to peptidyl resin, mix well then added coupling cocktail. Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], 1 min pre-activation, 1h, 45 ºC in DMF, *in situ* Fmoc removal 1x7min, 45 ºC.



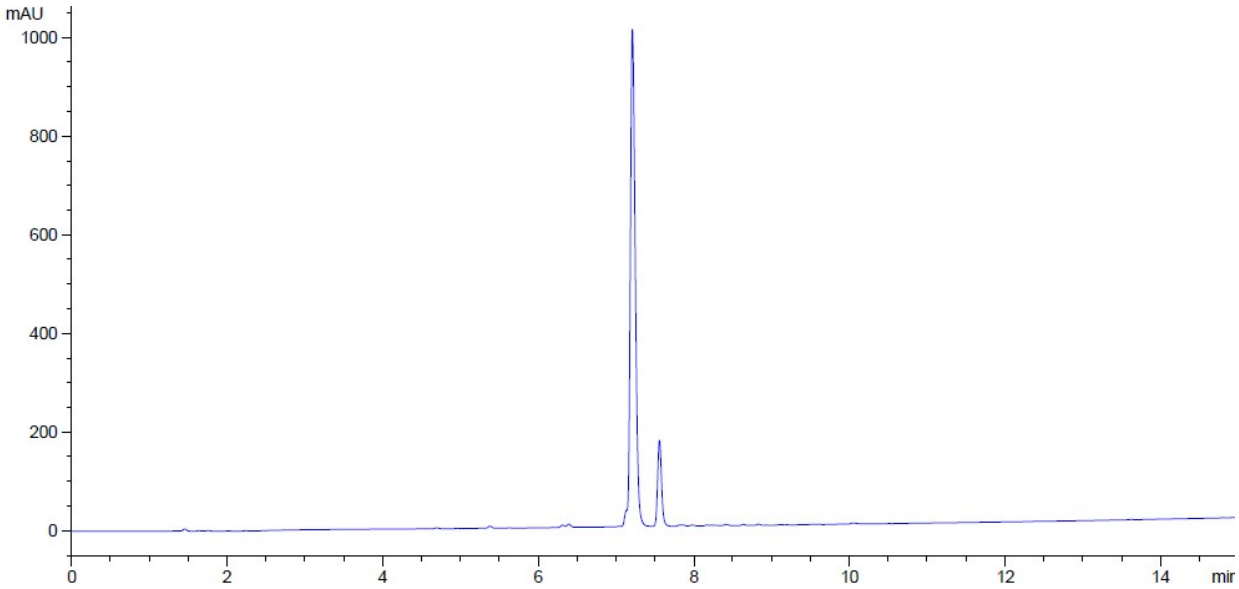
HPLC -S18. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Before coupling added 0.1 mL of 1% PIP/DMF to peptidyl resin, mix well then added coupling cocktail. Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], 1 min pre-activation, 1h, 45 ºC in DMF, *in situ* Fmoc removal 1x7min, 45 ºC.



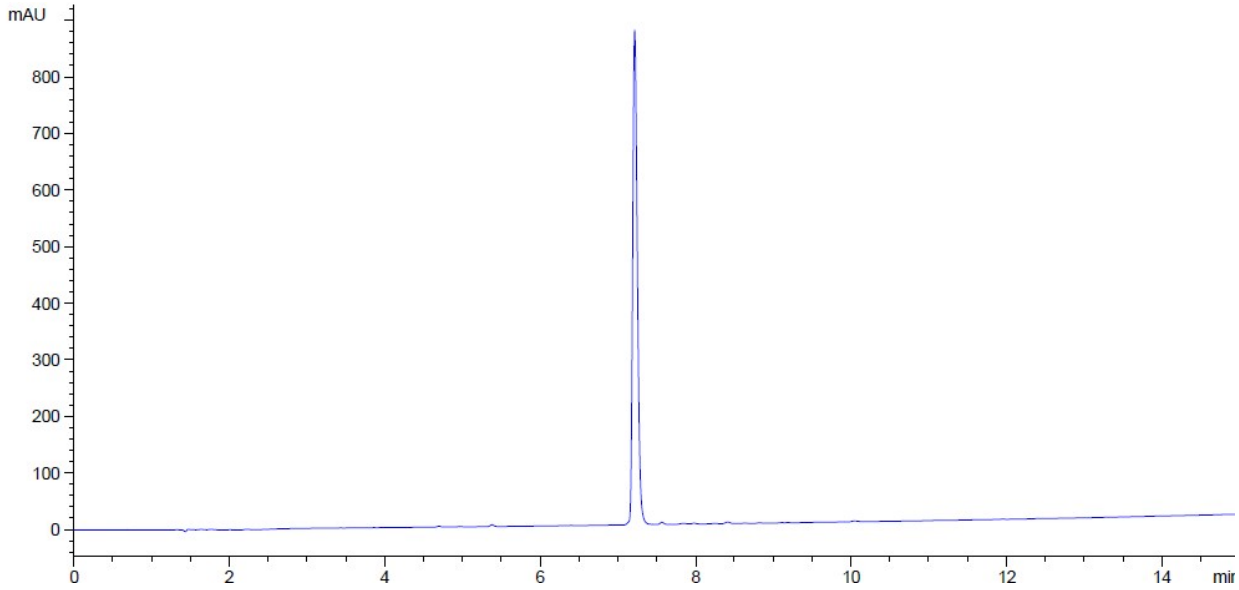
HPLC -S19. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Before coupling added 0.1 mL of 20% PIP/DMF to peptidyl resin, mix well and washed with 1x1 mL of 1% OxymaPure, then added coupling cocktail. Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 3.0 eq.], 1 min pre-activation, 1h, rt in DMF, *in situ* Fmoc removal 1x7min, rt.



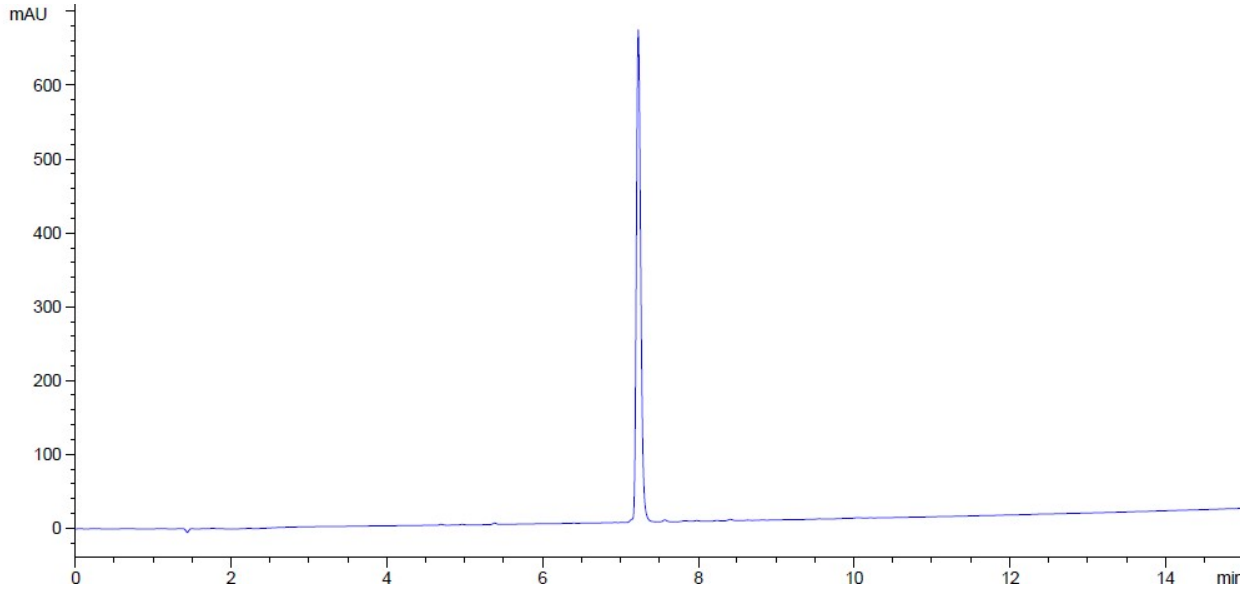
HPLC -S20. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Before coupling added 0.1 mL of 20% PIP/DMF to peptidyl resin, mix well and washed with 1x1 mL of 1% OxymaPure, then added coupling cocktail. Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], 1 min pre-activation, 1h, rt in DMF, *in situ* Fmoc removal 1x7min, rt.



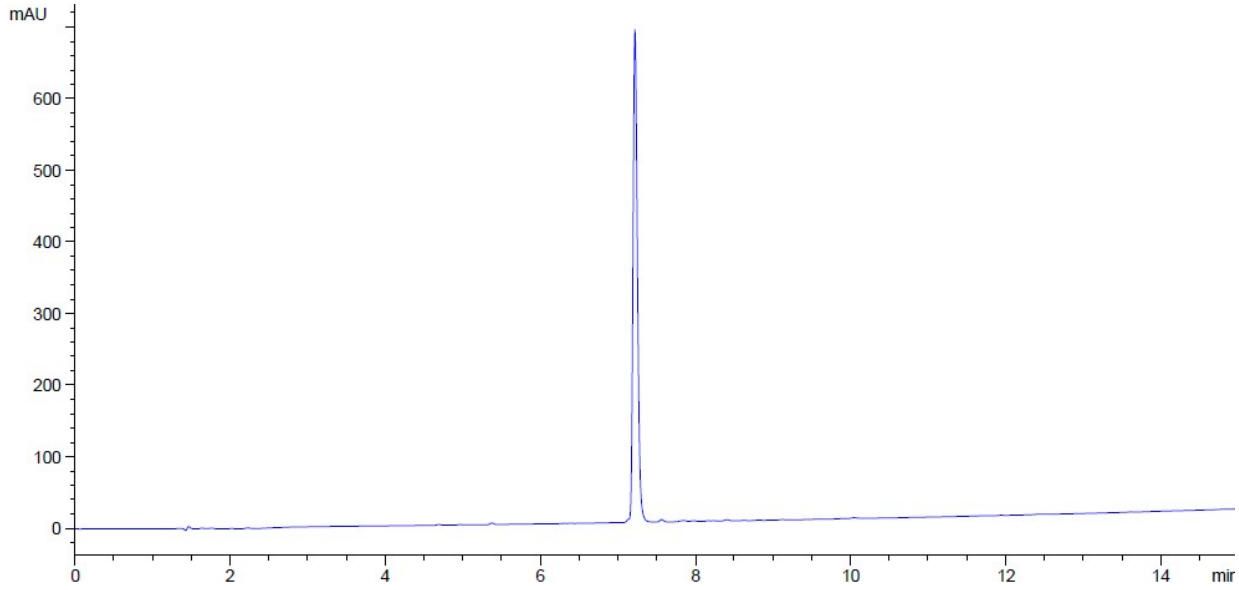
HPLC -S21. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Before coupling added 0.1 mL of 20% PIP/DMF to peptidyl resin, mix well and washed with 1x1 mL of 1% OxymaPure, then added coupling cocktail. Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], 1 min pre-activation, 1h, rt in DMF, *in situ* Fmoc removal 1x7min, 45 ºC.



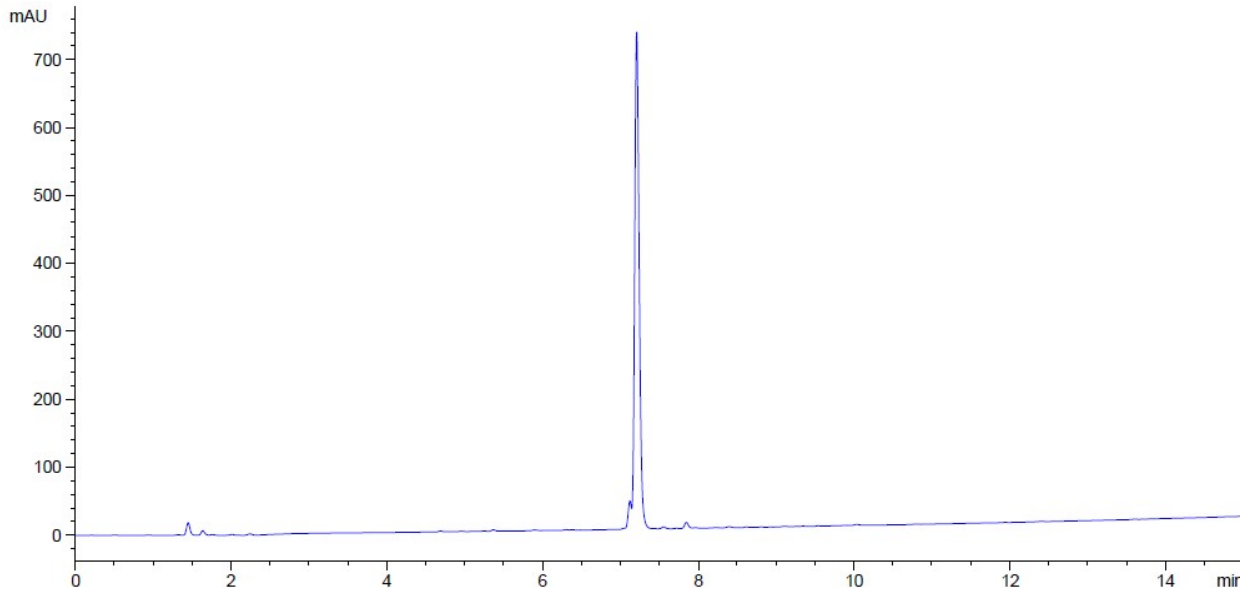
HPLC -S22. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Before coupling added 0.1 mL of 20% PIP/DMF to peptidyl resin, mix well and washed with 2x1 mL of 1% OxymaPure, then added coupling cocktail. Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], 1 min pre-activation, 1h, rt in DMF, *in situ* Fmoc removal 1x7min, rt.



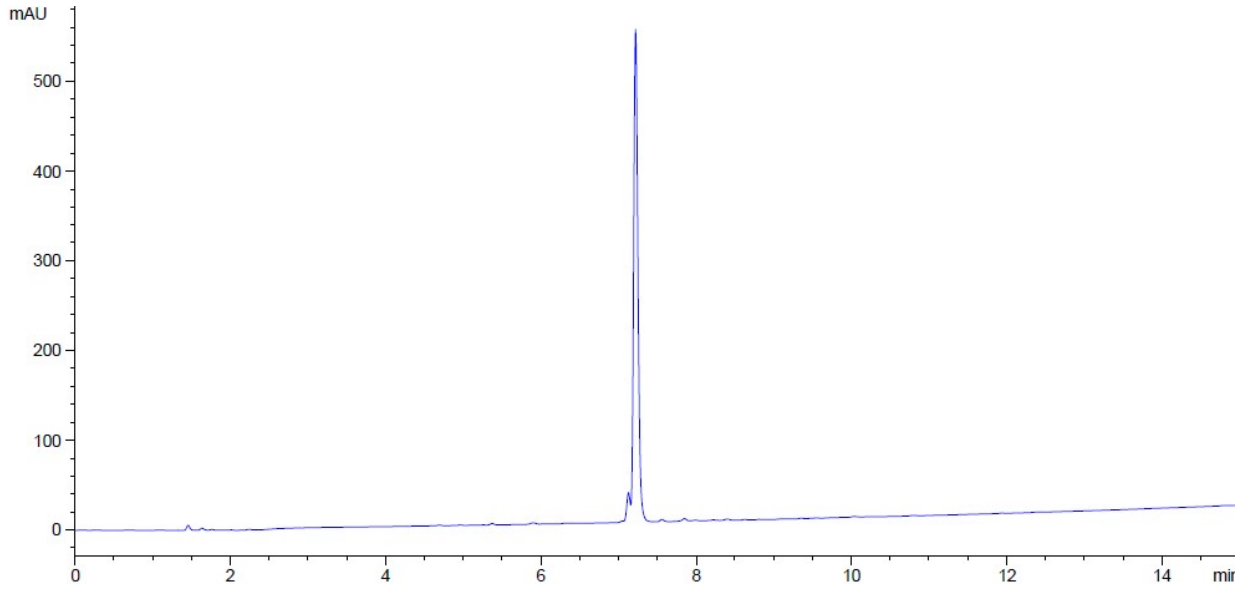
HPLC -S23. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Before coupling added 0.1 mL of 20% PIP/DMF to peptidyl resin, mix well and washed with 2x1 mL of 1% OxymaPure, then added coupling cocktail. Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], 1 min pre-activation, 1h, rt in DMF, *in situ* Fmoc removal 1x7min, 45 ºC.



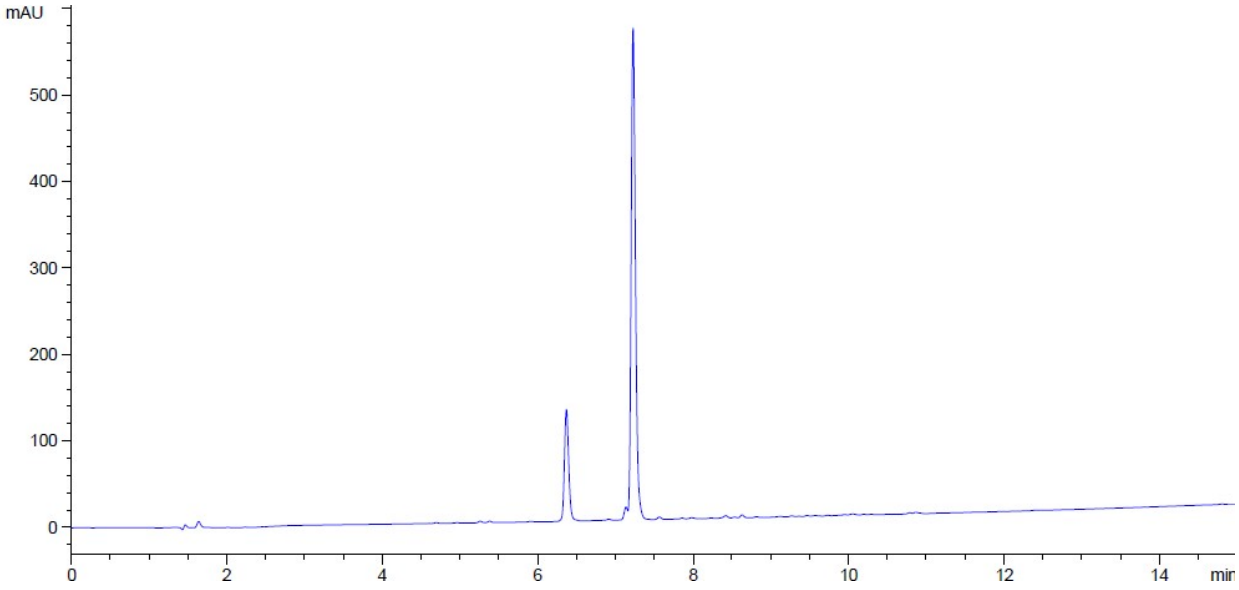
HPLC -S24. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Coupling as Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], 1 min pre-activation, 1h in DMF, *in situ* Fmoc removal 1x7min. Coupling and Fmoc removal at rt. Washing after Fmoc removal with 2x1 mL of 1% OxymaPure in DMF.



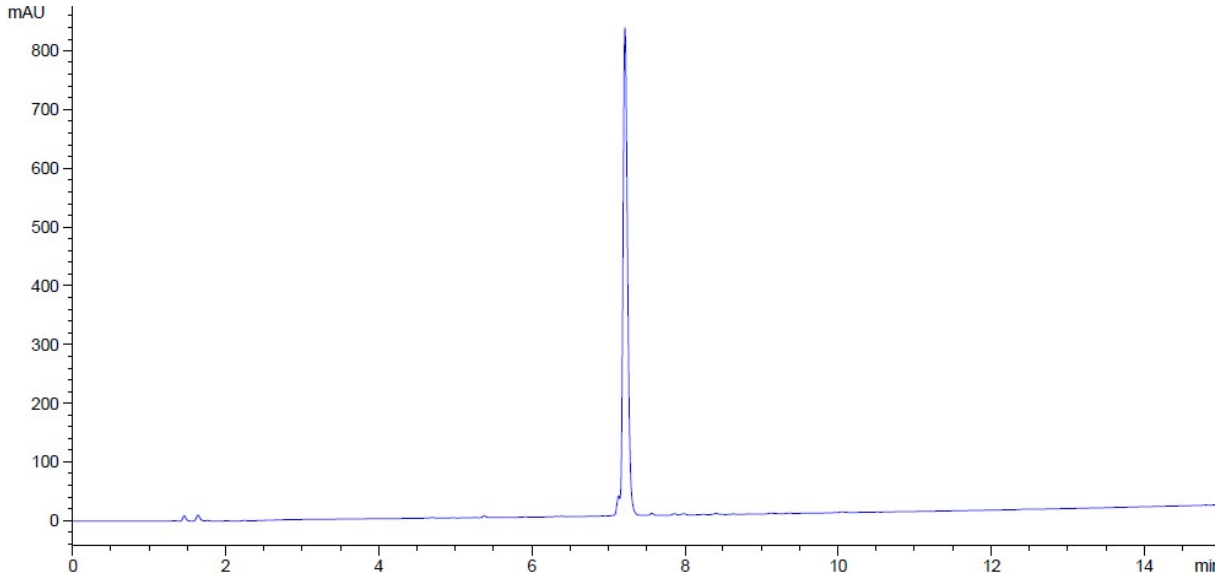
HPLC -S25. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Coupling as Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], 1 min pre-activation, 1h in DMF, *in situ* Fmoc removal 1x7min. Coupling and Fmoc removal at 45 ºC. Washing after Fmoc removal with 2x1 mL of 1% OxymaPure in DMF.



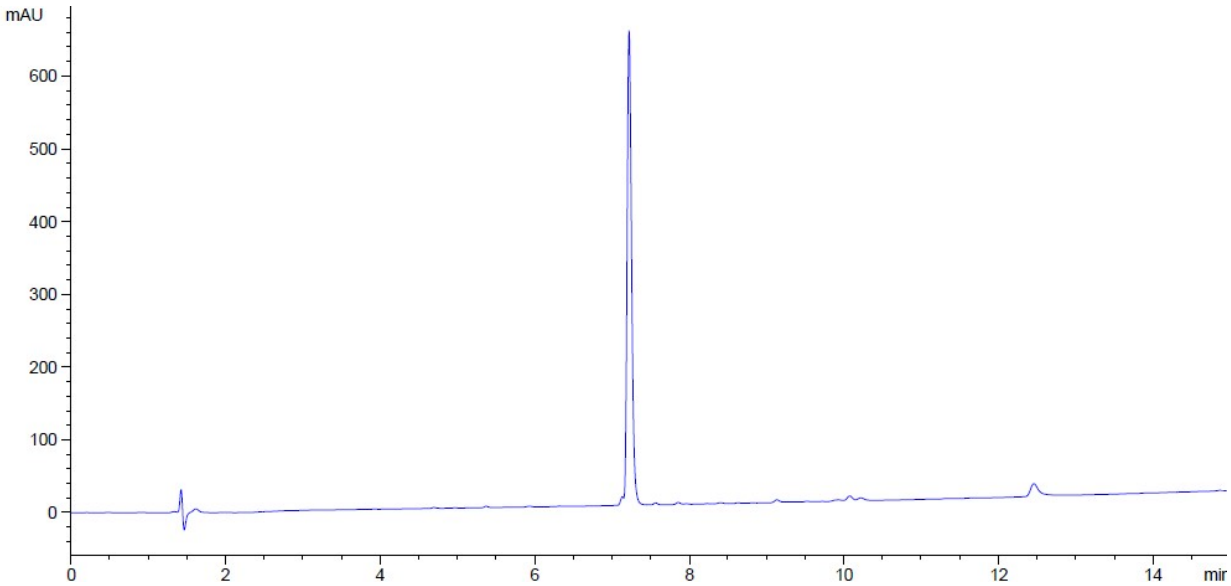
HPLC -S26. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Coupling as Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], 1 min pre-activation, 1h in DMF, *in situ* Fmoc removal 1x7min. Coupling and Fmoc removal at rt. Washing after Fmoc removal with 1x1 mL of 1% OxymaPure in DMF.



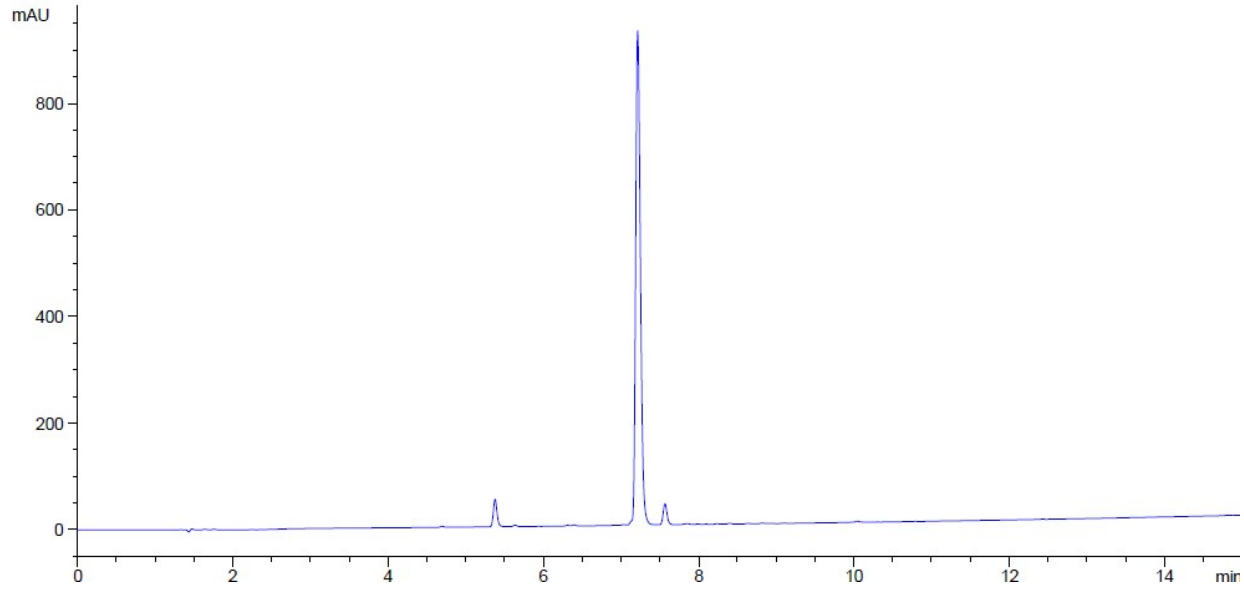
HPLC -S27. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Coupling as Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], 1 min pre-activation, 1h in DMF, *in situ* Fmoc removal 1x7min. Coupling and Fmoc removal at rt. Washing after Fmoc removal with 1x1mL DMF + 1x1 mL of 1% OxymaPure in DMF.



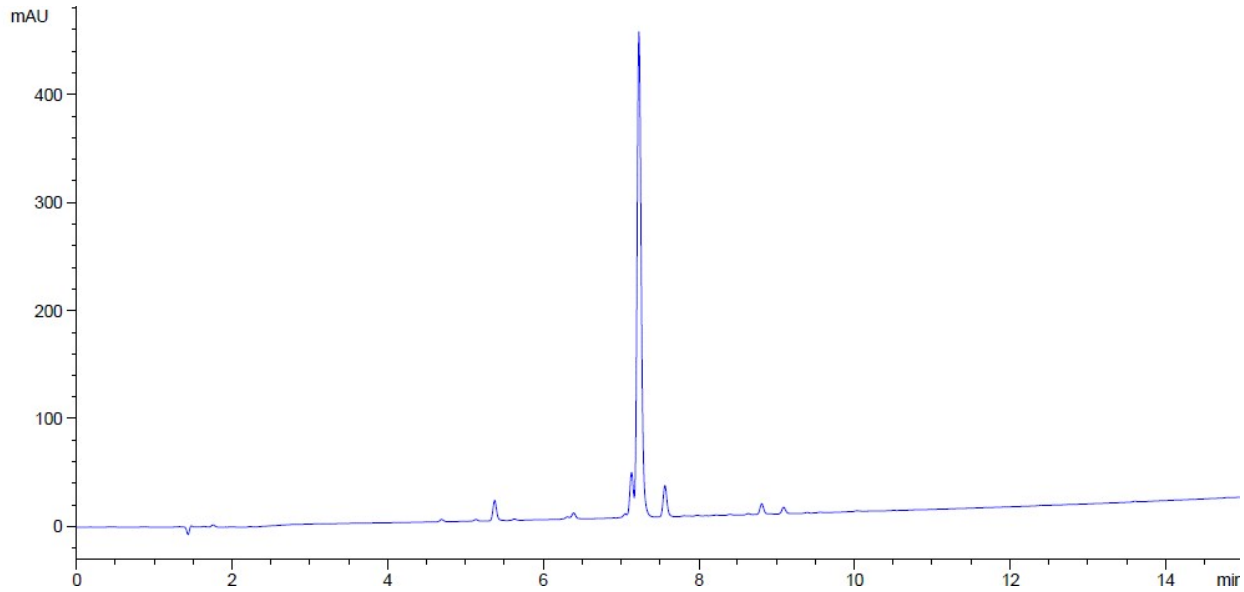
HPLC -S28. Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL). Coupling as Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], 1 min pre-activation, 1h in DMF, *in situ* Fmoc removal with 4-methyl piperidine (4-MP) 1x7min. Coupling and Fmoc removal at rt. Washing after Fmoc removal with 1x1mL DMF + 1x1 mL of 1% OxymaPure in DMF.

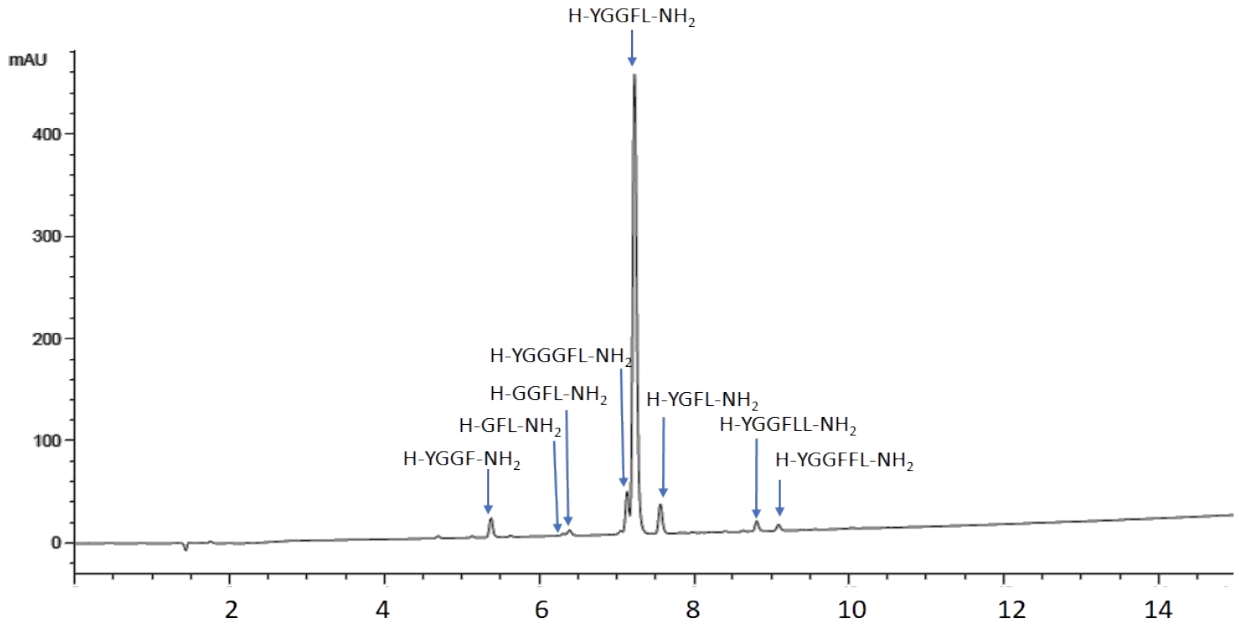


HPLC -S29. SPPS of Leu-Enkephalin pentapeptide (H-YGGFL-NH_2) as standard protocol (Coupling-Washing-Fmoc Removal-Washing) using Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], 1 min pre-activation, 1h in DMF.

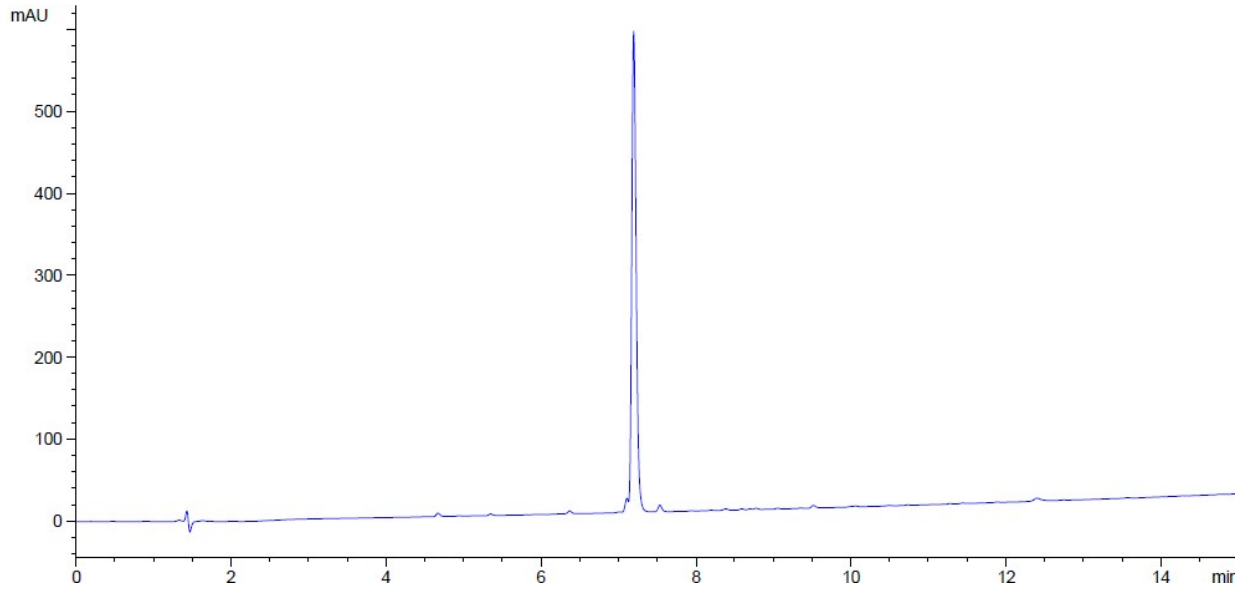


HPLC -S30. SPPS of Leu-Enkephalin pentapeptide (H-YGGFL-NH_2) as using Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], 1 min pre-activation, 1h in DMF. *In situ* Fmoc removal (with 4-MP). Washing after Fmoc removal with 1x1mL DMF + 1x1 mL of 1% OxymaPure in DMF.

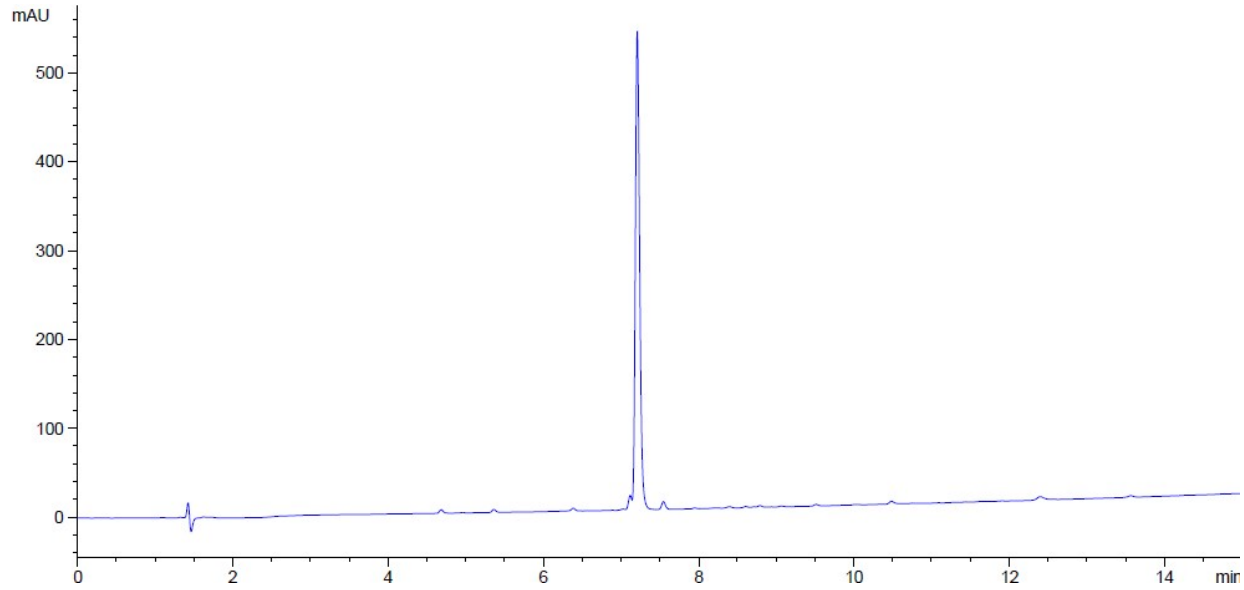




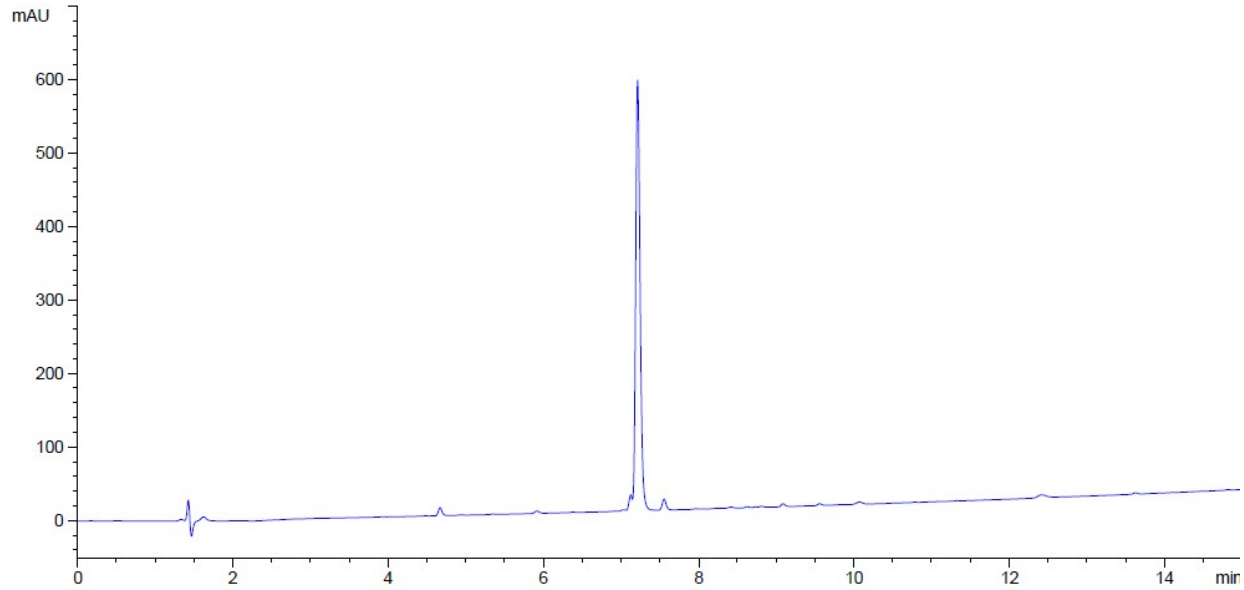
HPLC -S31. SPPS of Leu-Enkephalin pentapeptide (H-YGGFL-NH_2) as using Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], in situ-activation, 1h in DMF. *In situ* Fmoc removal (with 4-MP). Washing after Fmoc removal with 1x1mL DMF +1x1 mL of 1% OxymaPure in DMF.



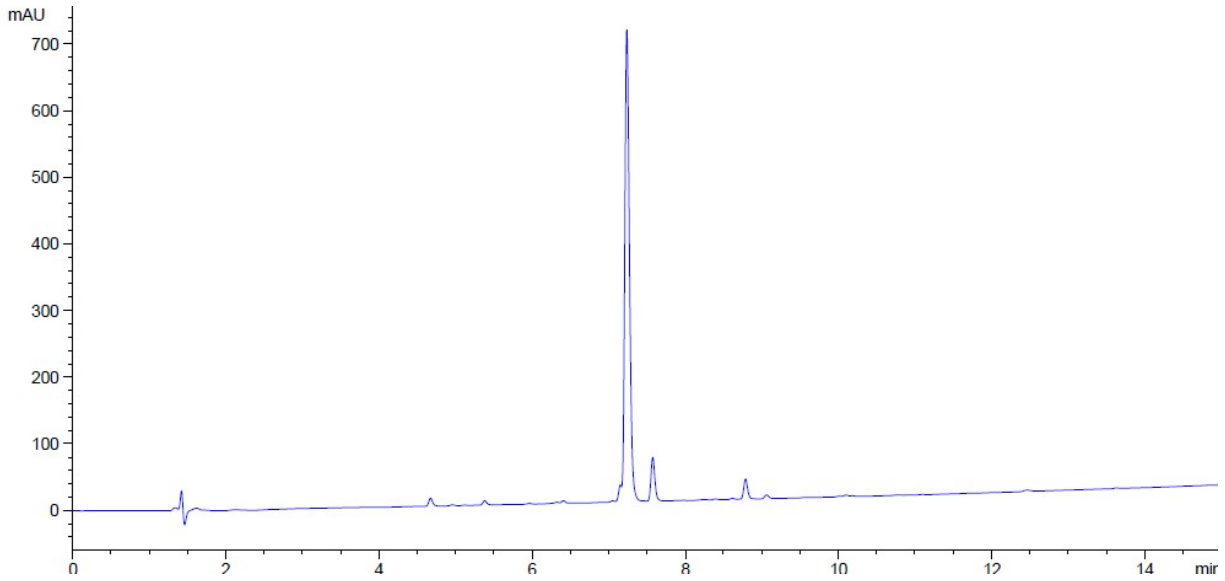
HPLC -S32. SPPS of Leu-Enkephalin pentapeptide (H-YGGFL-NH_2) as using Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], in situ-activation, half DIC at 1 min + half at 15 min, 1h in DMF. *In situ* Fmoc removal (with 4-MP). Washing after Fmoc removal with 1x1mL DMF + 1x1 mL of 1% OxymaPure in DMF.



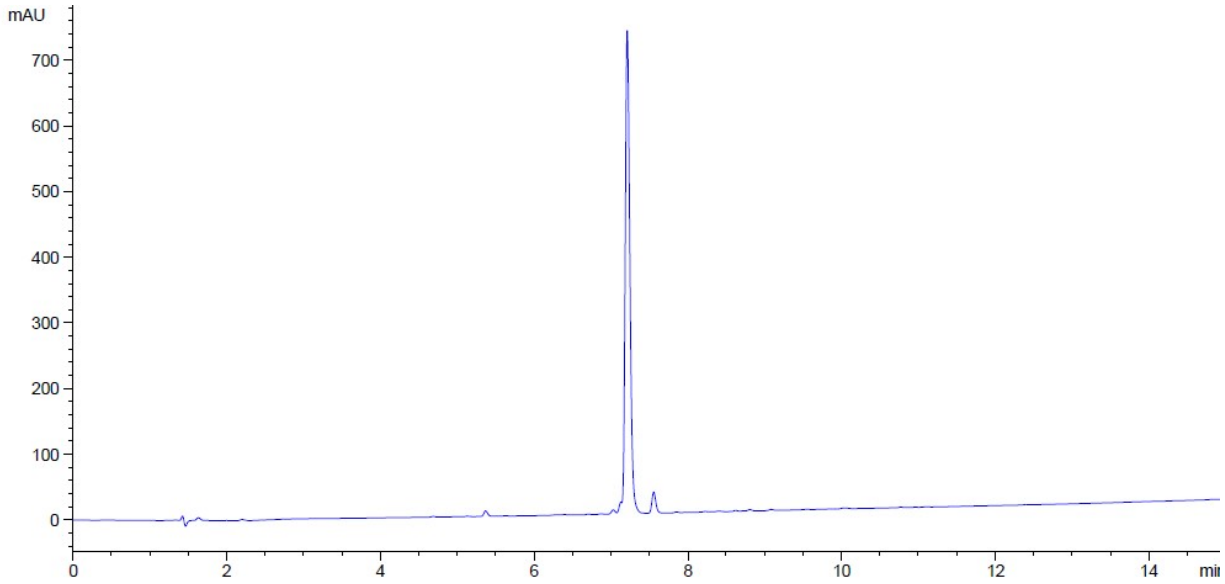
HPLC -S33. SPPS of Leu-Enkephalin pentapeptide (H-YGGFL-NH_2) as using Fmoc-AA-OH: DIC: OxymaPure [1:1:2, 1.5 eq.], in situ-activation, 1h in DMF. *In situ* Fmoc removal (with 4-MP). Washing after Fmoc removal with 1x1mL DMF + 1x1 mL of 1% OxymaPure in DMF.



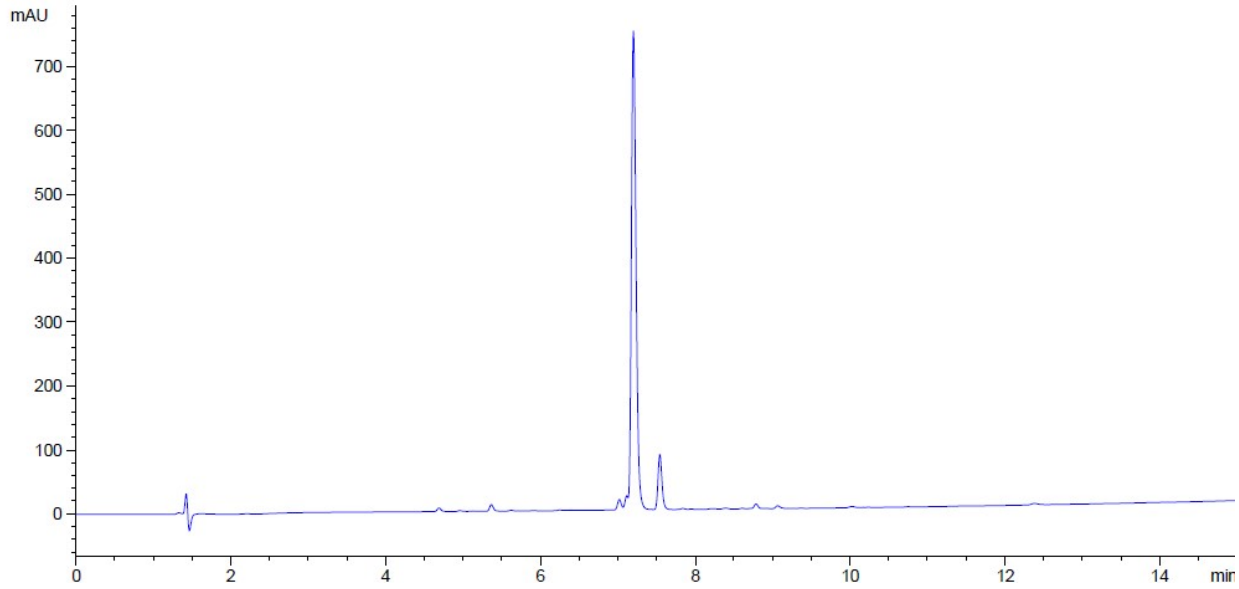
HPLC -S34. SPPS of Leu-Enkephalin pentapeptide (H-YGGFL-NH_2) as using Fmoc-AA-OH: DIC: OxymaPure [1:1:2, 1.5 eq.], in situ-activation, 1h in DMF. *In situ* Fmoc removal (with 4-MP) except of both Gly unit. After coupling of Gly filtered coupling cocktail then added 0.5 mL of 20% 4-MP in DMF. Washing after Fmoc removal with 1x1mL DMF + 1x1 mL of 1% OxymaPure in DMF.



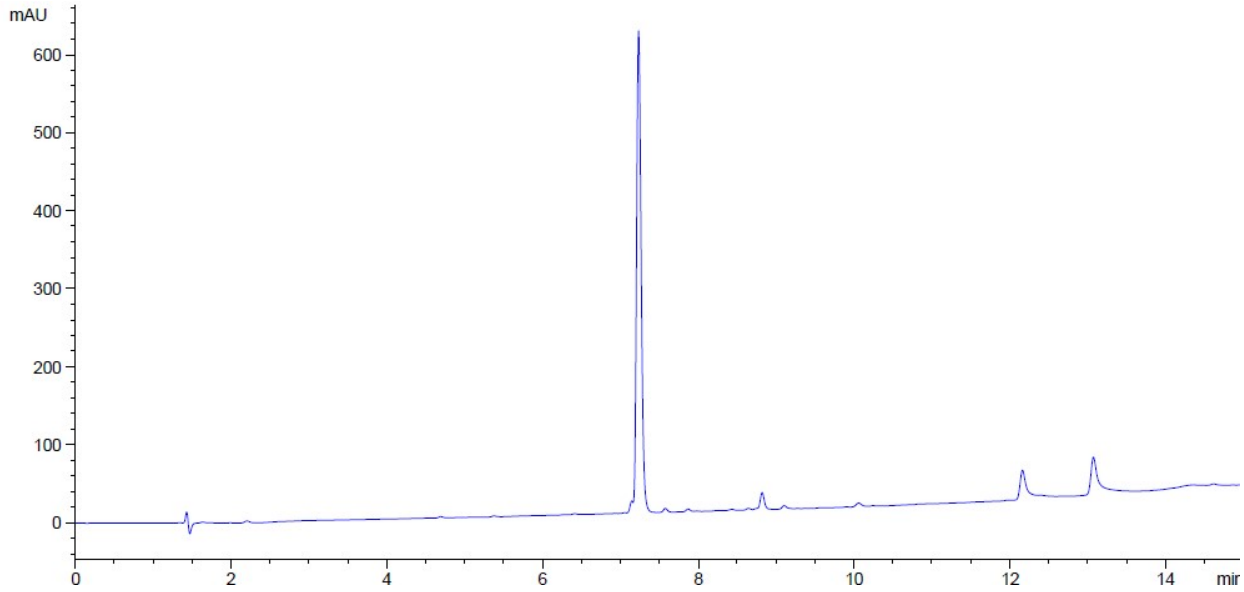
HPLC -S35. SPPS of Leu-Enkephalin pentapeptide (H-YGGFL-NH_2) as using Fmoc-AA-OH: DIC: OxymaPure [1:1:2, 1.5 eq.], in situ-activation, 1h in DMF. After coupling filtered coupling cocktail then added 0.5 mL of 20% 4-MP in DMF for 10 min. Washing after Fmoc removal with 1x1mL DMF + 1x1 mL of 1% OxymaPure in DMF.



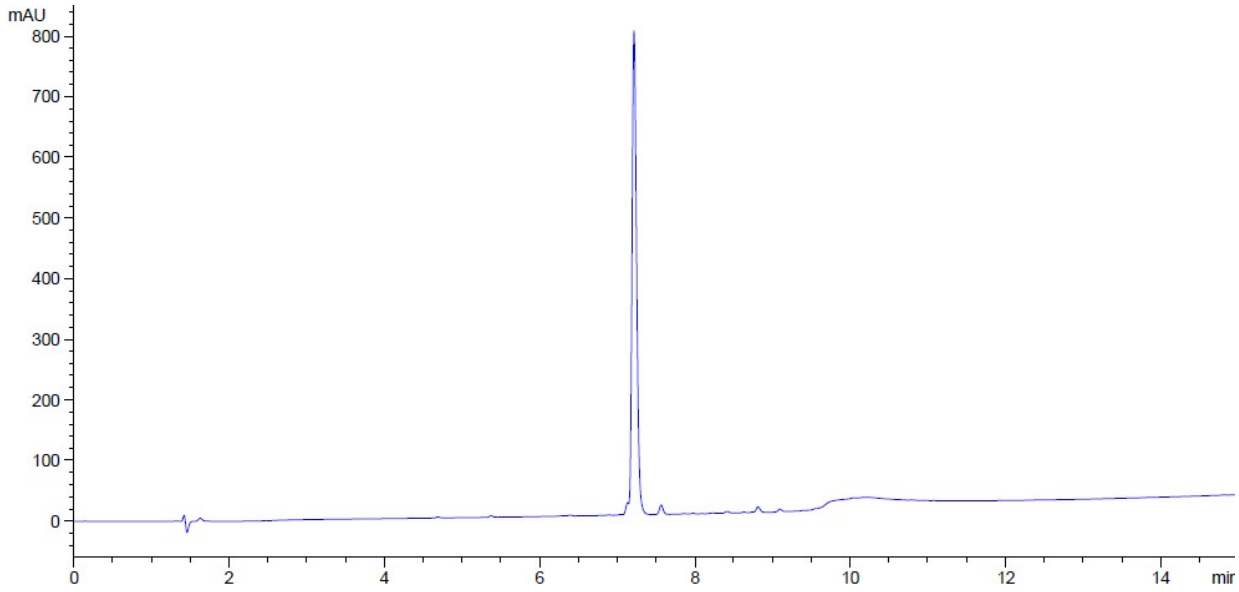
HPLC -S36. SPPS of Leu-Enkephalin pentapeptide (H-YGGFL-NH_2) as using Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], in situ-activation, 1h in DMF. *In situ* Fmoc removal (with 4-MP) for 10 min. Washing after Fmoc removal with 1x1mL DMF + 1x1 mL of 1% OxymaPure in DMF.



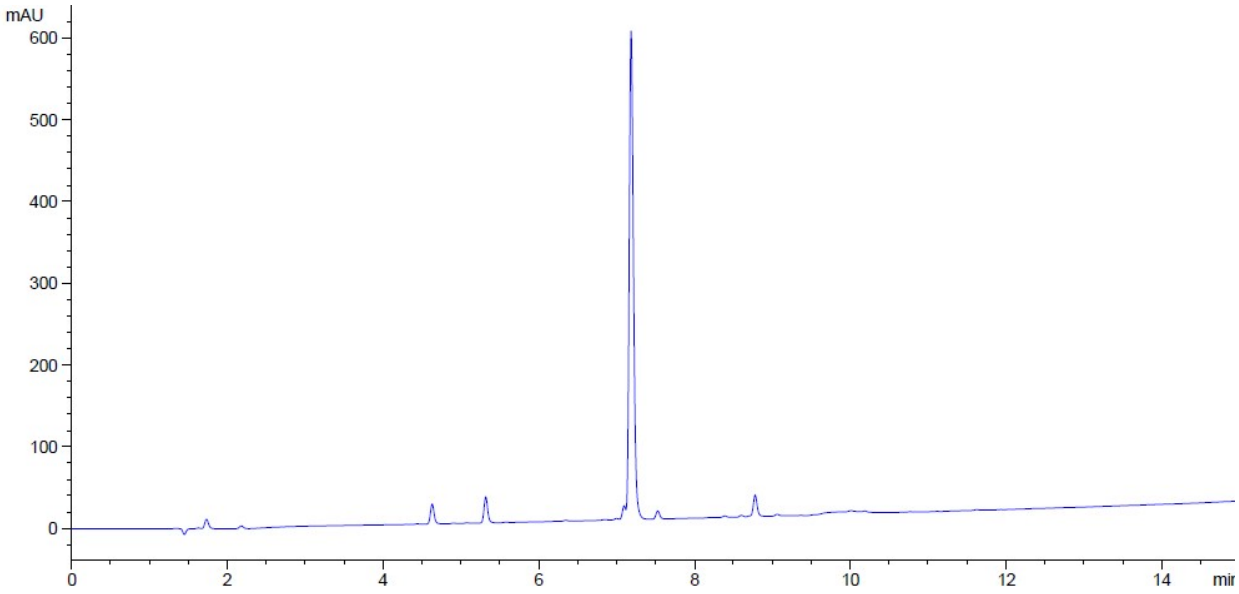
HPLC -S37. SPPS of Leu-Enkephalin pentapeptide (H-YGGFL-NH_2) as using Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], in situ-activation, 2h in DMF. *In situ* Fmoc removal (with 4-MP) for 20 min. Washing after Fmoc removal with 1x1mL DMF + 1x1 mL of 1% OxymaPure in DMF.



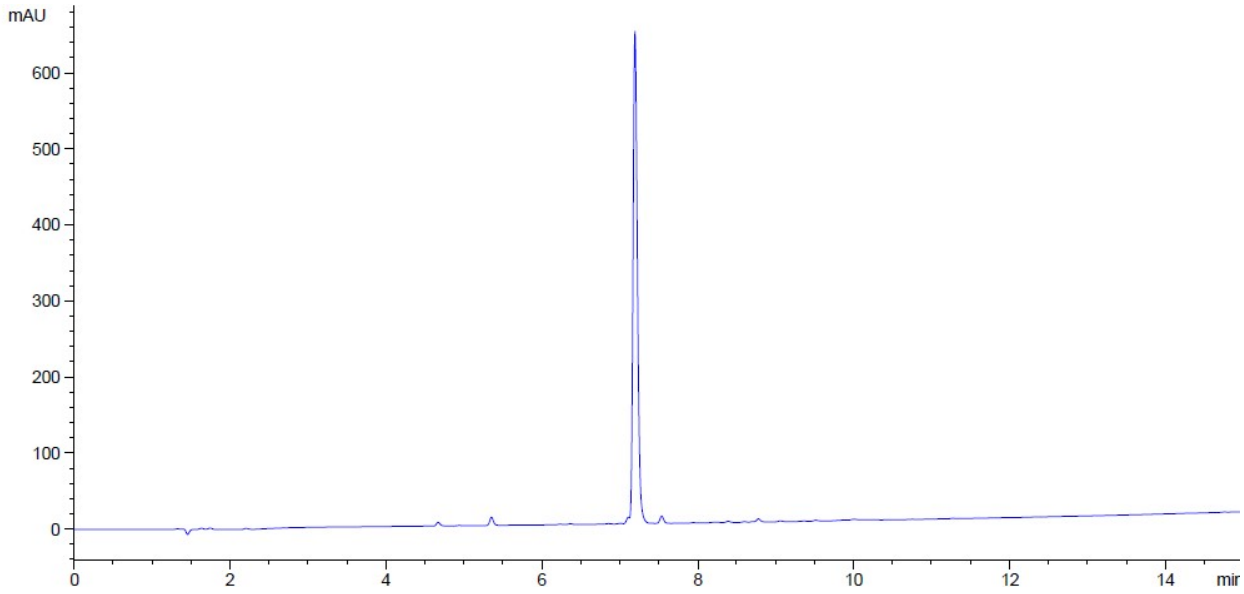
HPLC -S38. SPPS of Leu-Enkephalin pentapeptide (H-YGGFL-NH_2) as using Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], in situ-activation, 1h in DMF. *In situ* Fmoc removal (with 4-MP) for 10 min. Washing after Fmoc removal with 2x1 mL of 1% OxymaPure in DMF.



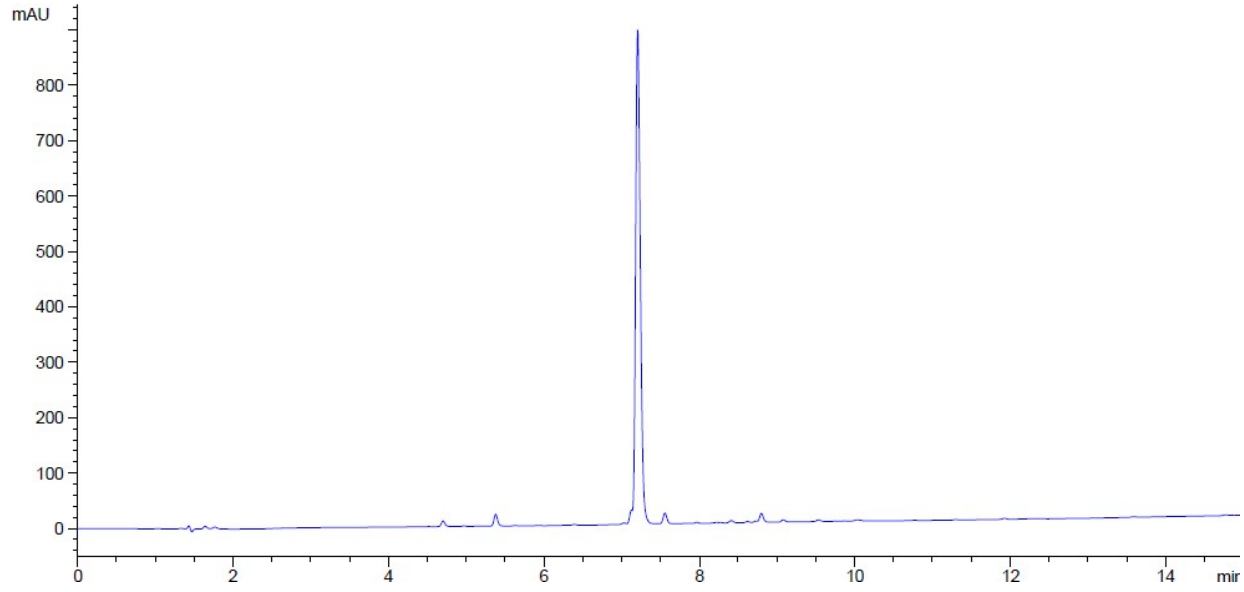
HPLC -S39. SPPS of Leu-Enkephalin pentapeptide (H-YGGFL-NH_2) as using Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], in situ-activation, 1h in DMF. After 30 min of coupling more 0.3 eq DIC was added during coupling. *In situ* Fmoc removal (with 4-MP) for 10 min. Washing after Fmoc removal with 2x1 mL of 1% OxymaPure in DMF.



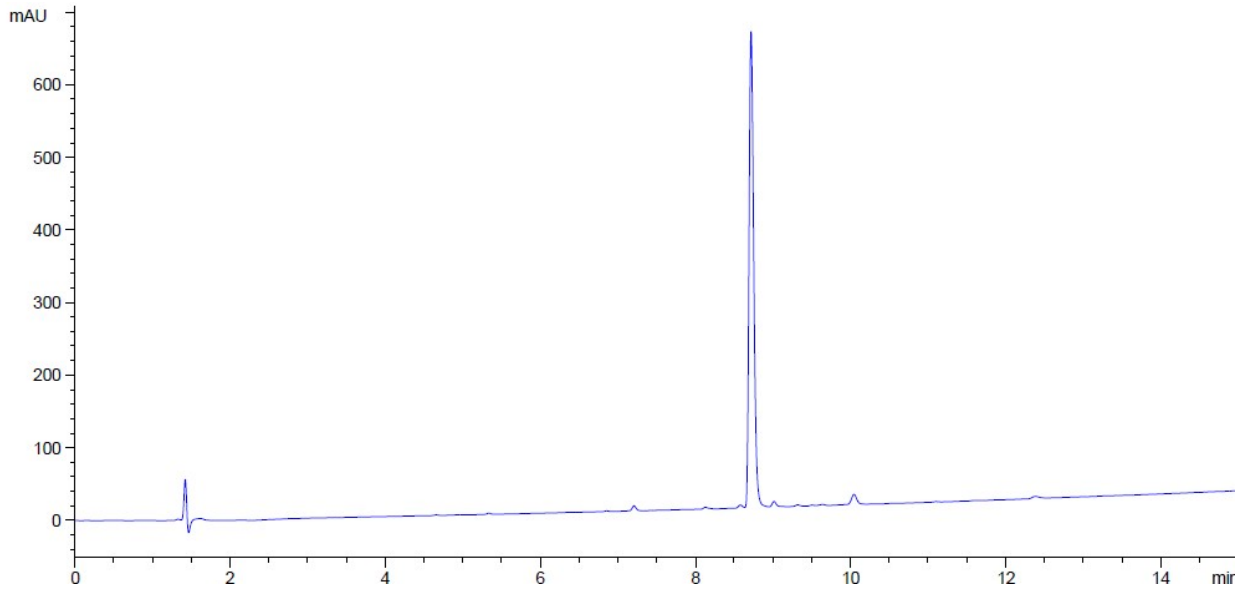
HPLC -S40. SPPS of Leu-Enkephalin pentapeptide (H-YGGFL-NH_2) as using Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], in situ-activation, 1.5h in DMF. After 30 min of coupling more 0.3 eq DIC was added during coupling. *In situ* Fmoc removal (with 4-MP) for 20 min. Washing after Fmoc removal with 2x1 mL of 1% OxymaPure in DMF.



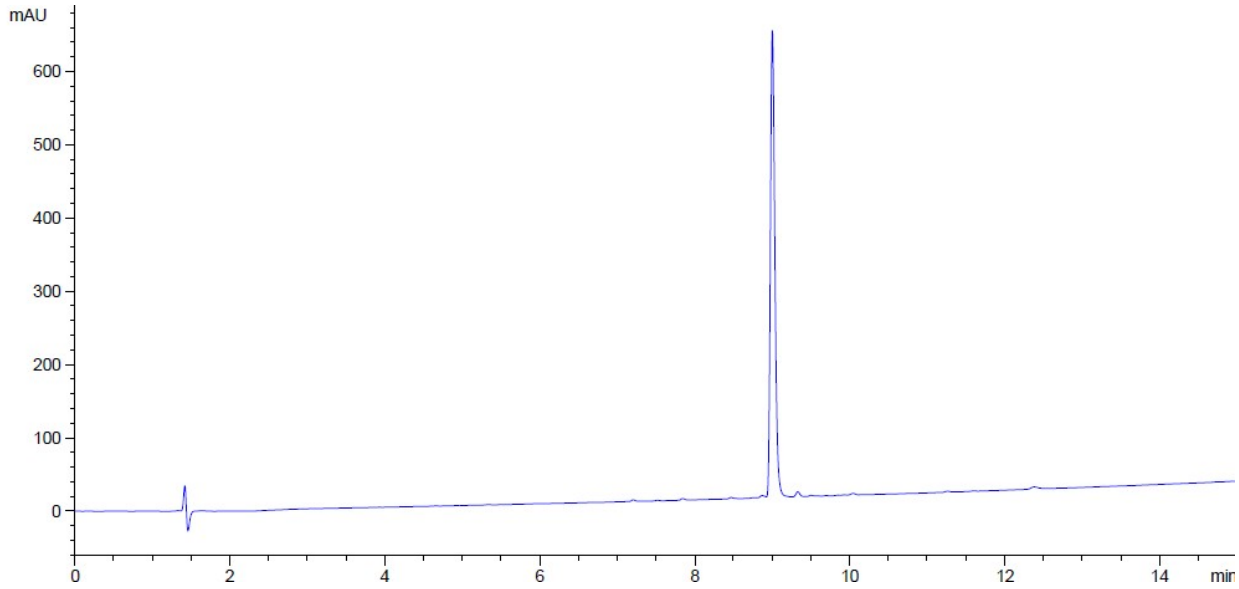
HPLC -S41. SPPS of Leu-Enkephalin pentapeptide (H-YGGFL-NH_2) as using Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], in situ-activation, 1.5h in DMF. After 30 min of coupling more 0.3 eq DIC was added during coupling. *In situ* Fmoc removal (with 4-MP) for 20 min. Washing after Fmoc removal with 2x1 mL of 1% OxymaPure in DMF.



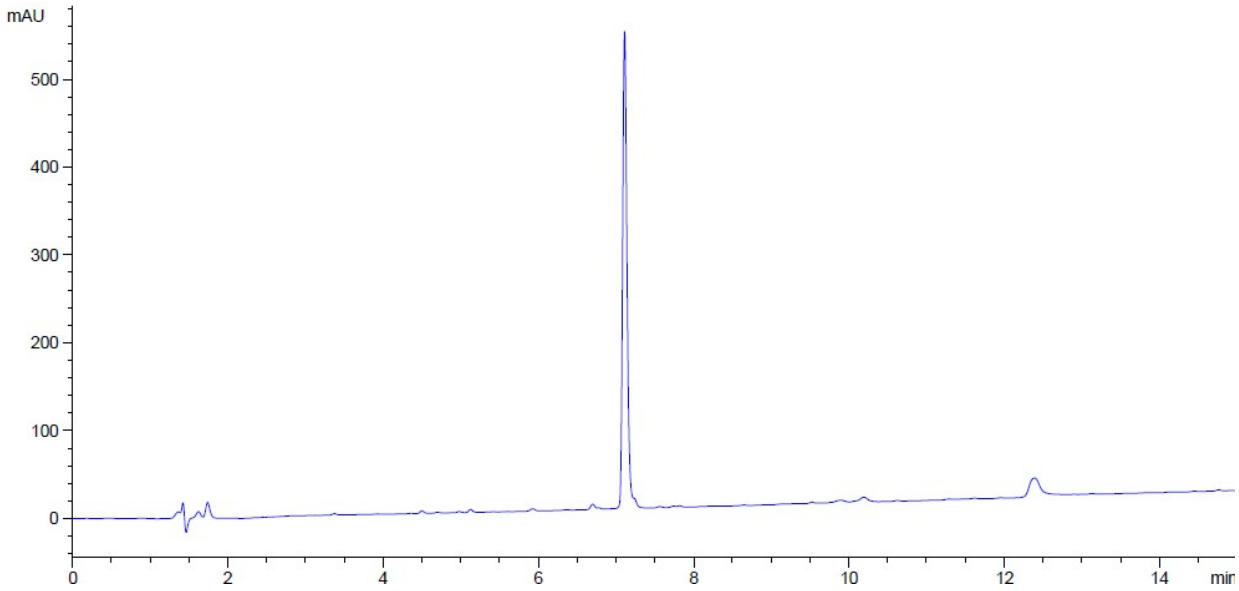
HPLC -S42. SPPS of H-Tyr-Gly-Gly-Phe-Leu-Leu-NH_2 (H-YGGFLL-NH_2).



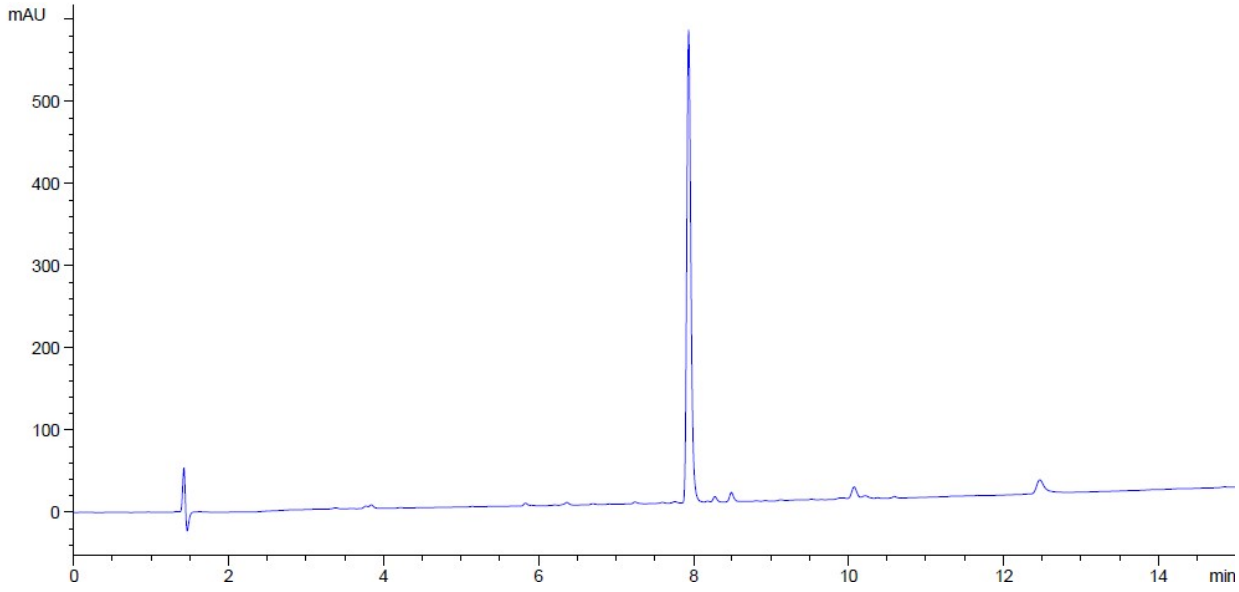
HPLC -S43. SPPS of H-Tyr-Gly-Gly-Phe-Phe-Leu-NH_2 (H-YGGFFL-NH_2).



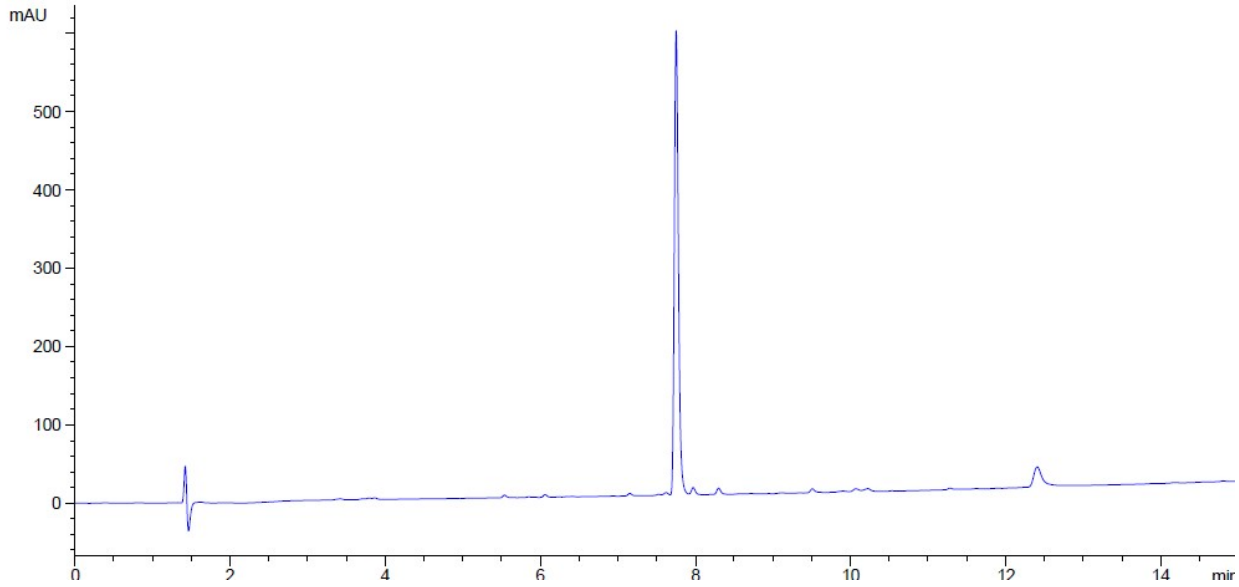
HPLC -S44. SPPS of H-Tyr-Gly-Gly-Gly-Phe-Leu-NH_2 (H-YGGGFL-NH_2).



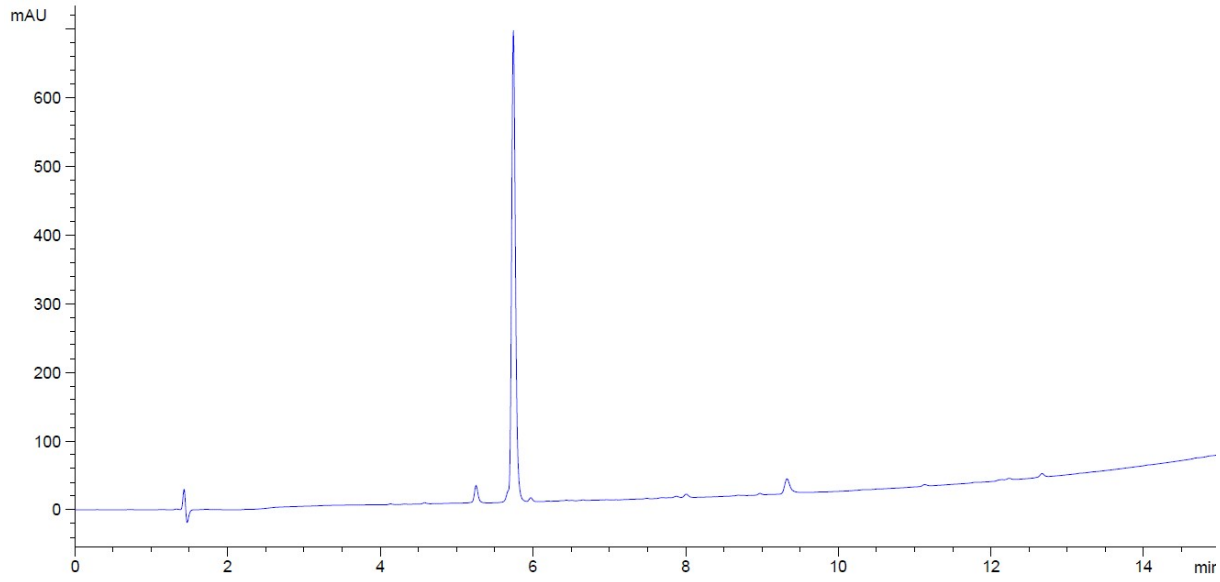
HPLC -S45. SPPS of H-Tyr-Tyr-Gly-Gly-Phe-Leu-NH_2 (H-YYGGFL-NH_2).



HPLC -S46. SPPS of H-Tyr-Tyr-Gly-Gly-Gly-Phe-Leu-NH_2 (H-YYGGGFL-NH_2).



HPLC -S47 (Comparable to experiments carried out in DMF in Table 6). **(**5-95% B into A in 15 min). Coupling and Fmoc removal of Gly and Tyr unit on tripeptidyl resin (GFL) at 45 °C. Coupling as Fmoc-AA-OH: DIC: OxymaPure [1:1:1, 1.5 eq.], 1 min pre-activation, 1h in NBP, *in situ* Fmoc removal with 4-methyl piperidine (4-MP) 1x7min. Coupling and Fmoc removal at 45 °C. Washing after Fmoc removal with 1x1mL NBP + 1x1 mL of 1% OxymaPure in NBP.



LCMS-S48: Leu-Enkephalin pentapeptide (H-Tyr-Gly-Gly-Phe-Leu-NH_2)