Supporting Information

Successful Development of a Method for the Incorporation of Fmoc-Arg(Pbf)-OH in Solid-Phase Peptide Synthesis using N Butylpyrrolidinone (NBP) as Solvent

Beatriz G. de la Torre, [*a] Ashish Kumar,[a,b] Mahama Alhassan,[b] Christoph Bucher,[c] Fernando Albericio, [*b,d,e] John Lopez [*c,]

[a]KwaZulu-Natal Research Innovation and Sequencing Platform (KRISP), School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban 4041, South Africa

[b]Peptide Science Laboratory, School of Chemistry and Physics, University of KwaZulu-Natal, Durban 4001, South Africa

[c]Novartis Pharma AG, Novartis Campu Campus, WSJ-145-5.51, CH-4056 Basel, Switzerland; [d]CIBER-BBN, Networking Centre on Bioengineering, Biomaterials and Nanomedicine, Barcelona Science Park, University of Barcelona, 08028 Barcelona, Spain

[e]Department of Organic Chemistry, University of Barcelona, 08028 Barcelona, Spain

Table of content

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 0.74 mmol/g) and 2-CTC resin were purchased from Iris Biotech. NBP (TamiSolveNxG) was purchased from Eastman, OxymaPure was a gift from Luxembourg Biotech., and *N*,*N*-Diisopropylethylamine (DIEA) and 4-methylpiperidine were supplied by Sigma-Aldrich. Organic solvents Dimethylformamide (DMF), 2-Methyltetrahydrofuran (2-MeTHF), cyclopenthylmethyl eter (CPME) 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. Solvent A: 0.1% TFA in H_2O; Solvent B: 0.1% TFA in CH_3CN.

HPLC Method:

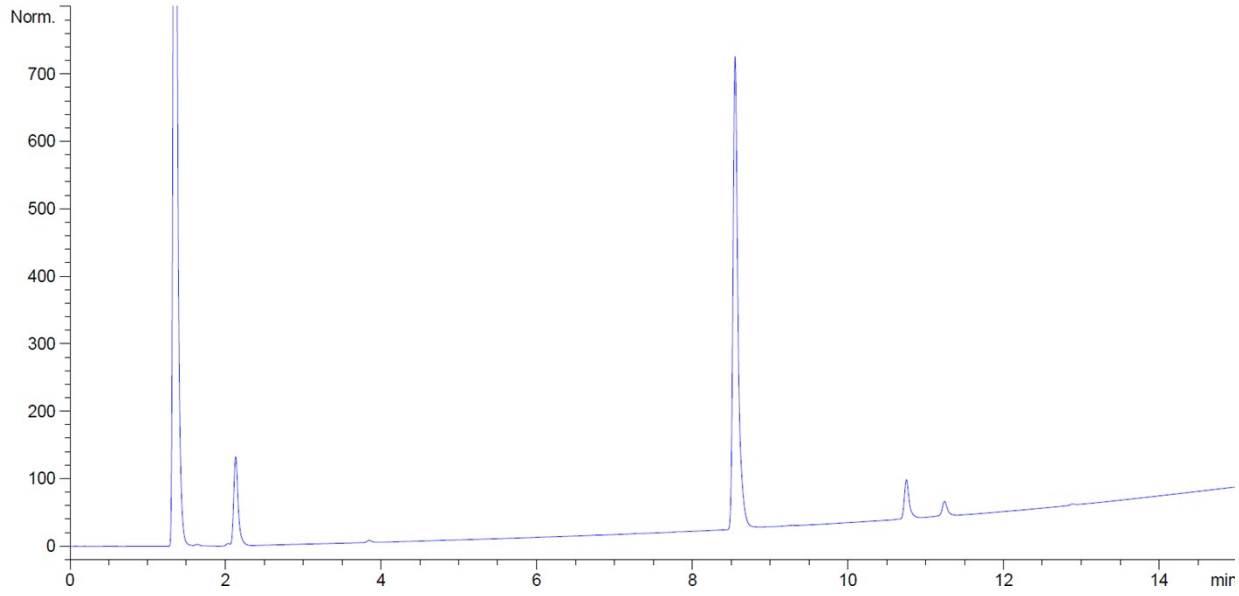
Supernatant analysis of δ-lactamization study: 30–95% B into A in 15 min.

Coupling quantification: 10–25% B into A

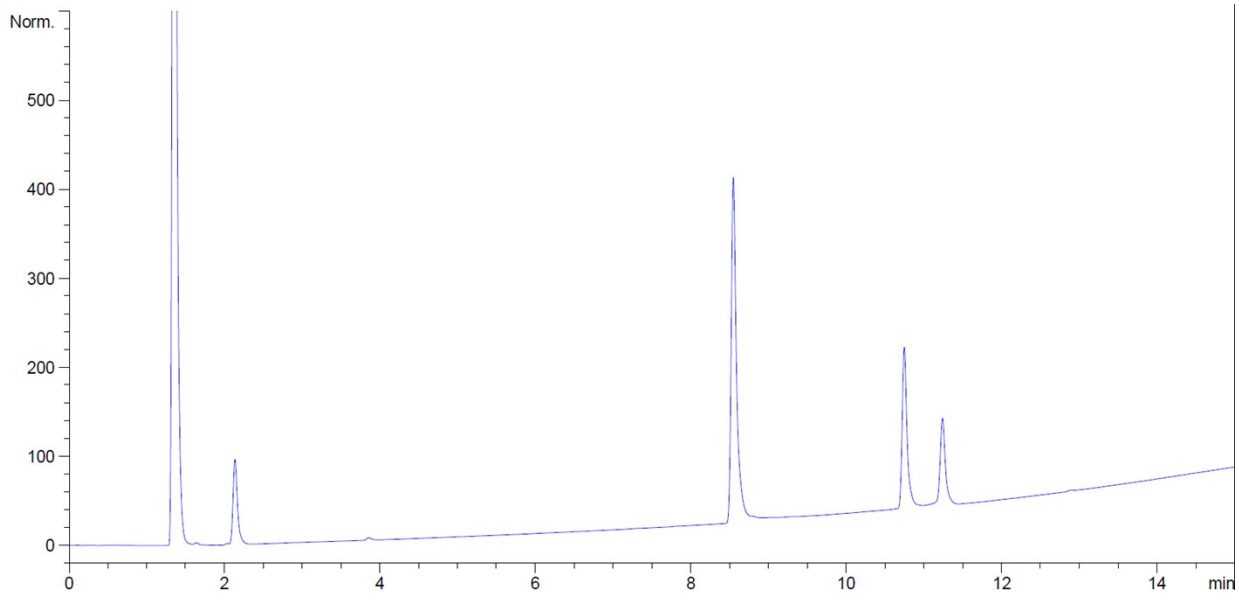
Linear precursor of RGD Peptide (H-DfKRG-OH): 5–20% B into A

HPLC-S1. Fmoc-Arg(Pbf)-OH : DIC : OxymaPure [1:1:1], at RT, in DMF, in absence of nucleophile.

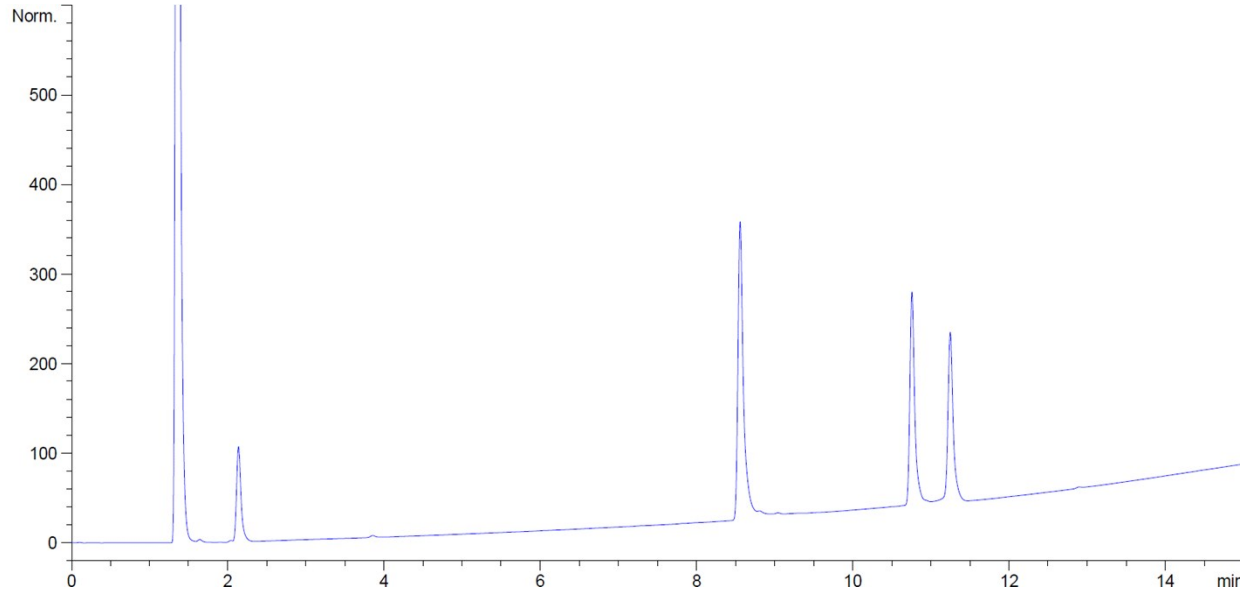
0 min



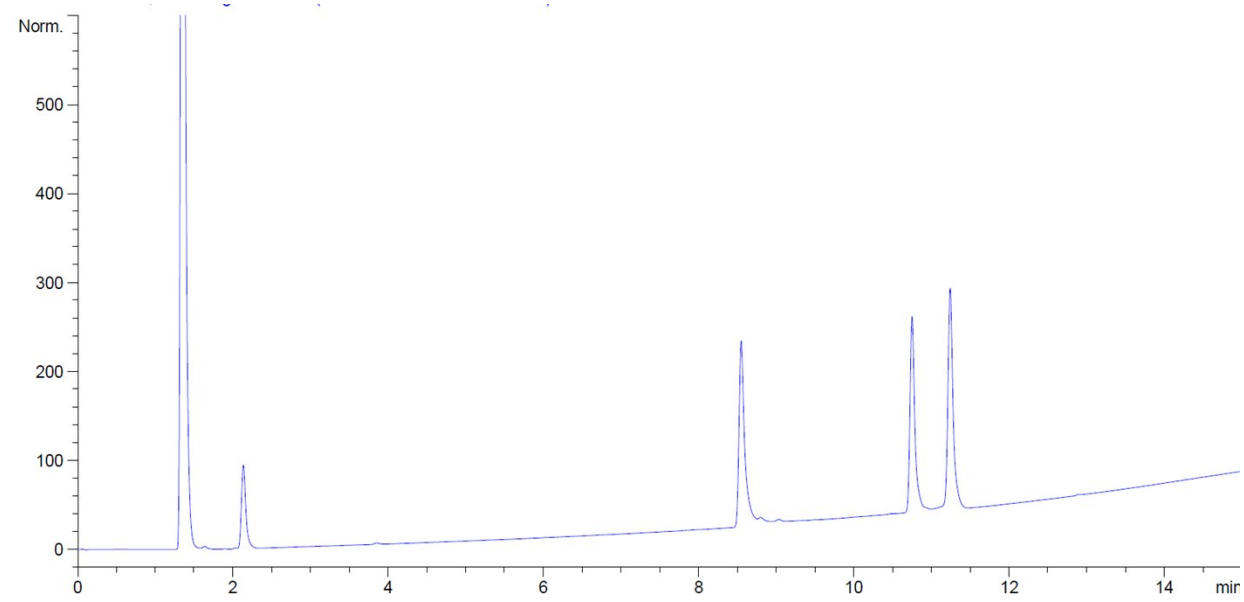
30 min



60 min

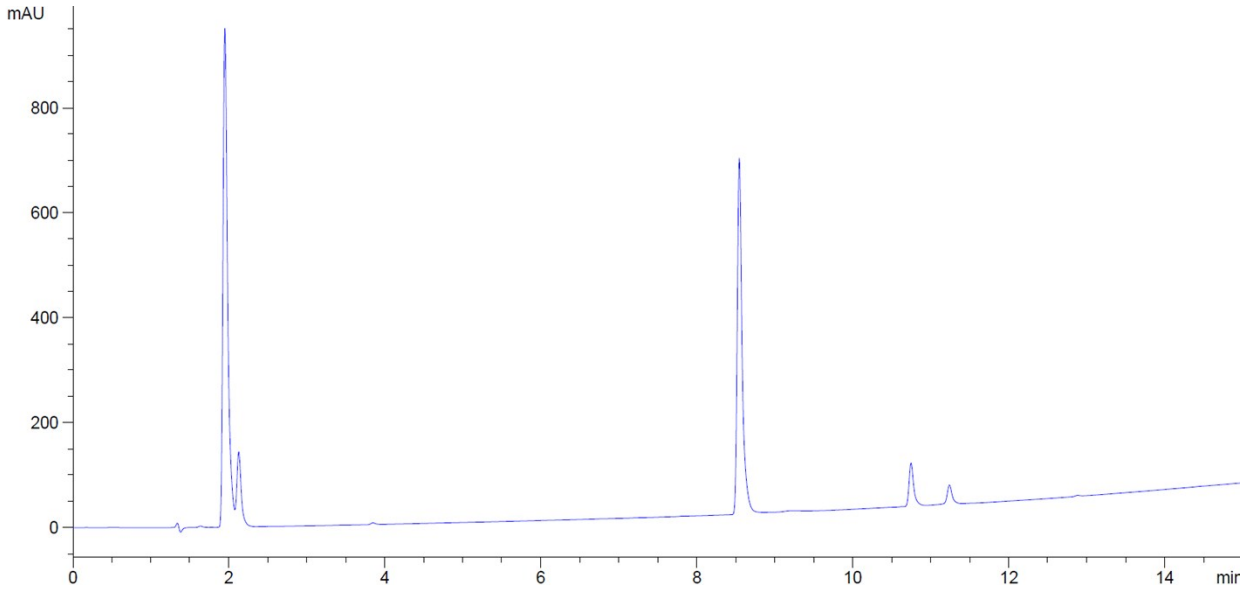


120 min

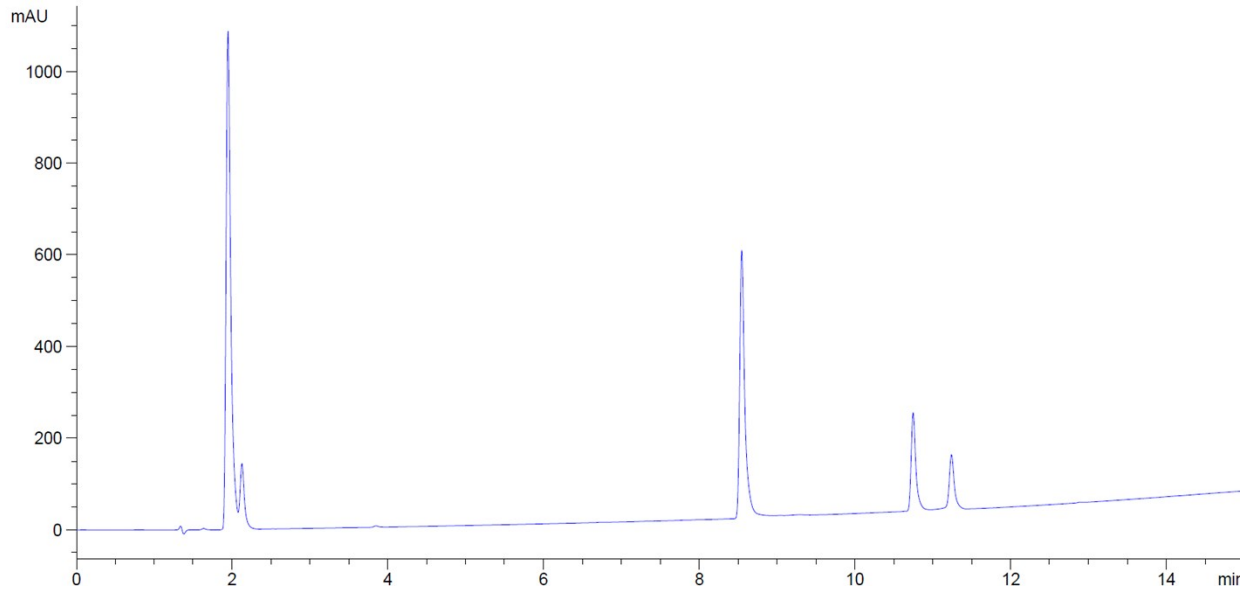


HPLC-S2. Fmoc-Arg(Pbf)-OH : DIC : OxymaPure [1:1:1], at RT, in NBP, in absence of nucleophile.

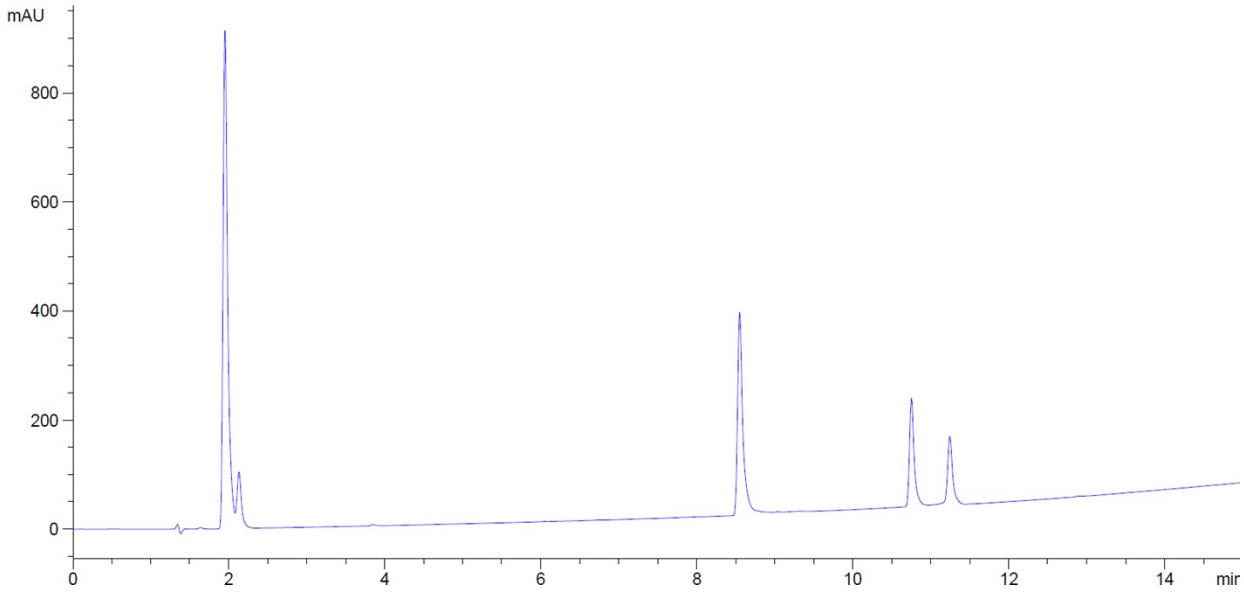
0 min



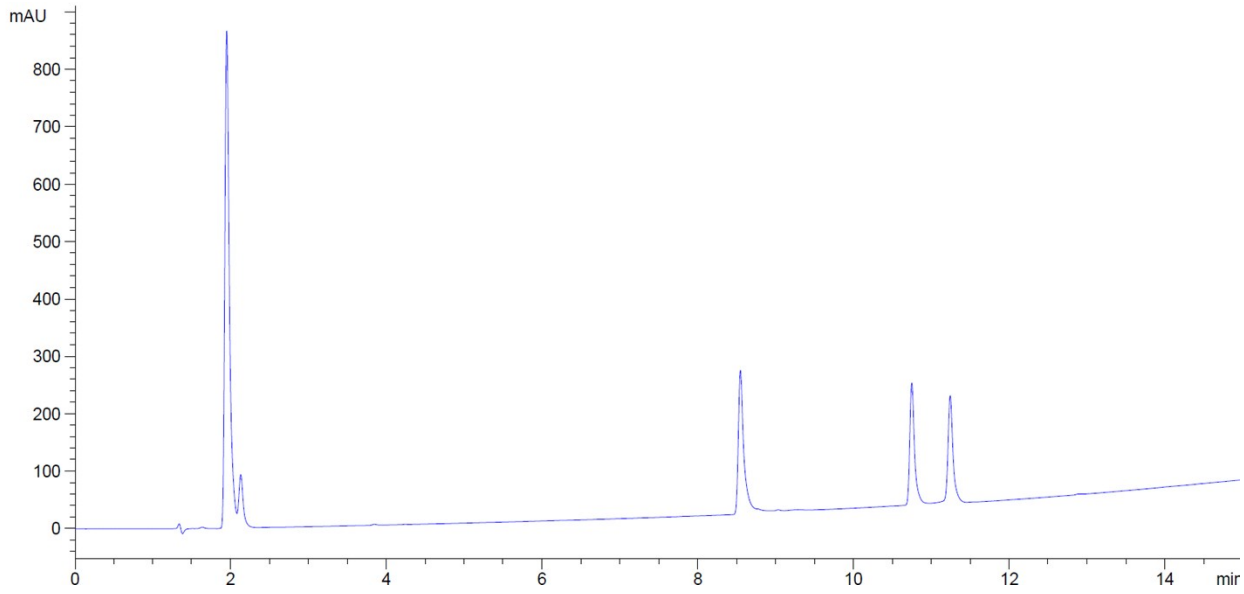
30 min



60 min

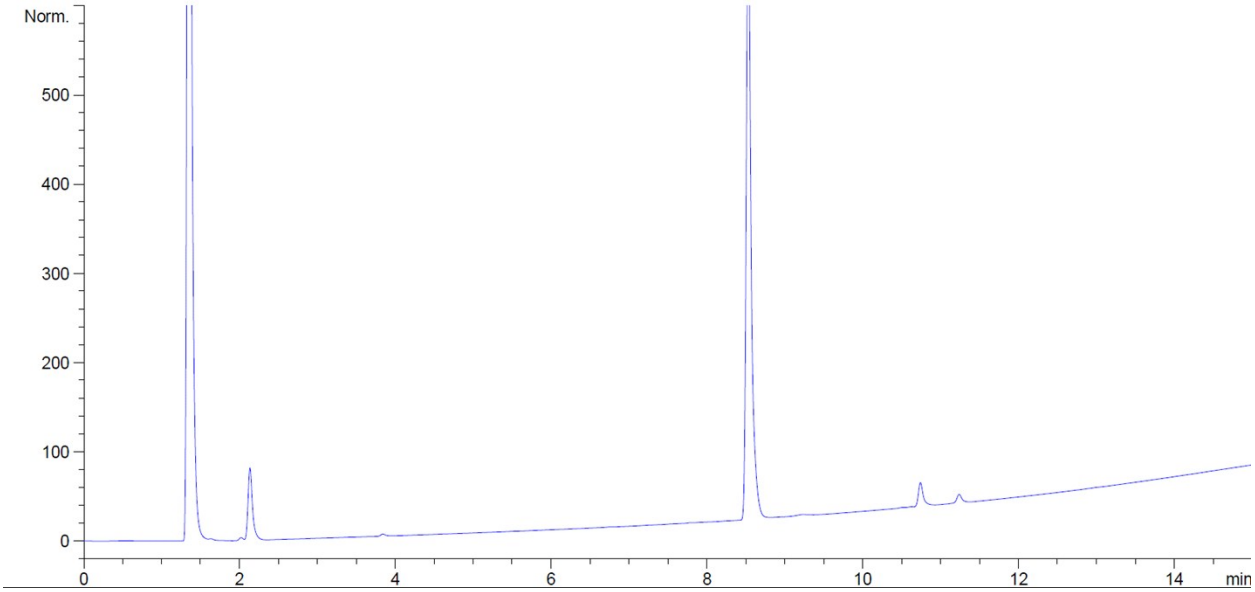


120 min

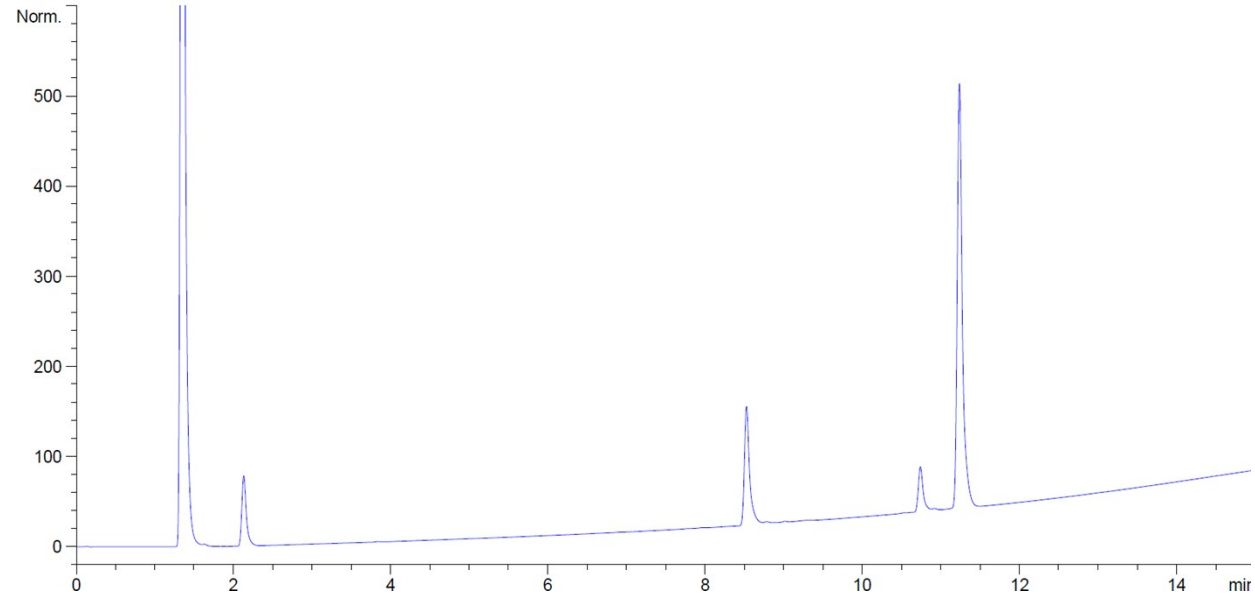


HPLC-S3. Fmoc-Arg(Pbf)-OH : DIC : OxymaPure [1:1:1], at 60 ºC, in DMF, in absence of nucleophile.

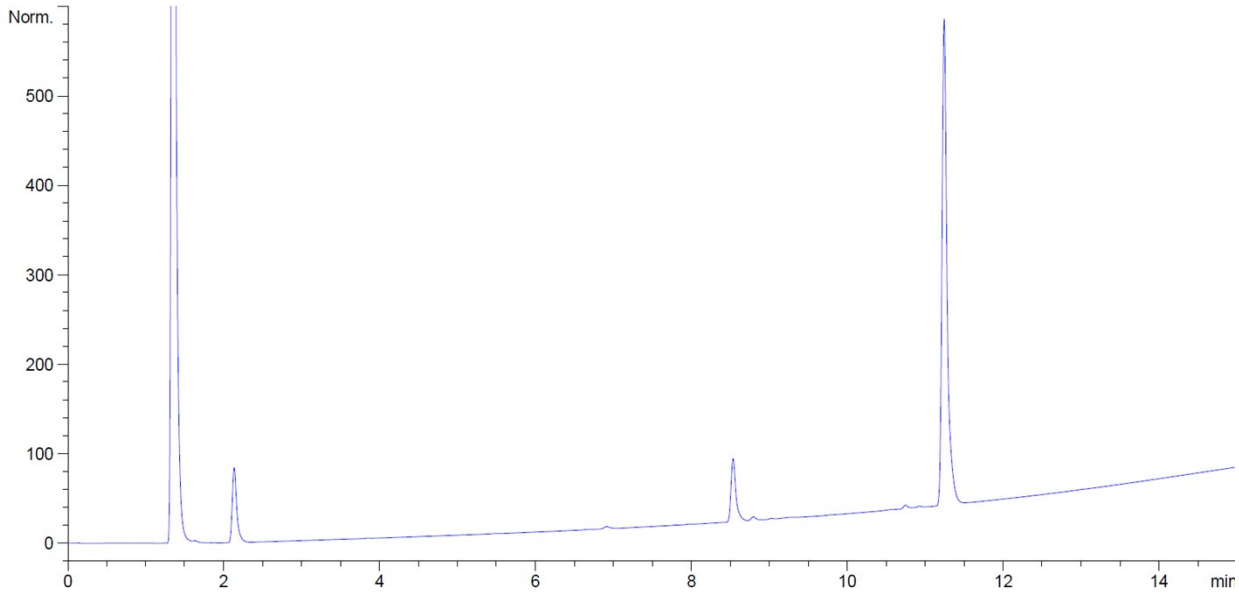
0 min



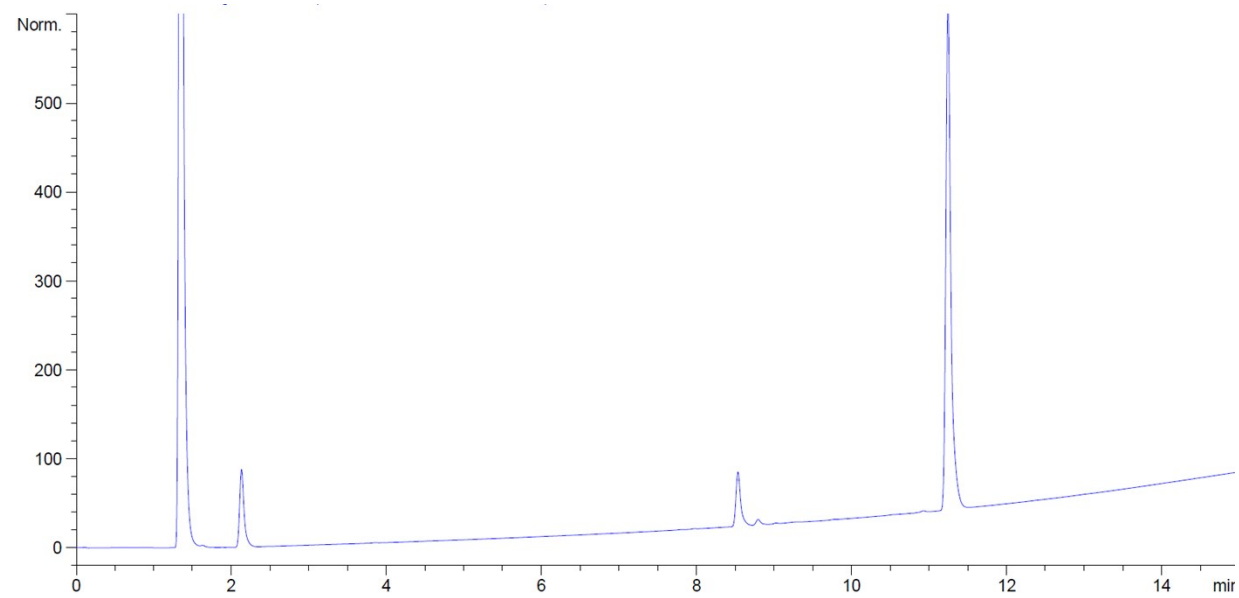
30 min



60 min

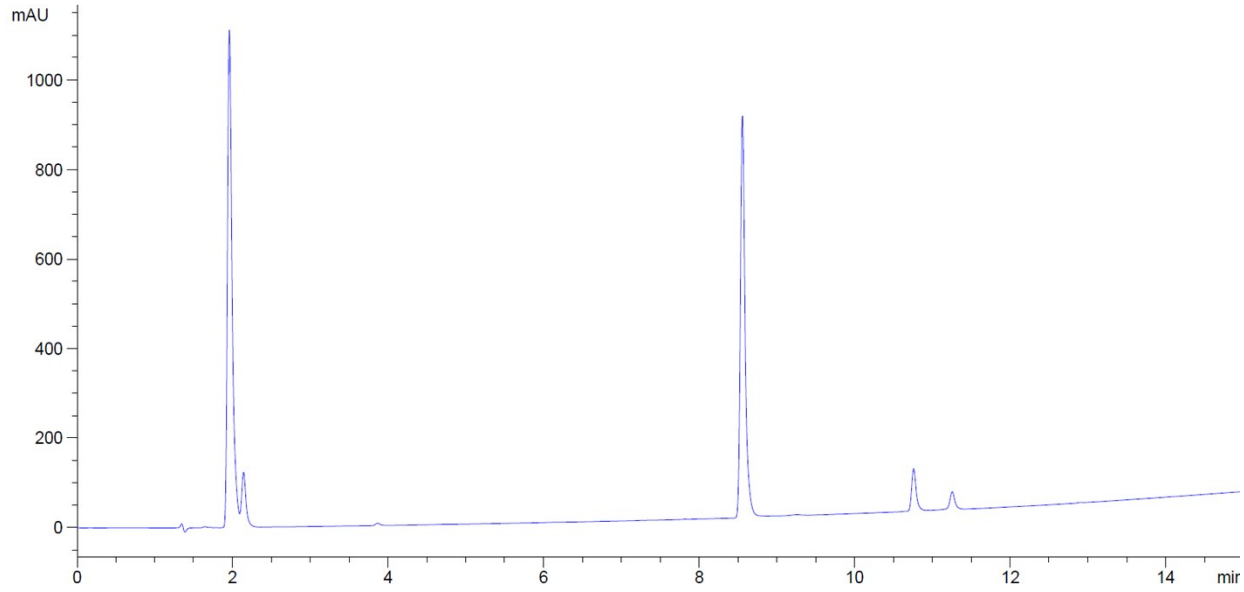


120 min

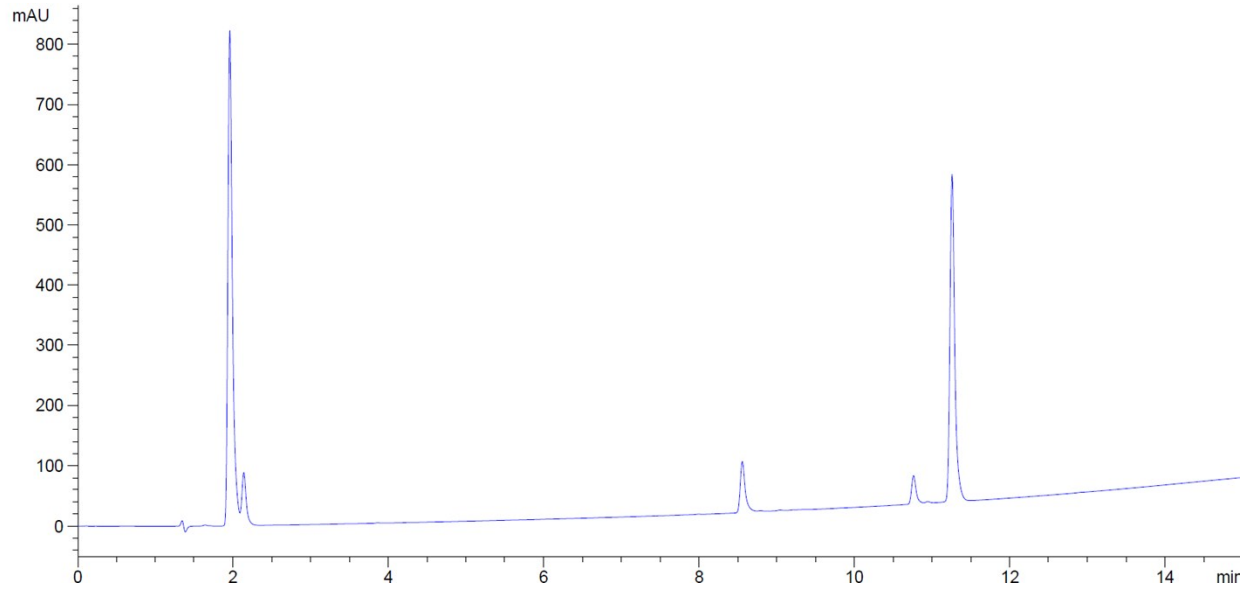


HPLC-S4. Fmoc-Arg(Pbf)-OH : DIC : OxymaPure [1:1:1], at 60 ºC, in NBP, in absence of nucleophile.

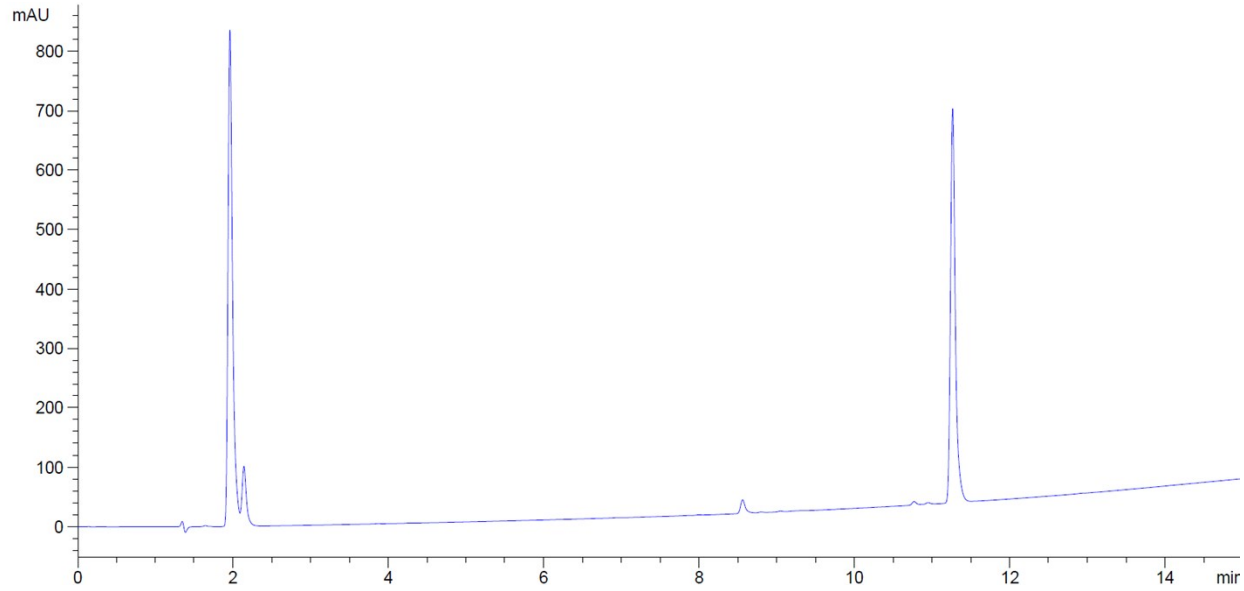
0 min



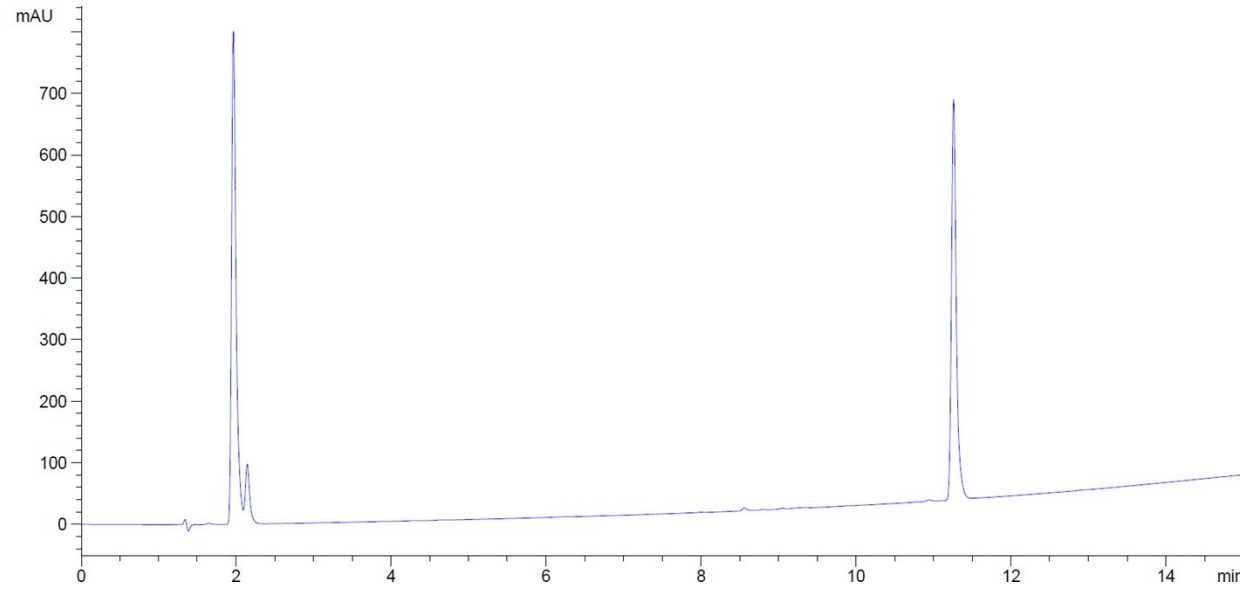
30 min



60 min

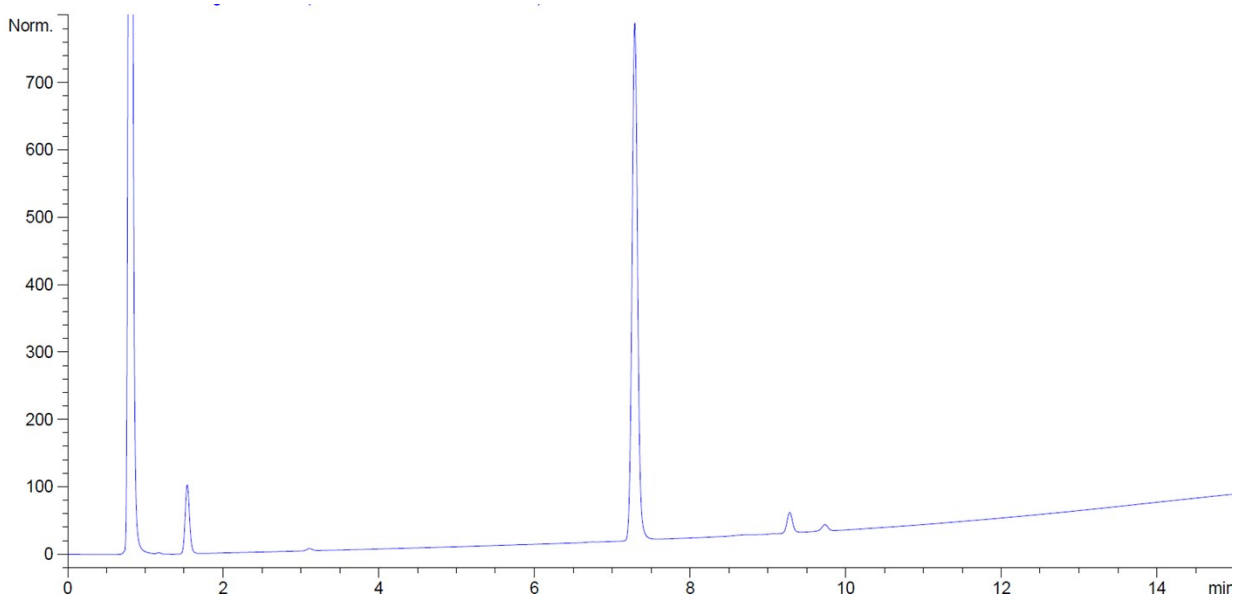


120 min

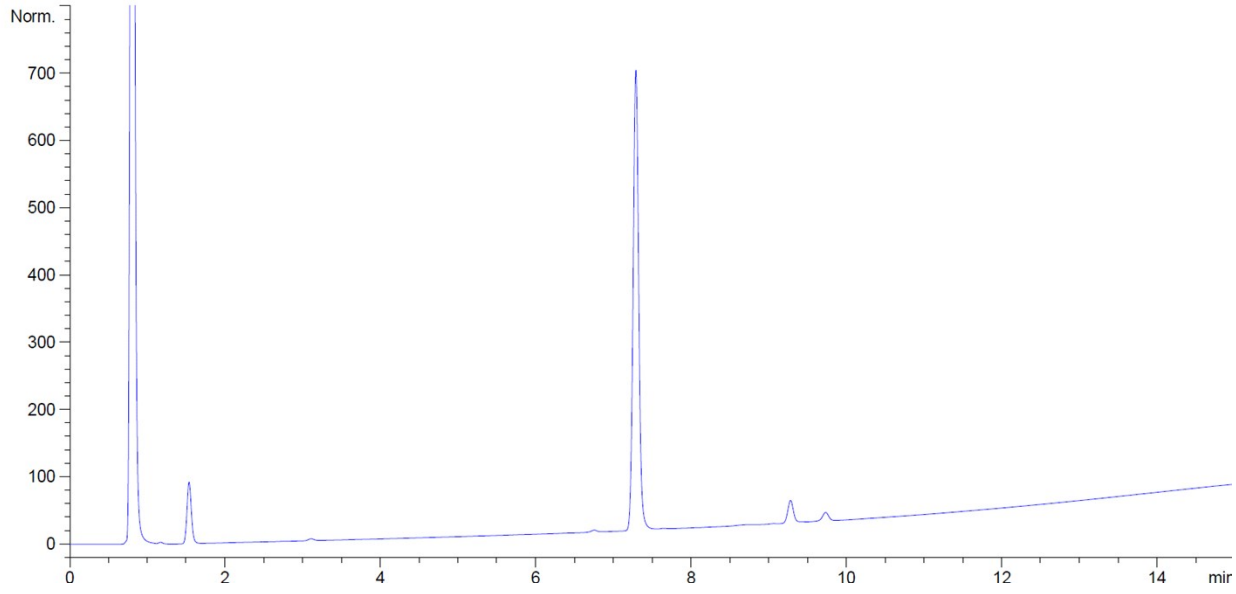


HPLC-S5. Fmoc-Arg(Pbf)-OH : DIC : OxymaPure [1:1:1], at RT, in DMF, in presence of nucleophile (tripeptidyl resin), 0.075M.

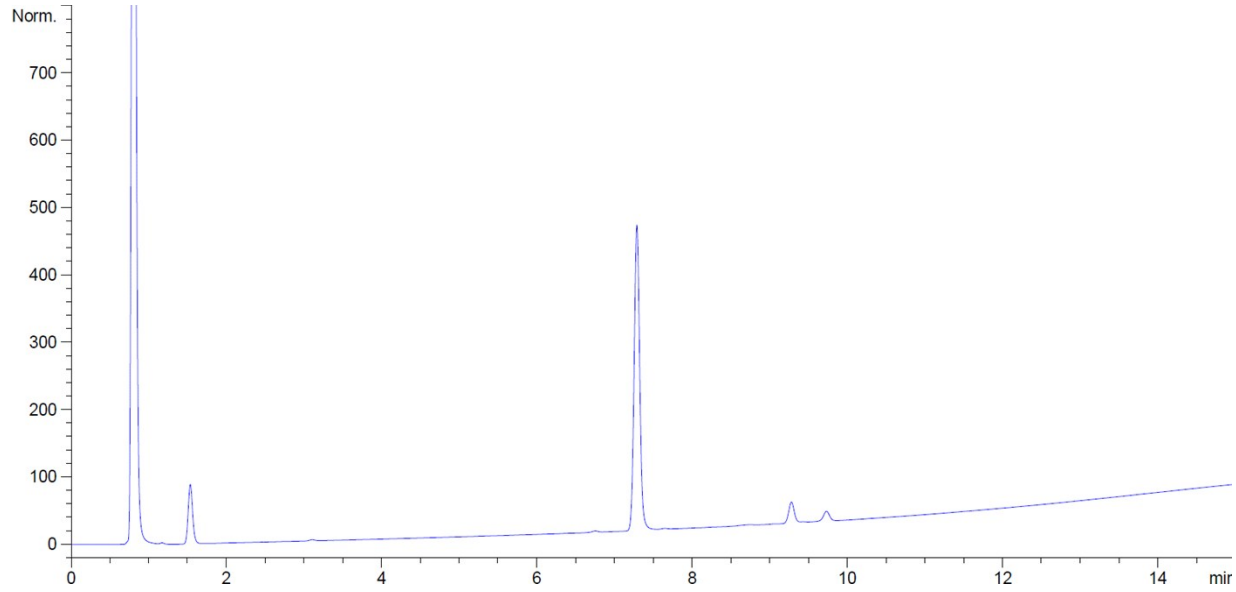
0 min



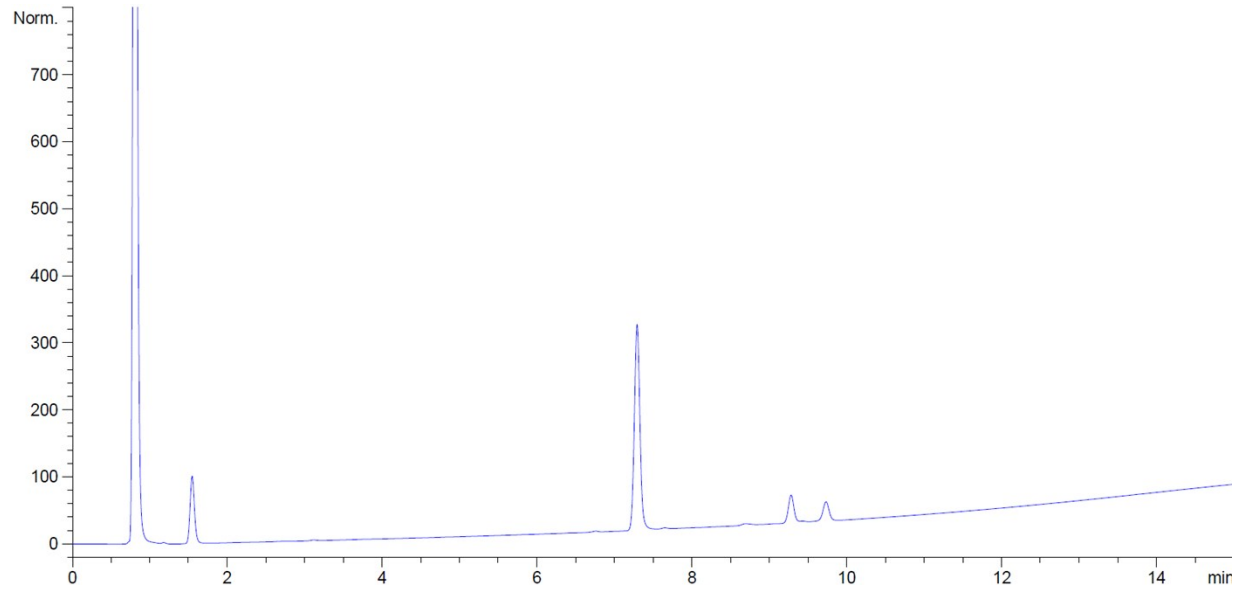
30 min



60 min

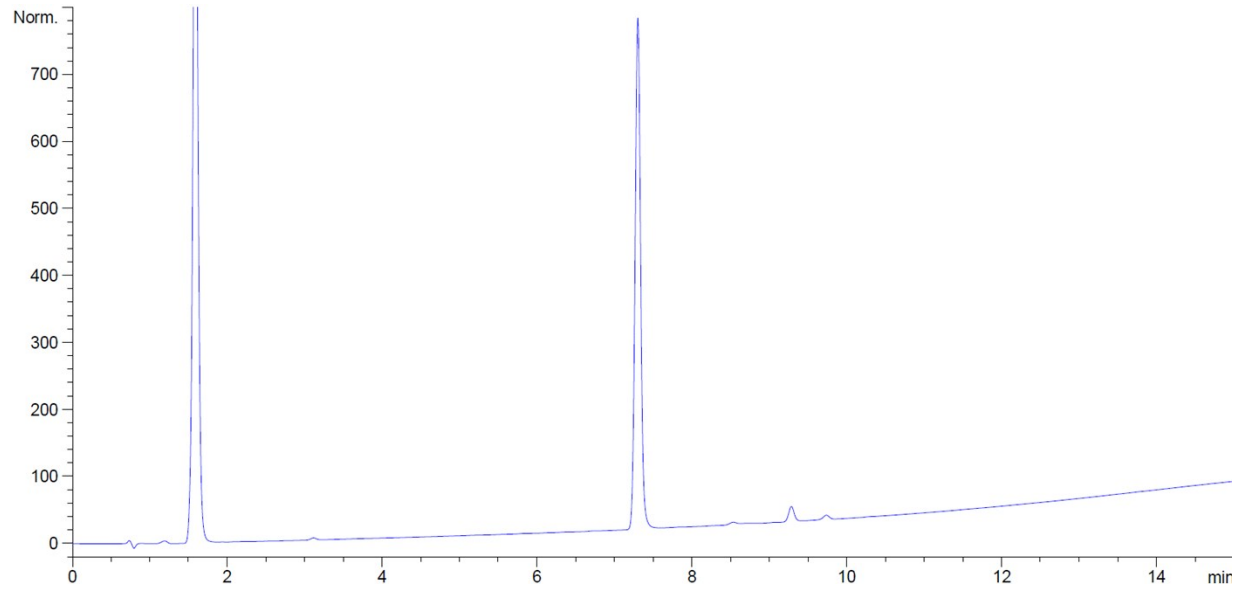


120 min

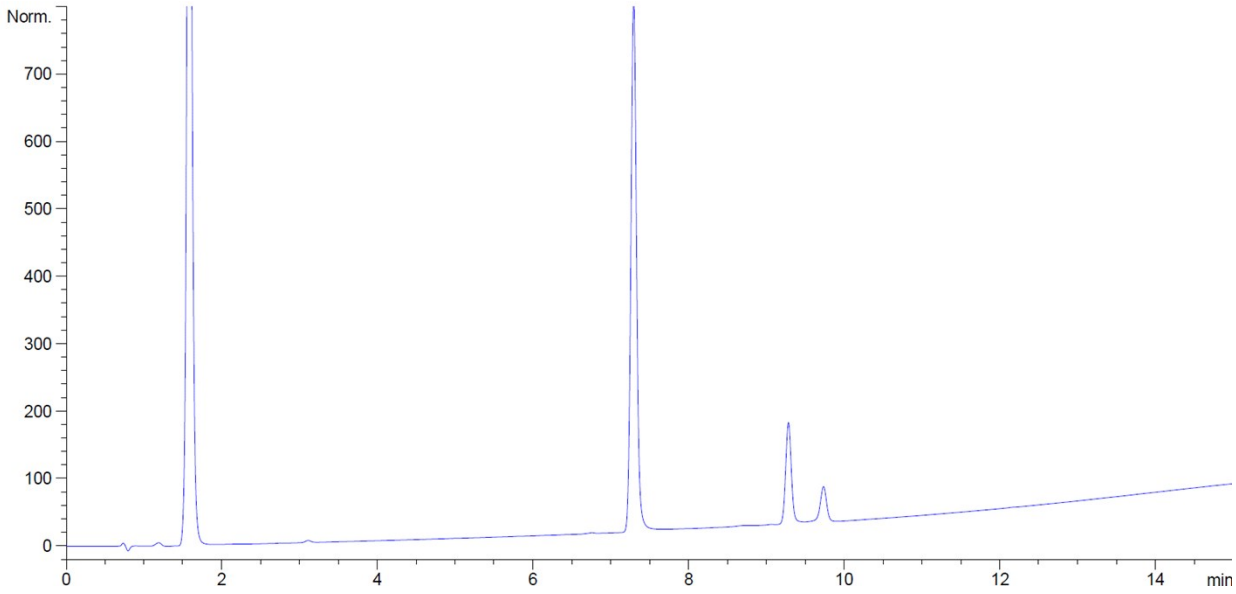


HPLC-S6. Fmoc-Arg(Pbf)-OH : DIC : OxymaPure [1:1:1], at RT, in NBP, in presence of nucleophile (tripeptidyl resin), 0.075M.

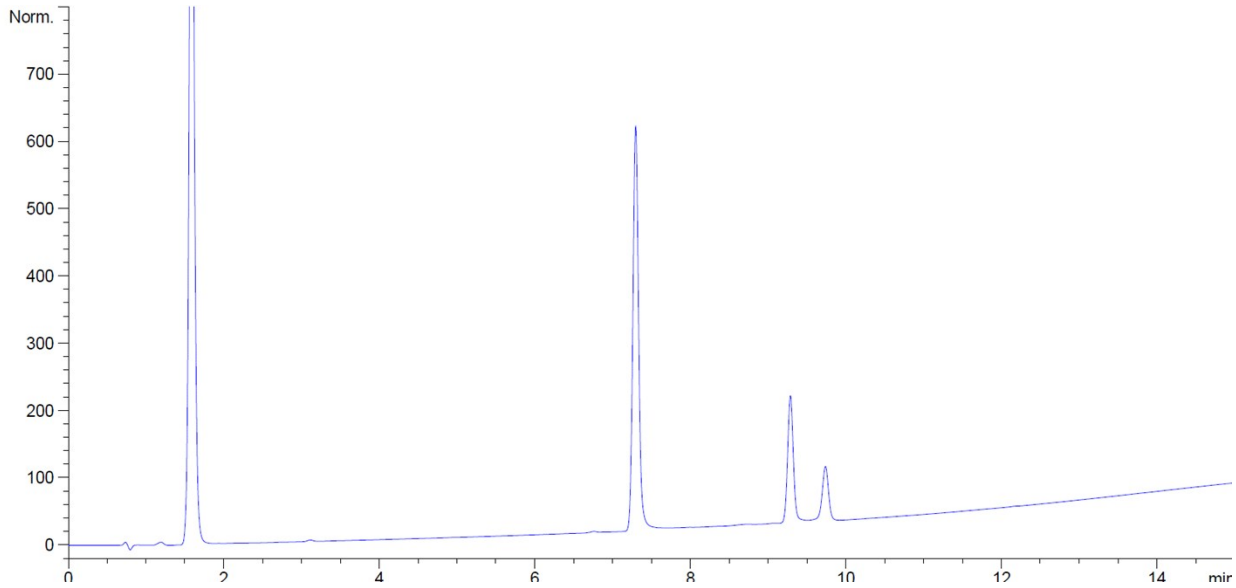
0 min



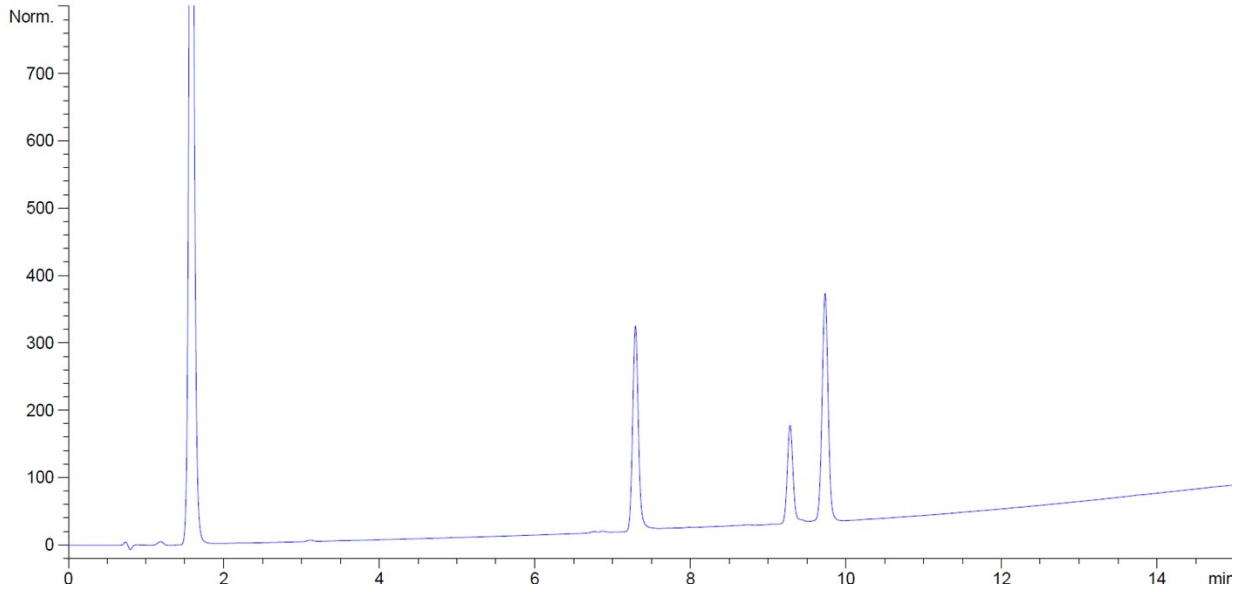
30 min



60 min

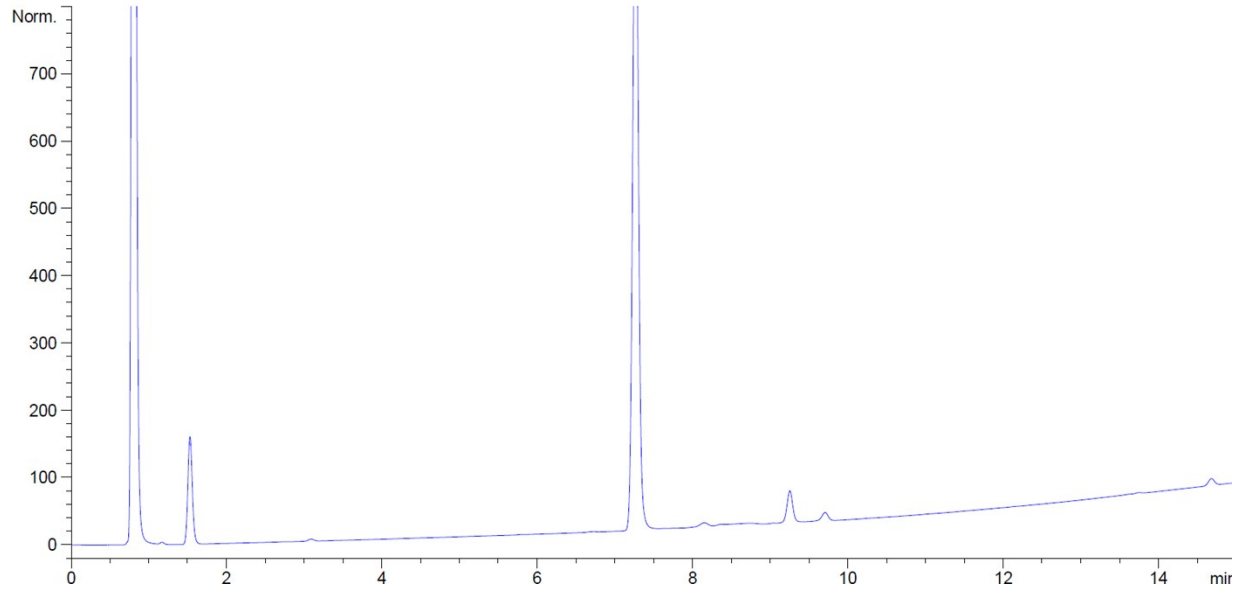


120 min

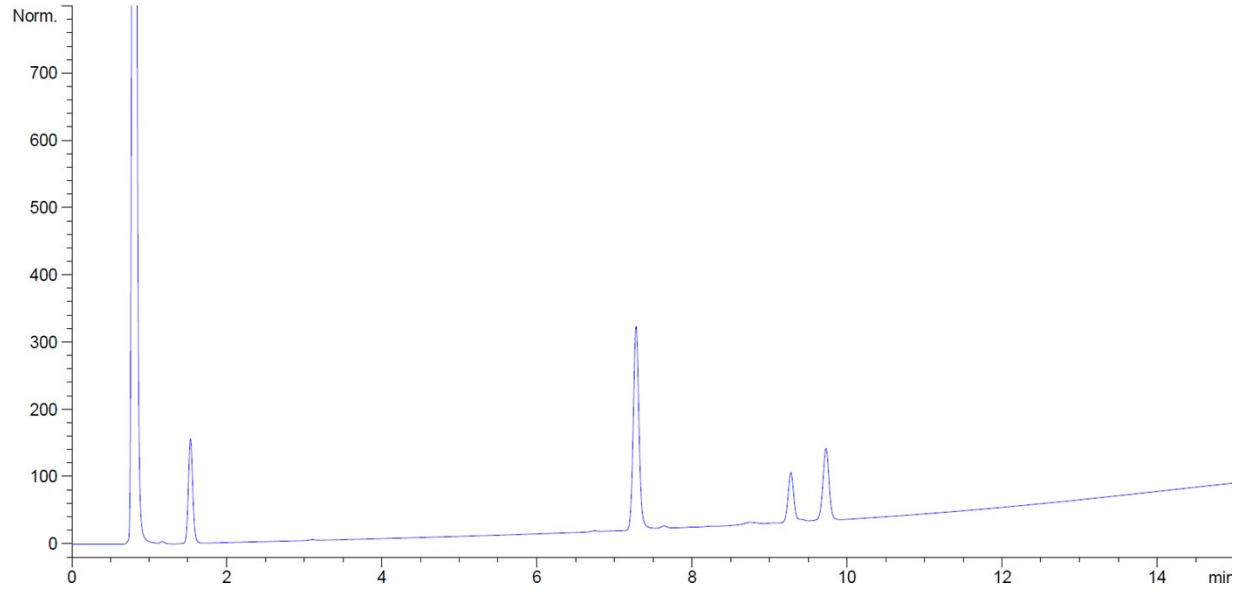


HPLC-S7. Fmoc-Arg(Pbf)-OH : DIC : OxymaPure [1:1:1], at 45 ºC, in DMF, in presence of nucleophile (tripeptidyl resin), 0.075M

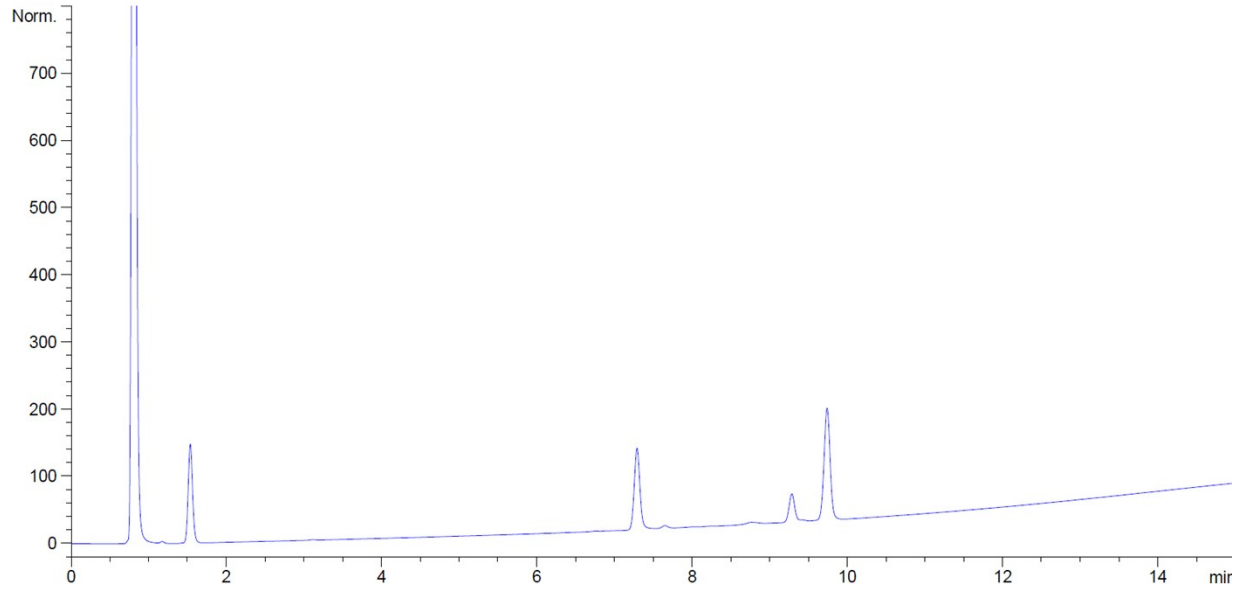
0 min



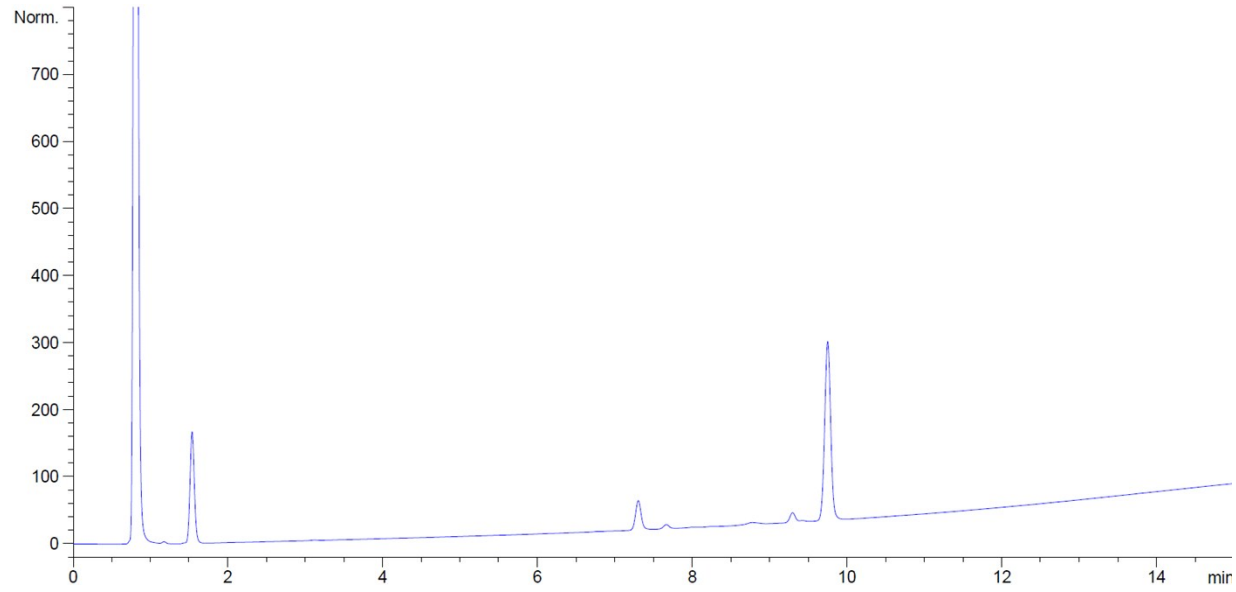
30 min



60 min

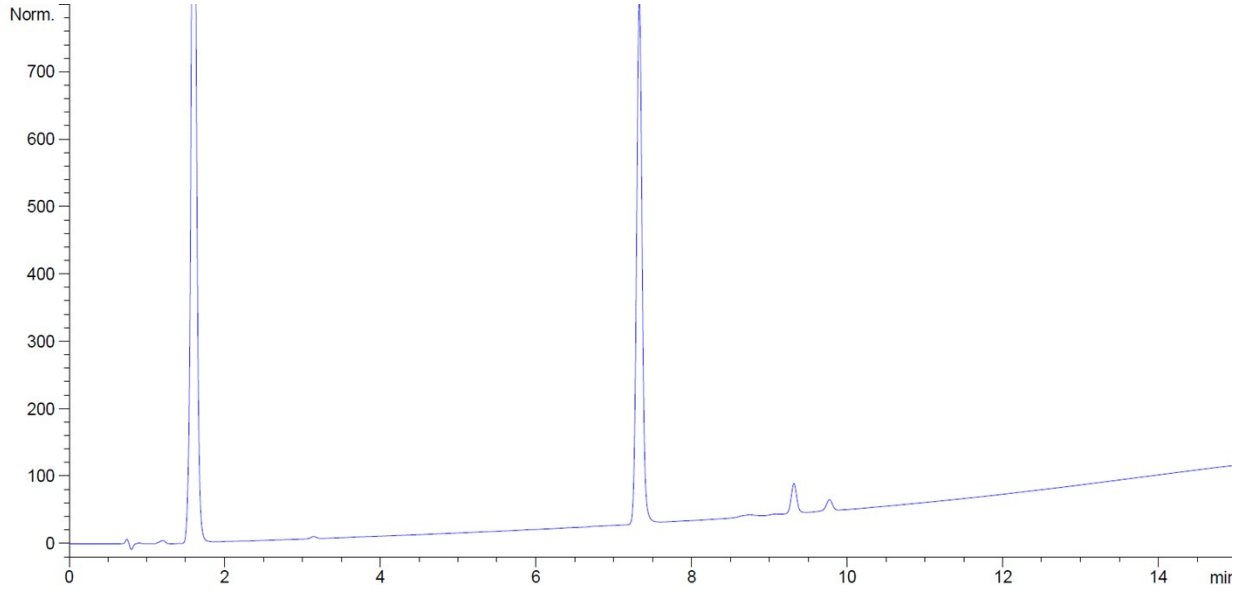


120 min

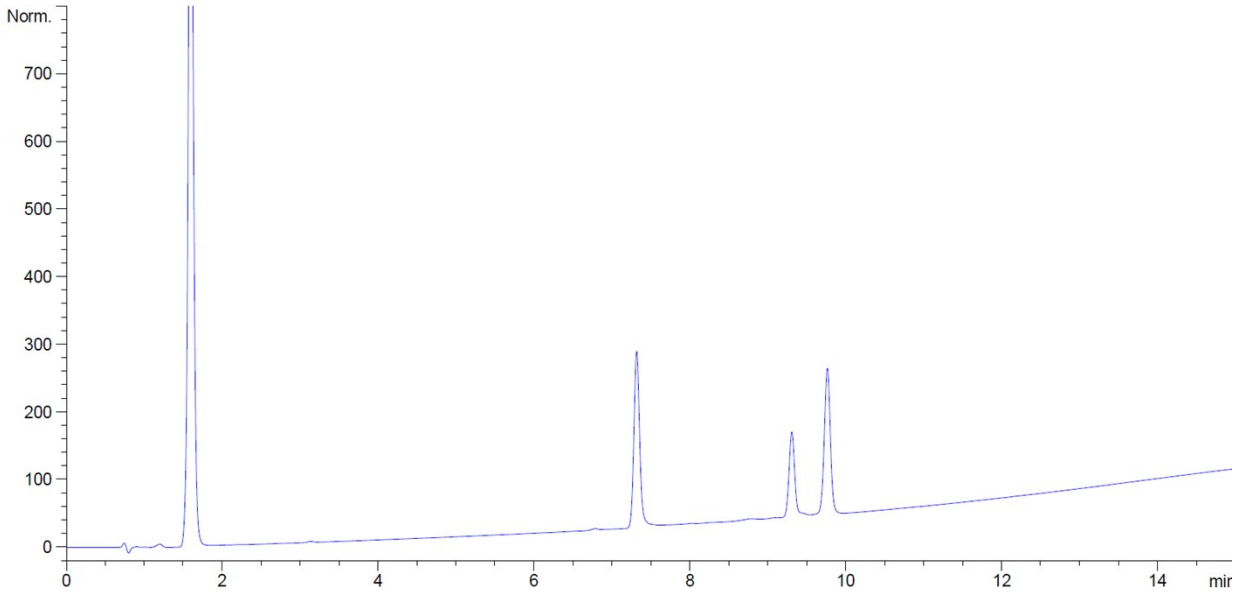


HPLC-S8. Fmoc-Arg(Pbf)-OH : DIC : OxymaPure [1:1:1], at 45 ºC, in NBP, in presence of nucleophile (tripeptidyl resin), 0.075M.

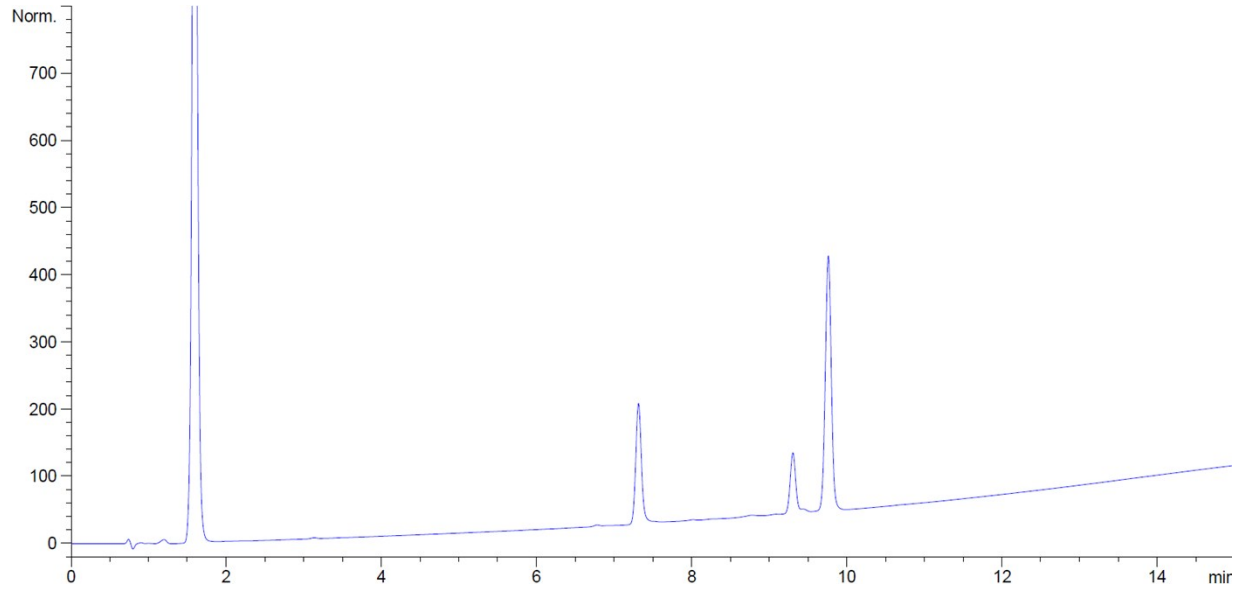
0 min



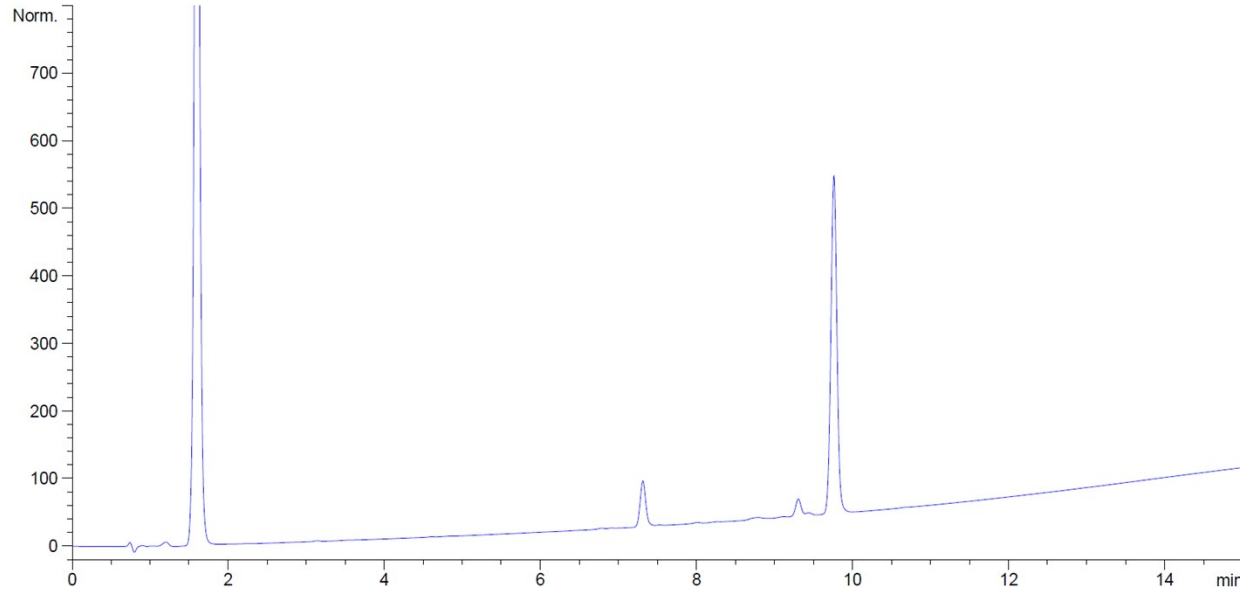
30 min



60 min

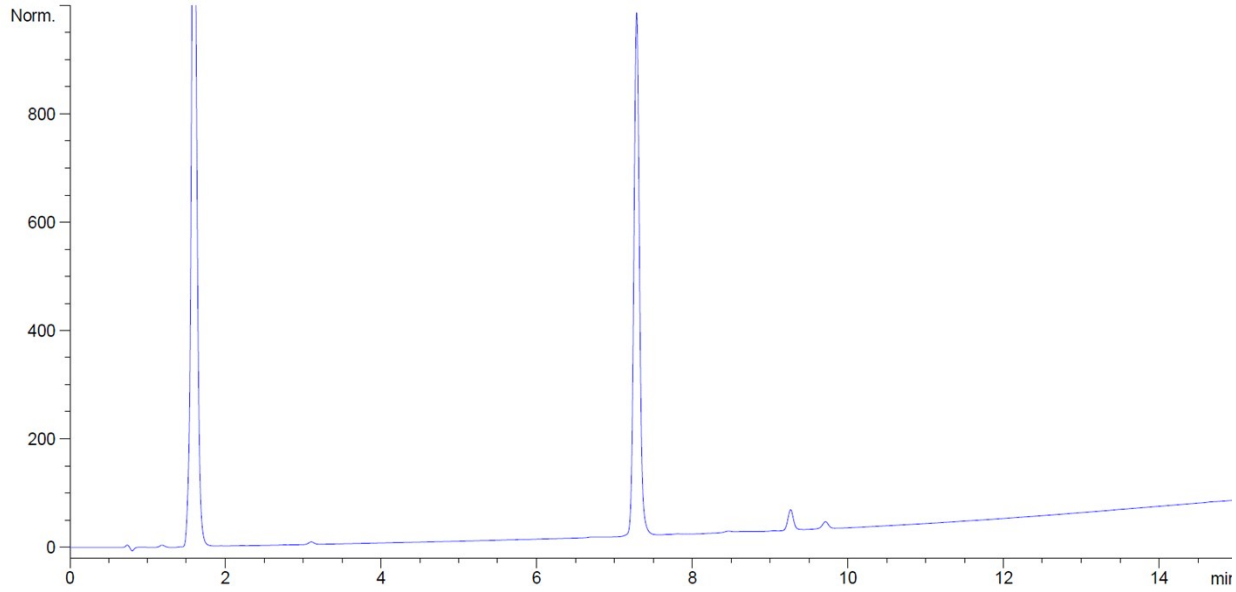


120 min

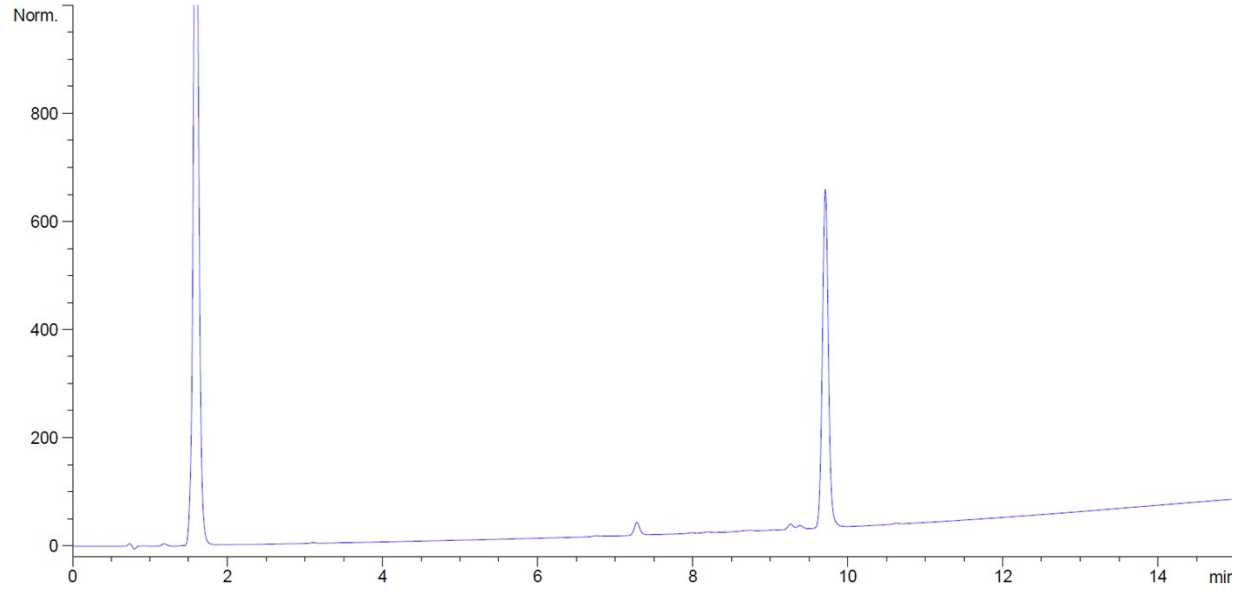


HPLC-S9. Fmoc-Arg(Pbf)-OH : DIC : OxymaPure [1:1:1], at 60 ºC, in NBP, in presence of nucleophile (tripeptidyl resin), 0.075M.

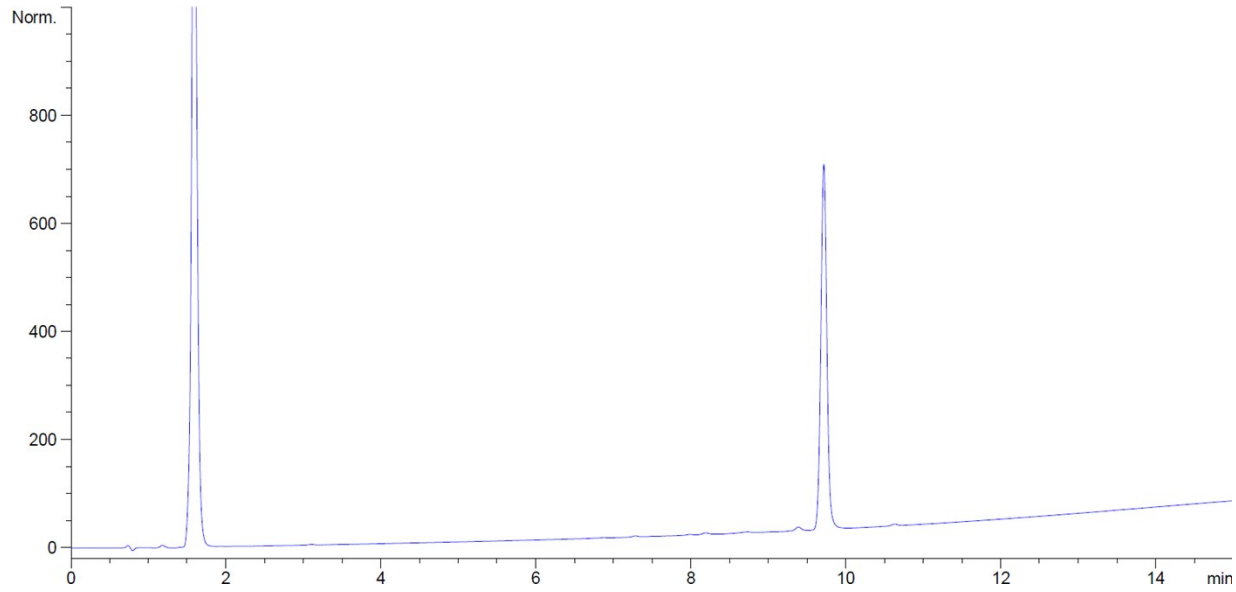
0 min



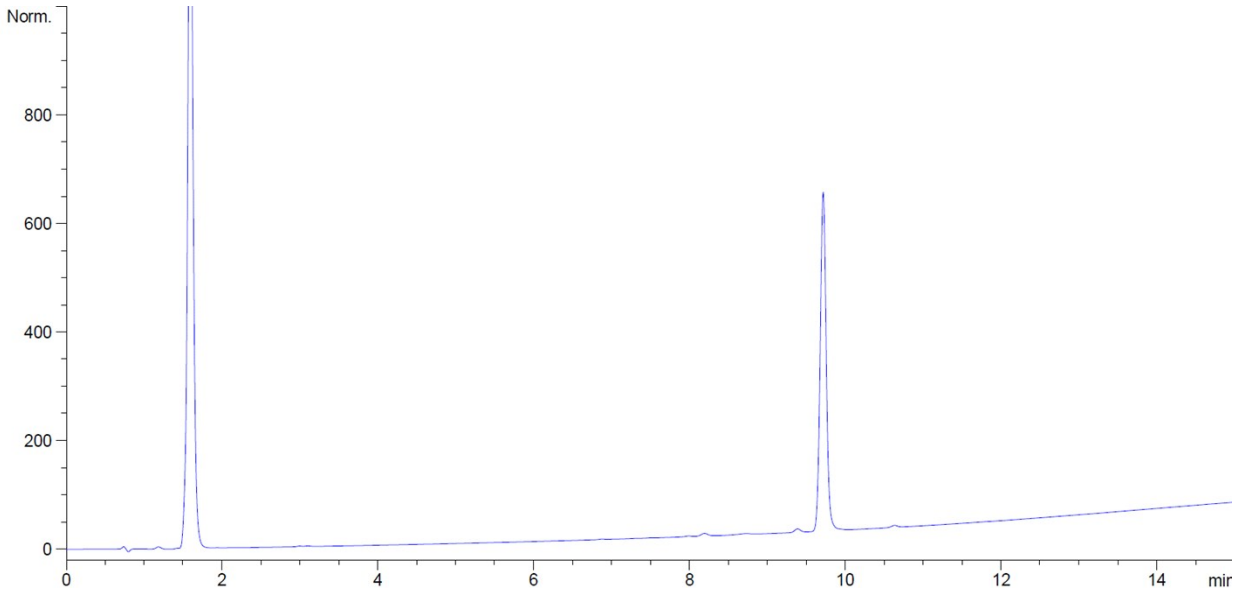
30 min



60 min

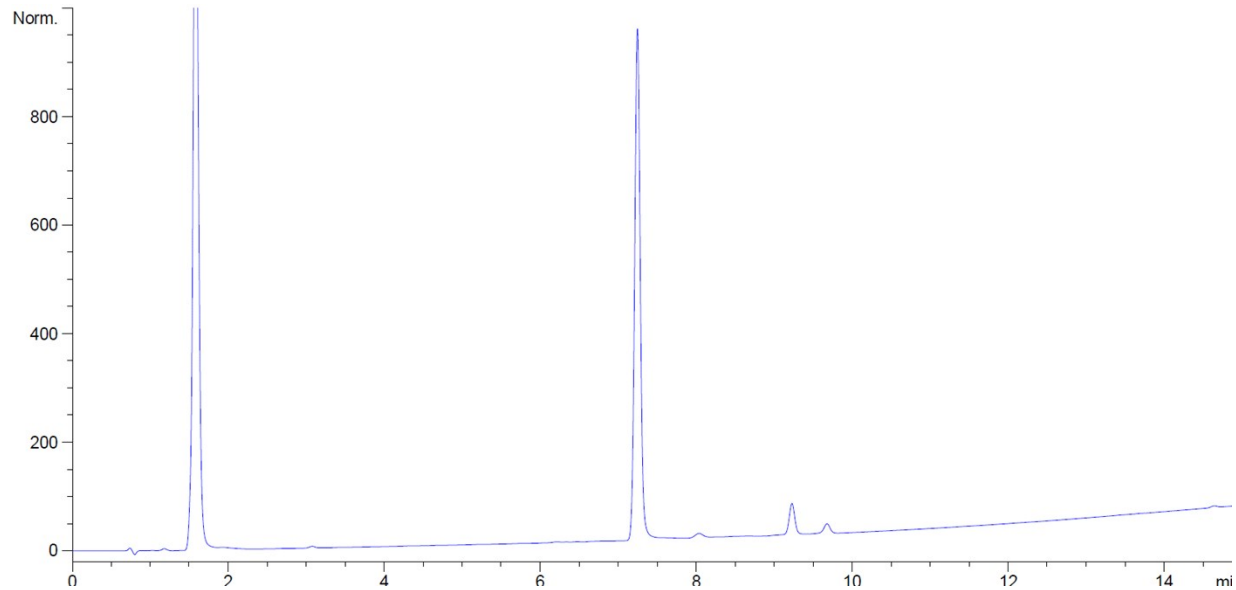


120 min

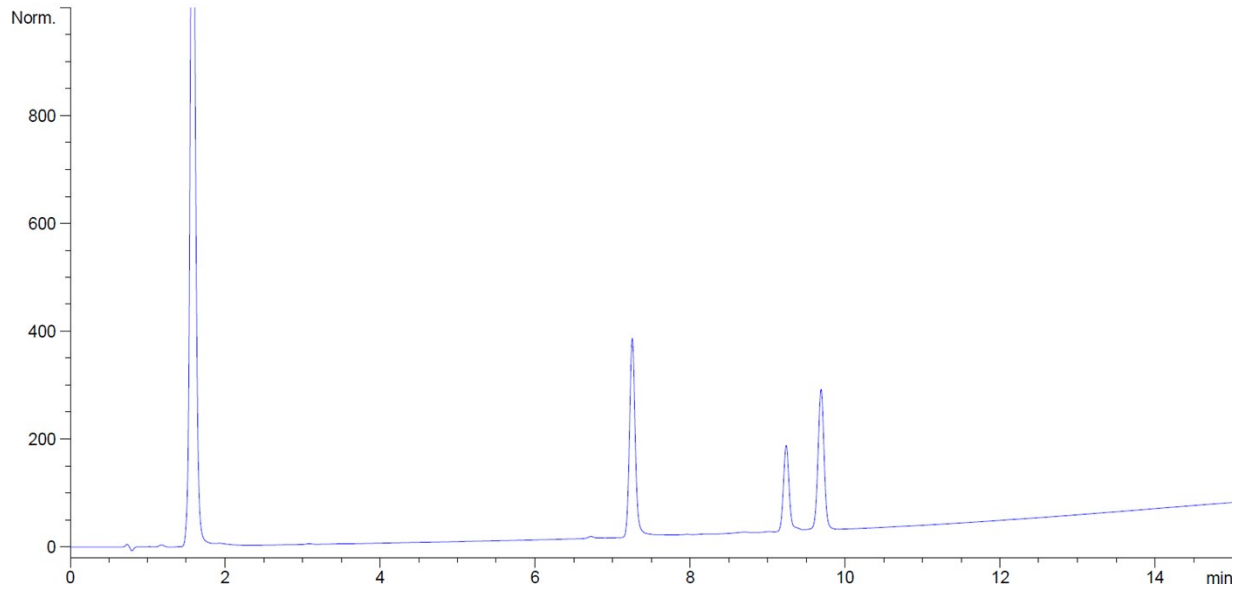


HPLC-S10. Fmoc-Arg(Pbf)-OH : DIC : OxymaPure [1:1:1], at 45 ºC, in NBP, in presence of nucleophile (tripeptidyl resin), Pre-conditioned of tripeptidyl resin, 0.075M.

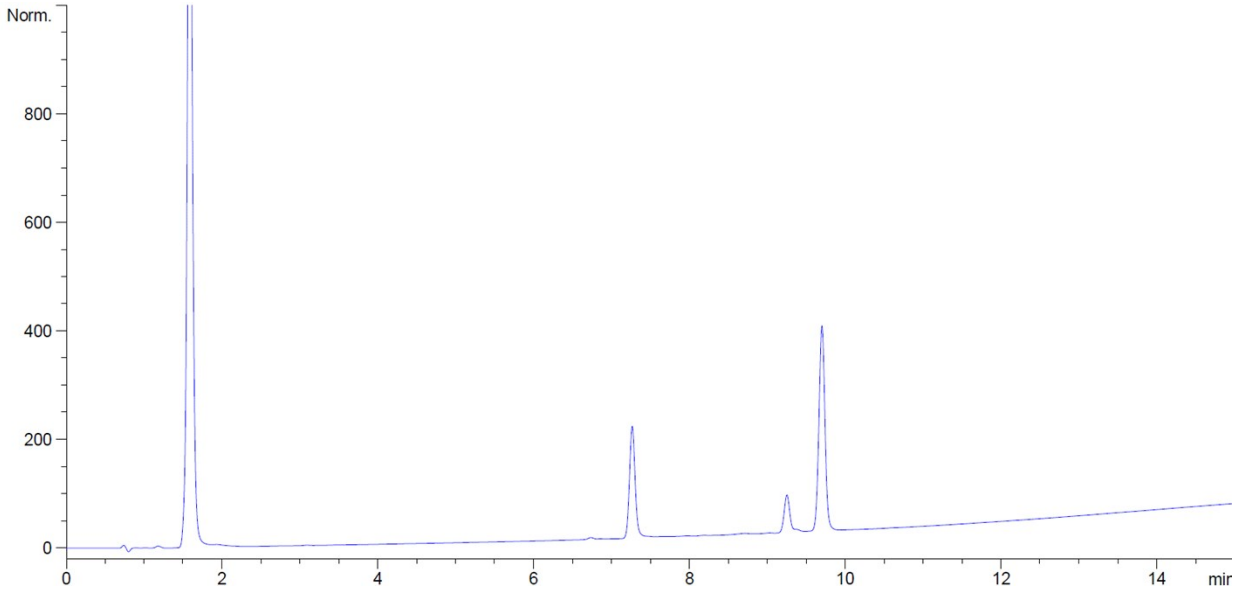
0 min



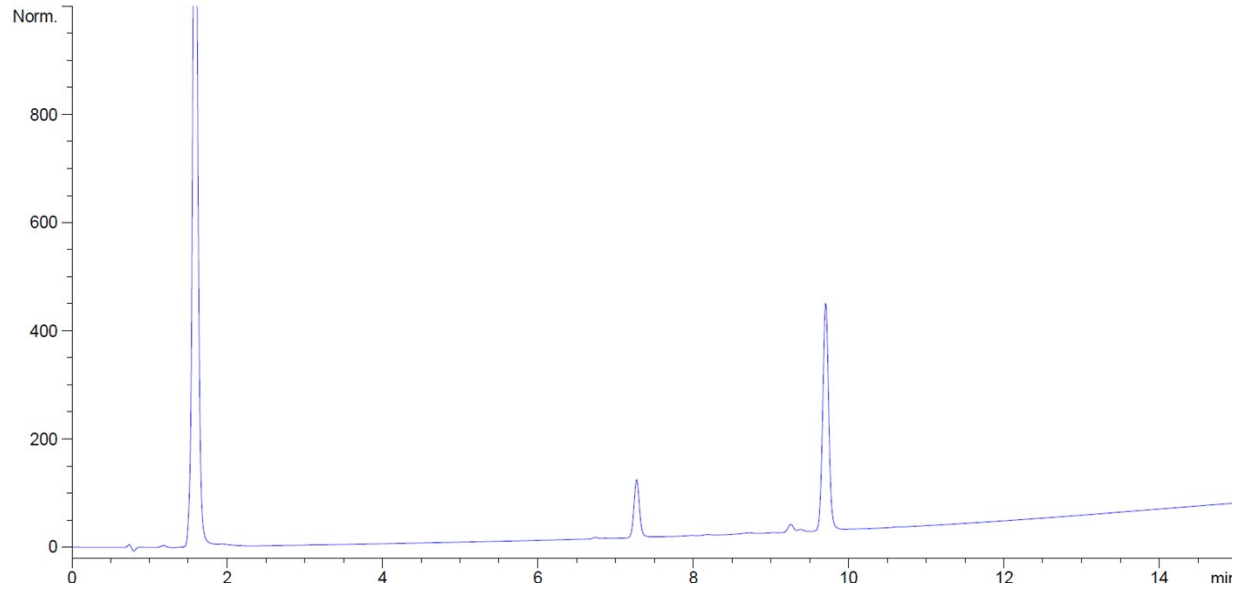
30 min



60 min

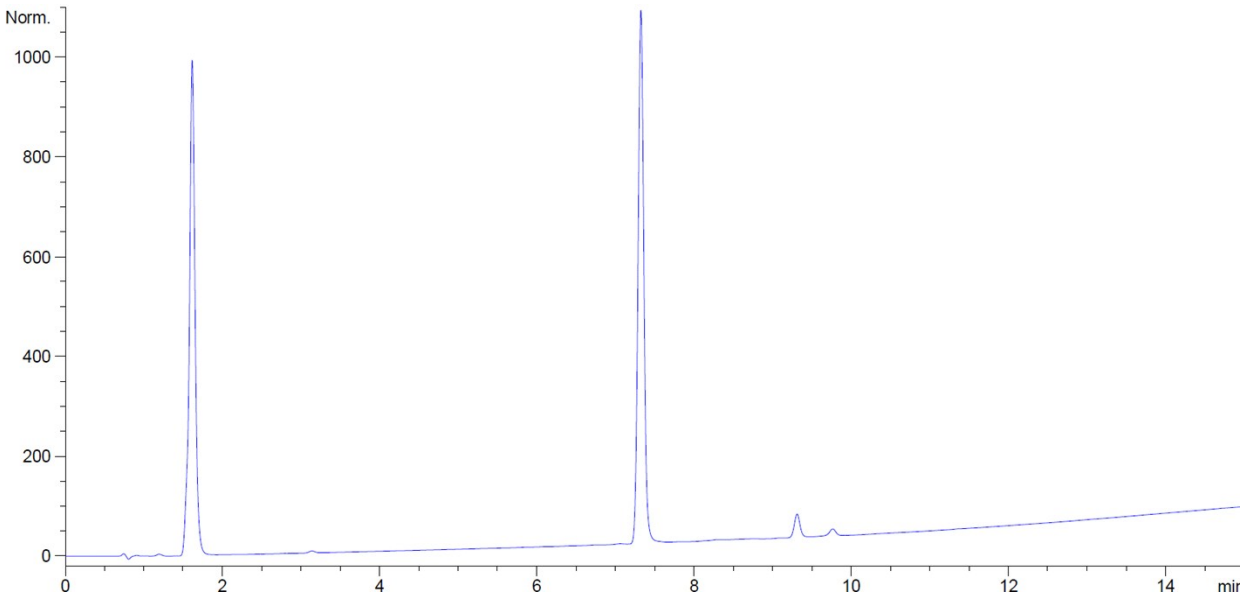


120 min

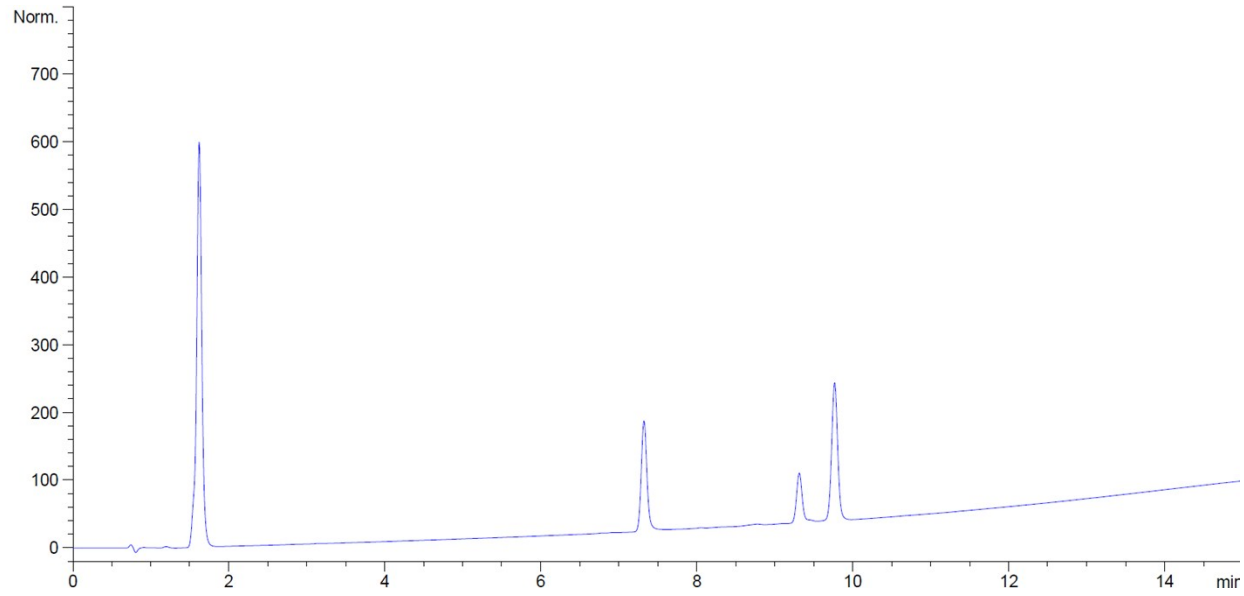


HPLC-S11. Fmoc-Arg(Pbf)-OH : DIC : OxymaPure [1:1:1], at 45 ºC, in NBP, in presence of nucleophile (tripeptidyl resin), In-situ activation, 0.15M.

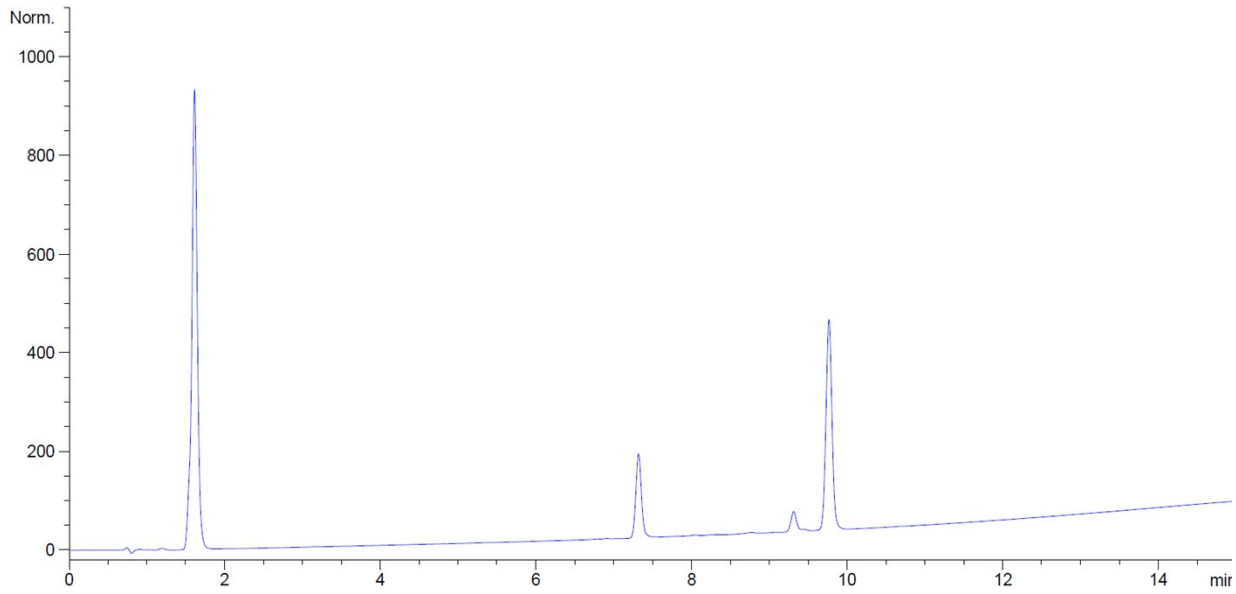
0 min



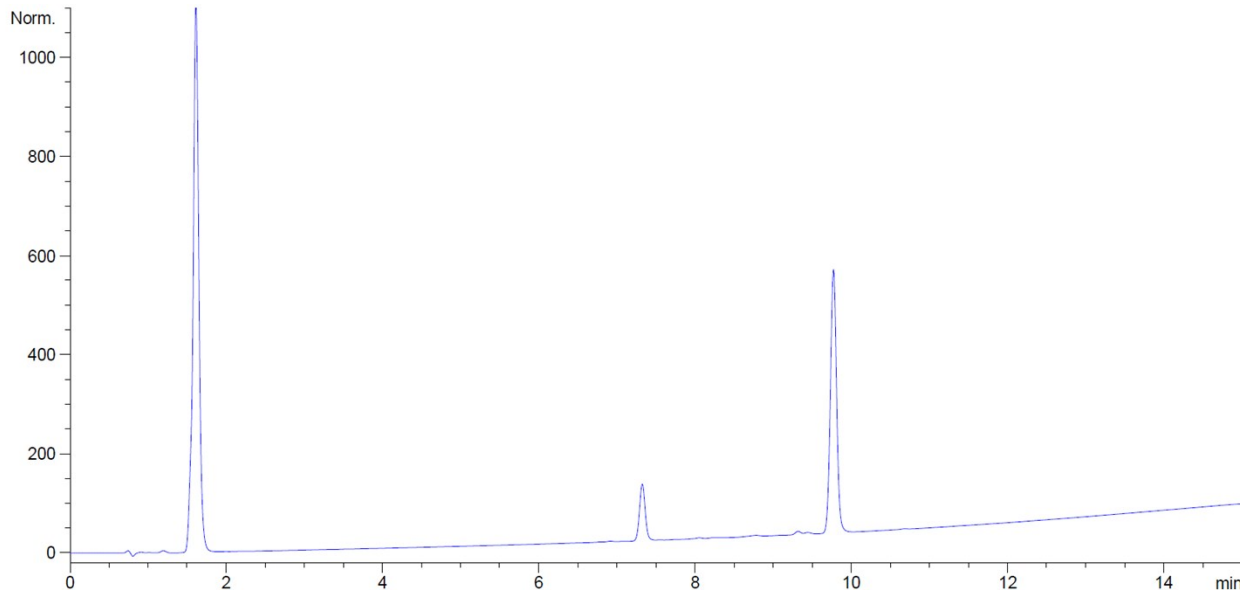
30 min



60 min

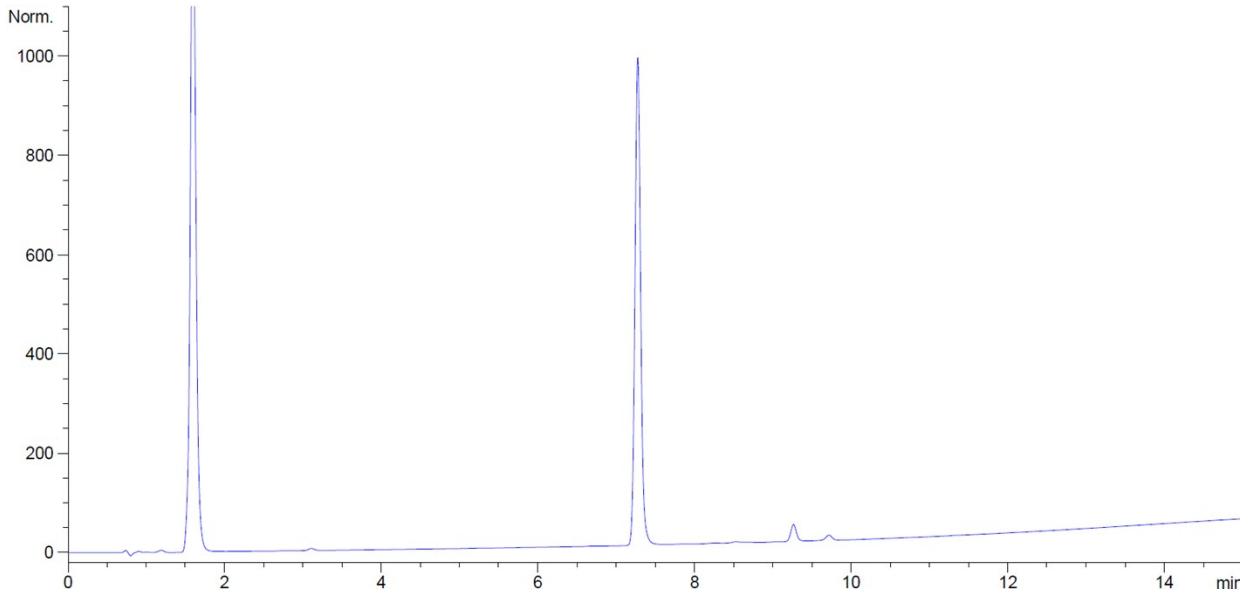


120 min

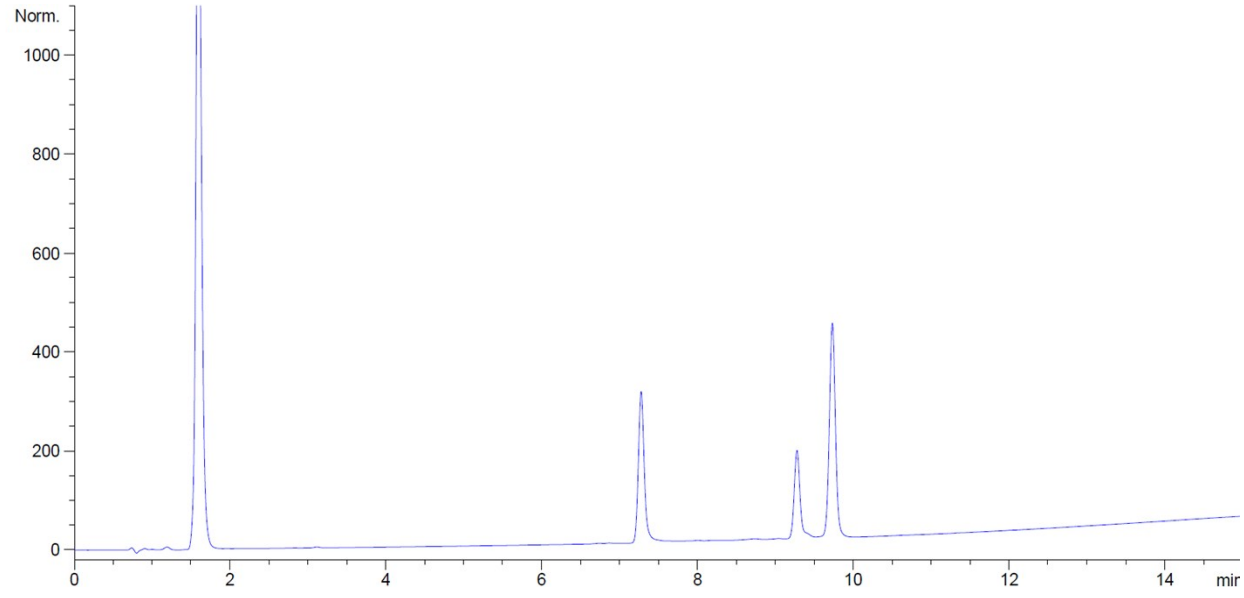


HPLC-S12. Fmoc-Arg(Pbf)-OH : DIC : OxymaPure [1:1:1], at 45 ºC, in NBP, in presence of nucleophile (tripeptidyl resin), In-situ activation, 0.075M.

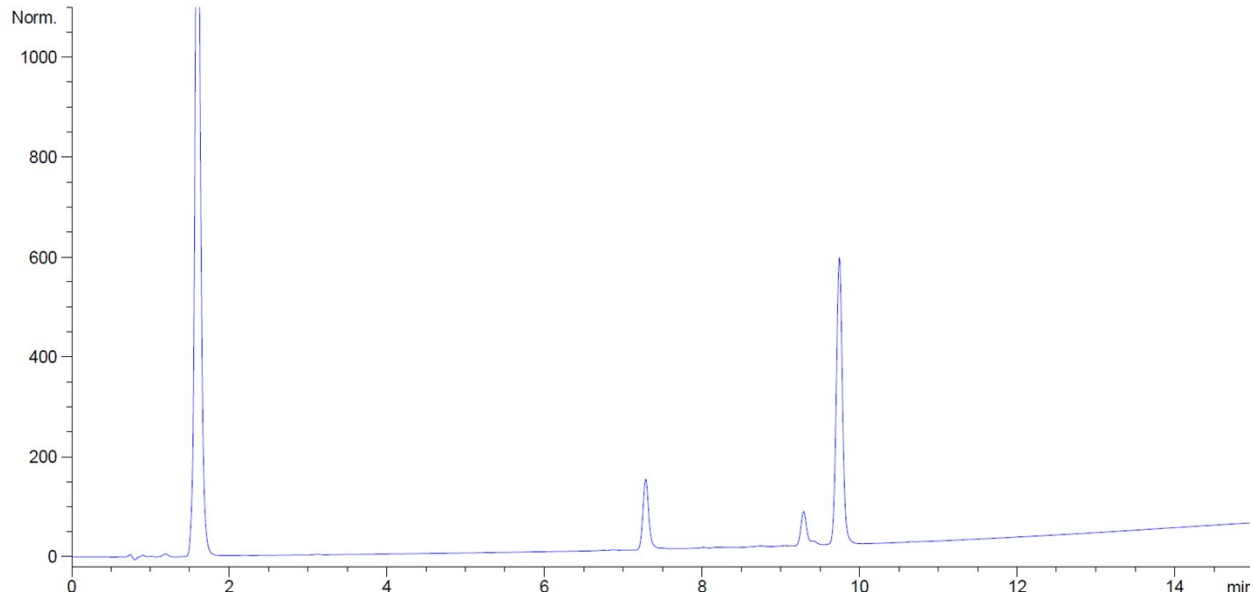
0 min



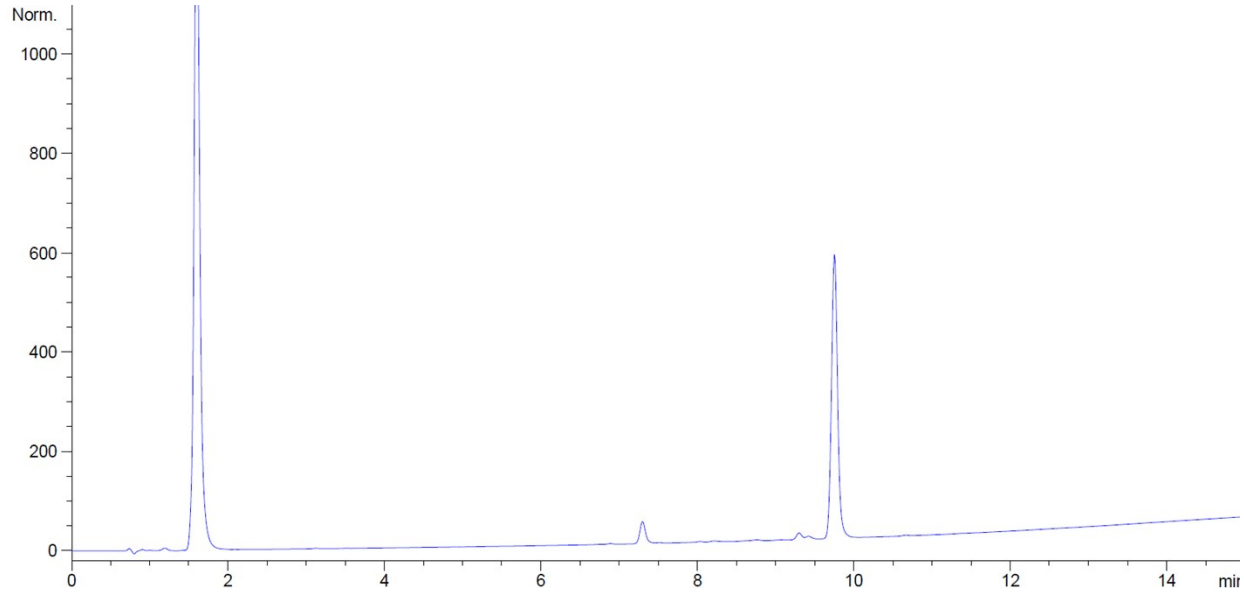
30 min



60 min

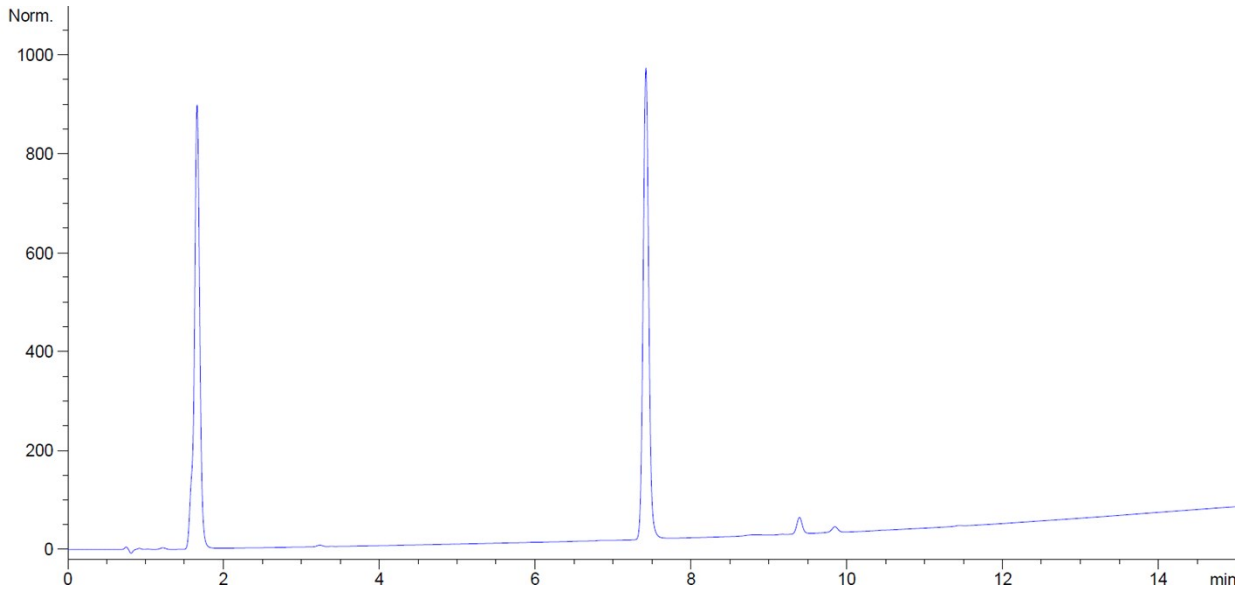


120 min

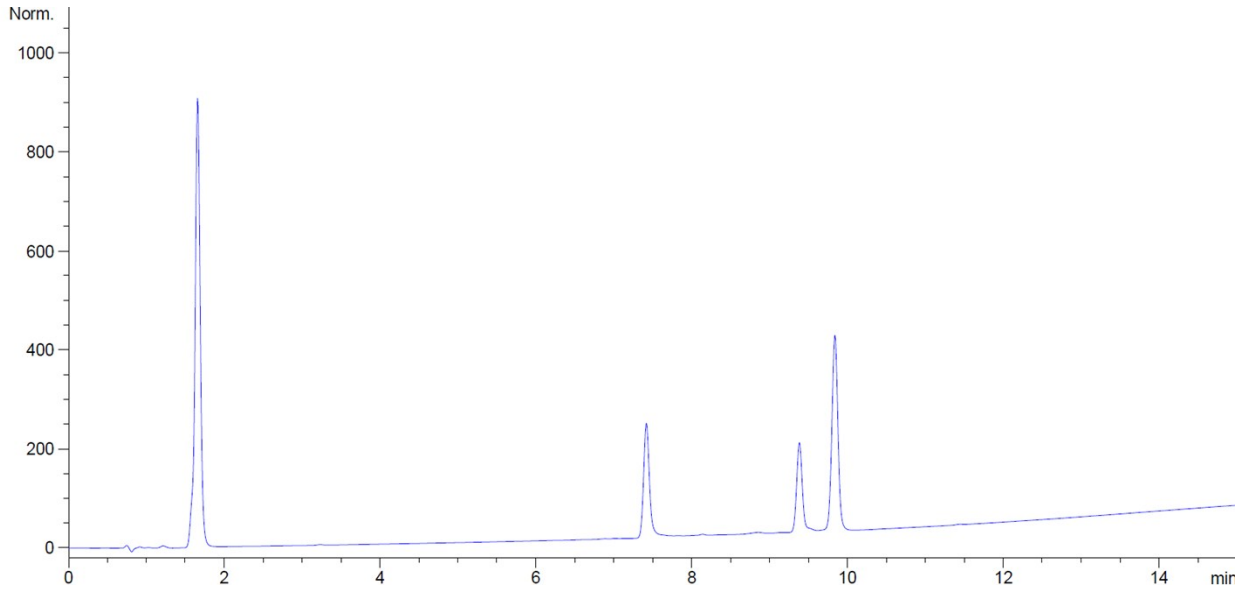


HPLC-S13. Fmoc-Arg(Pbf)-OH : DIC : OxymaPure [1:1.2:1], at 45 ºC, in NBP, in presence of nucleophile (tripeptidyl resin), In-situ activation, 0.15M.

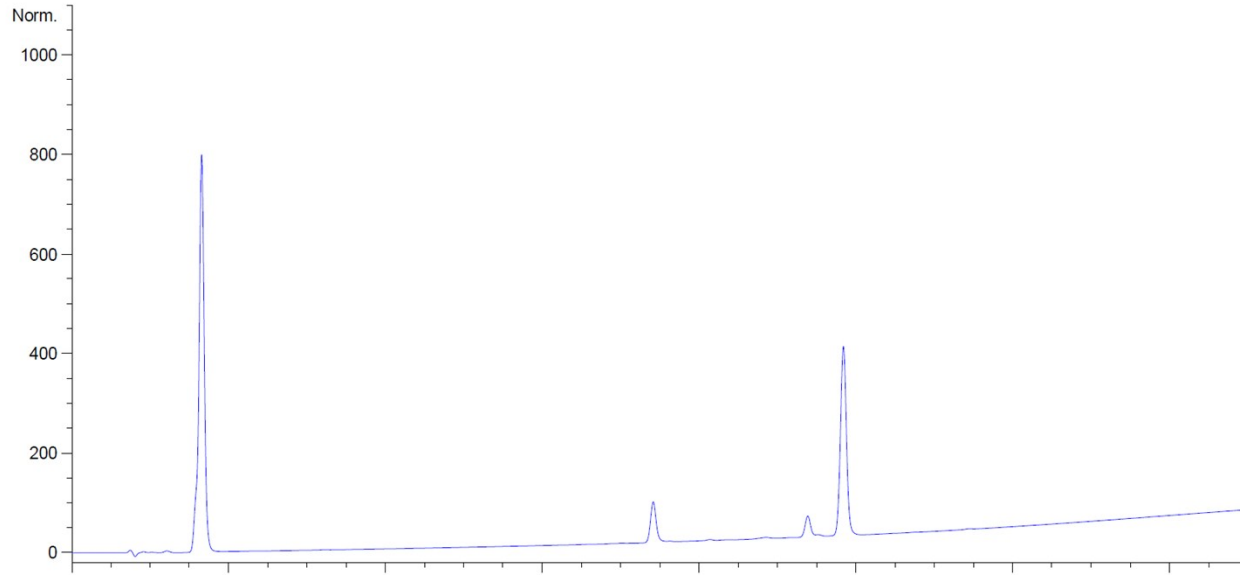
0 min



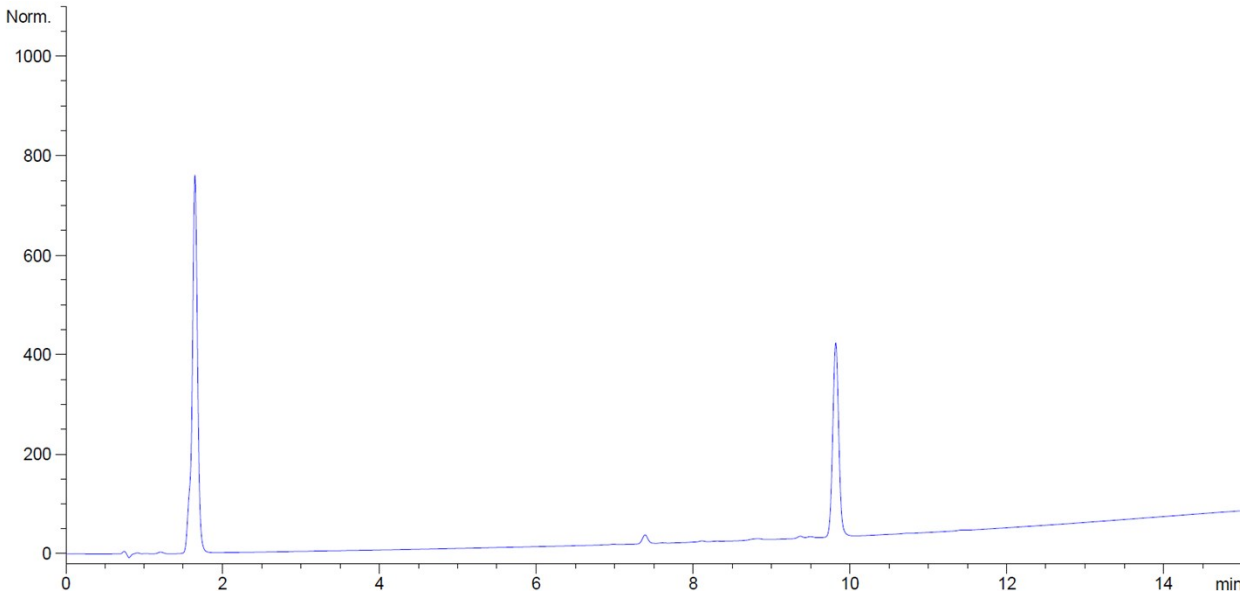
30 min



60 min

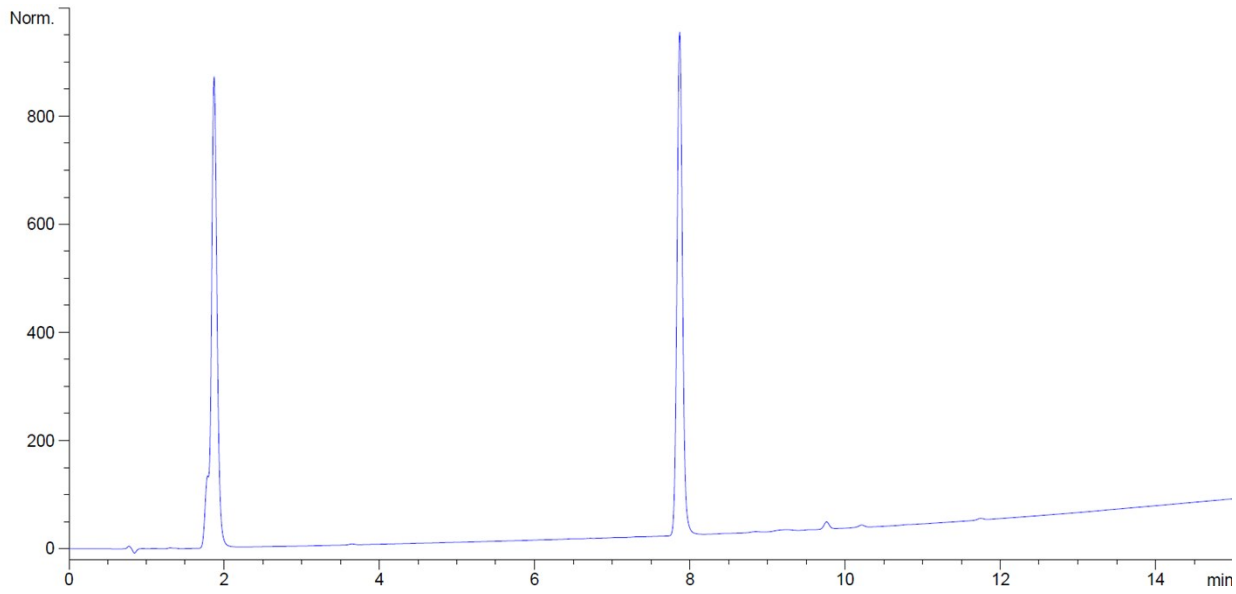


120 min

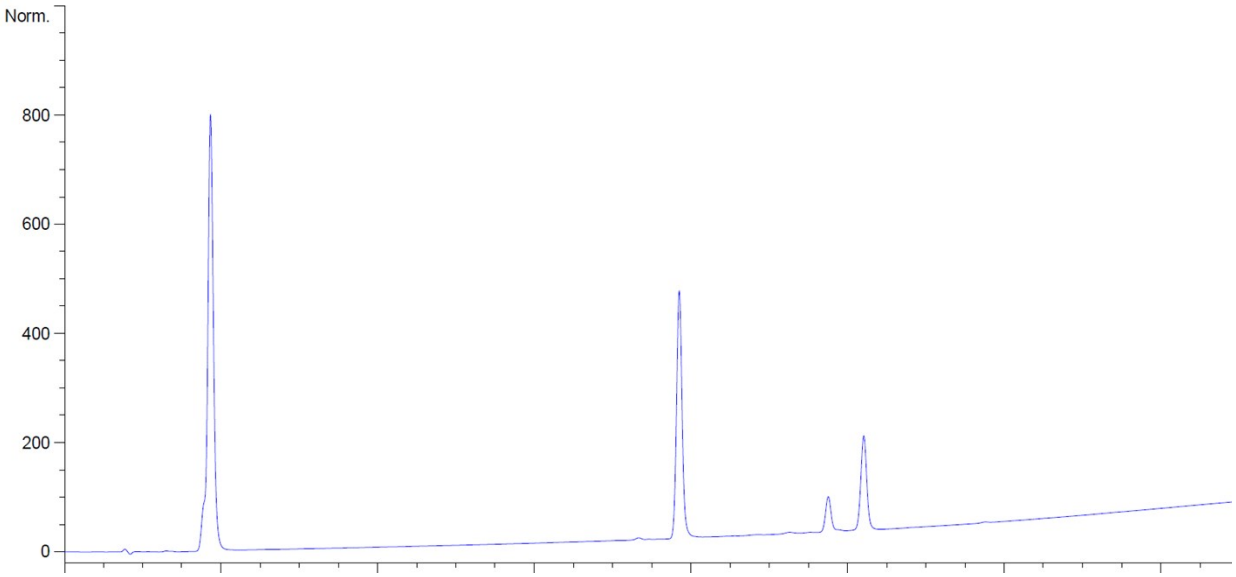


HPLC-S14. Fmoc-Arg(Pbf)-OH : DIC : OxymaPure [1:1.2:1], at 45 ºC, in NBP, in presence of nucleophile (tripeptidyl resin), In-situ activation, half DIC at 0 min and half at 30 min, 0.15M.

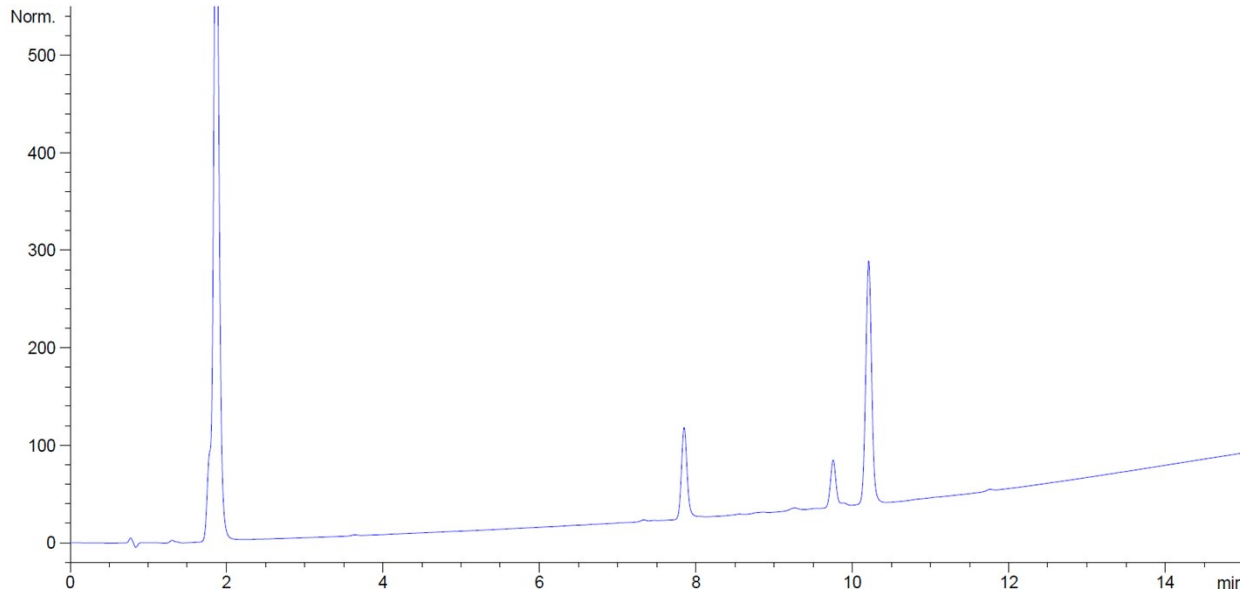
0 min



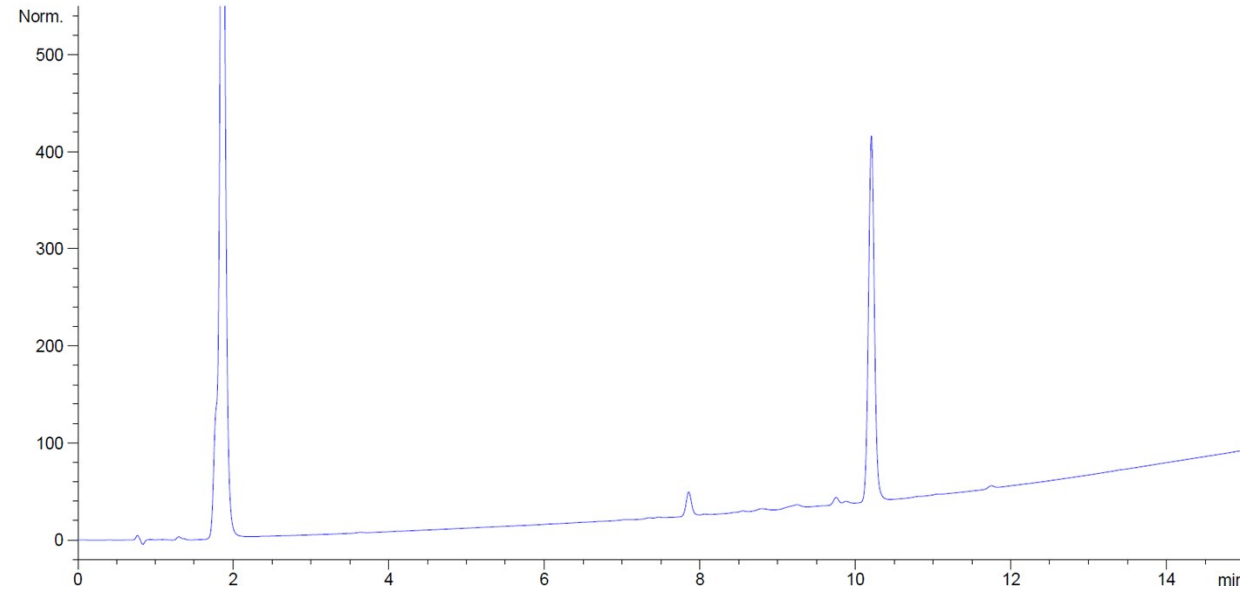
30 min



60 min

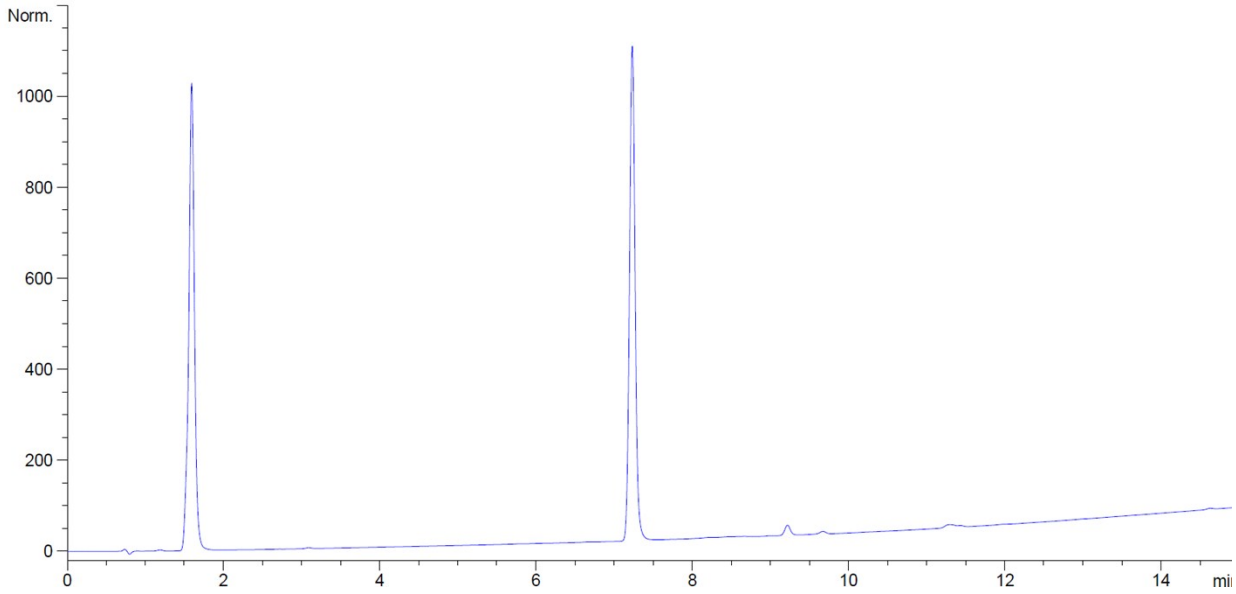


120 min

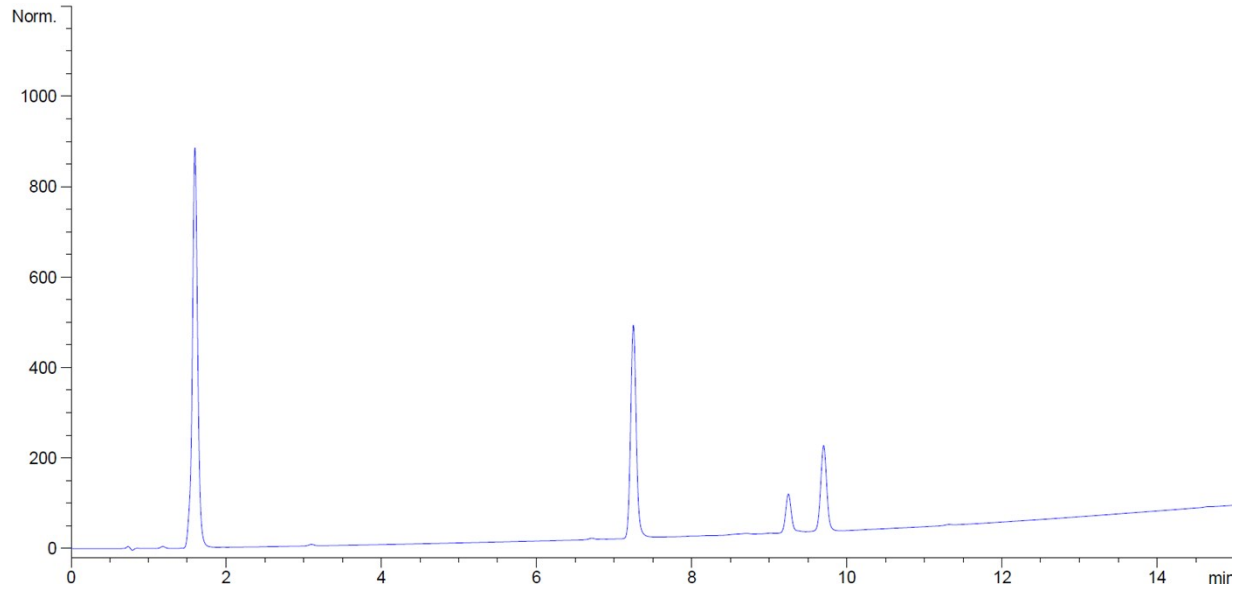


HPLC-S15. Fmoc-Arg(Pbf)-OH : DIC : OxymaPure [1:1.2:1], at 45 ºC, in NBP, in presence of nucleophile (tripeptidyl resin), In-situ activation, half DIC at 0 min and half DIC + 0.25 equiv. Fmoc-Arg(Pbf)-OH at 30 min, 0.15M.

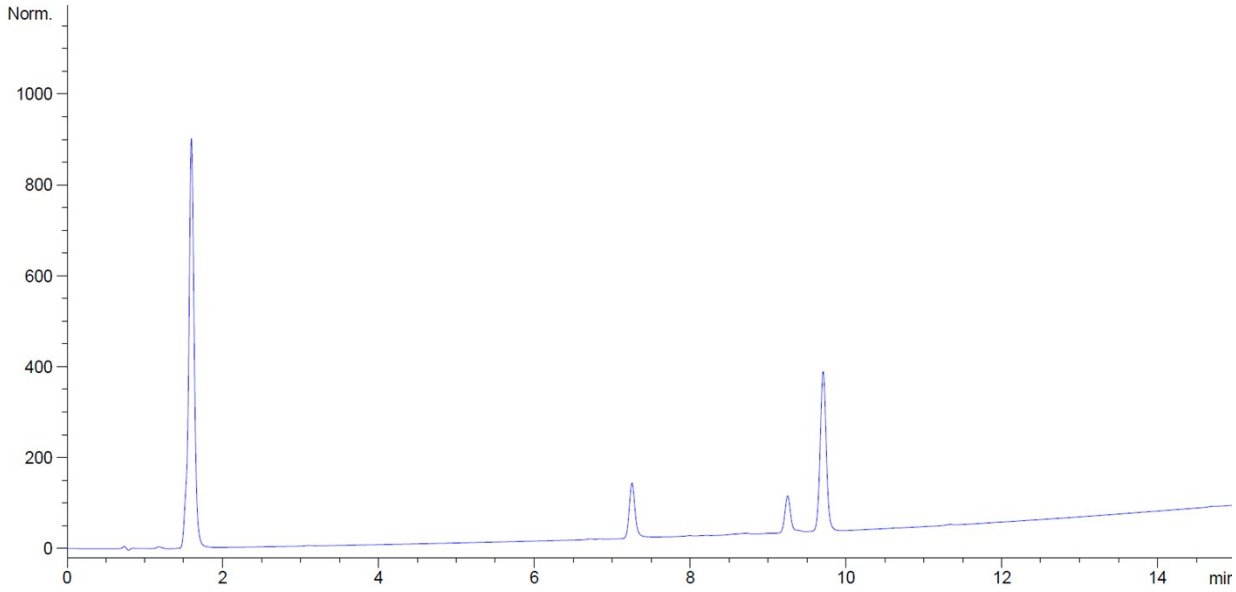
0 min



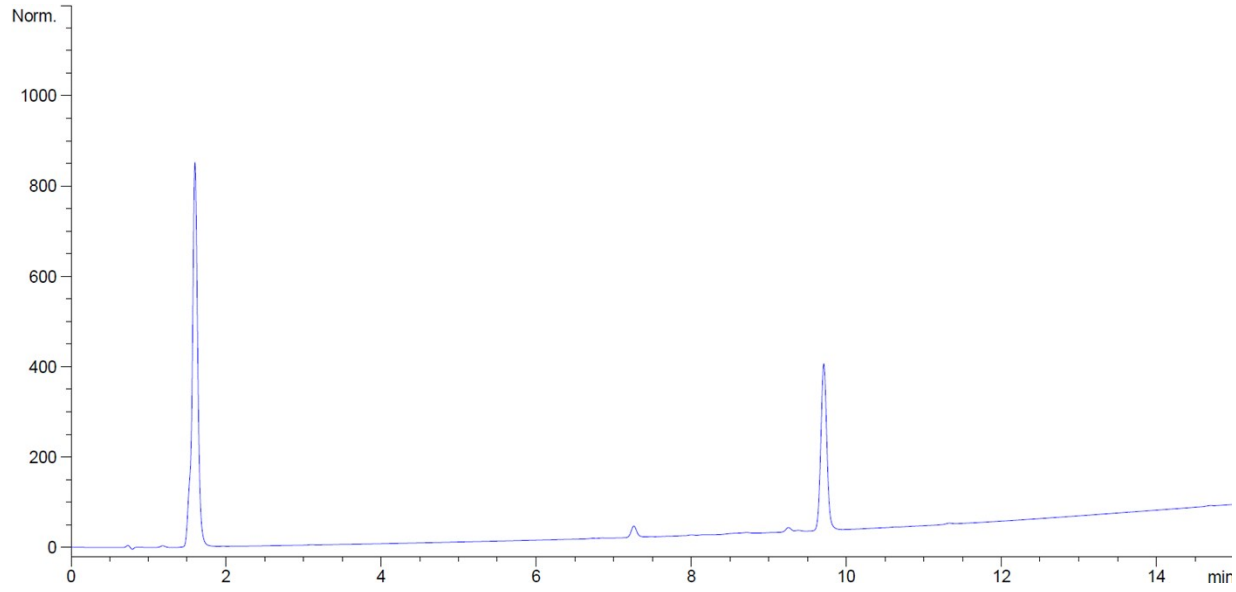
30 min



60 min

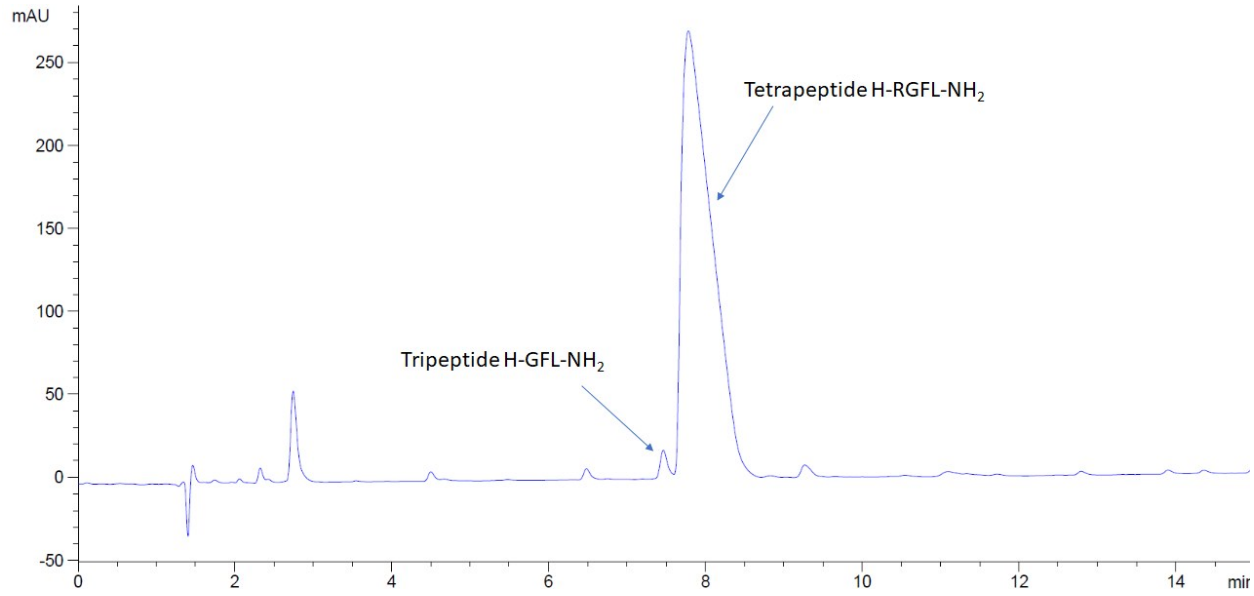


120 min

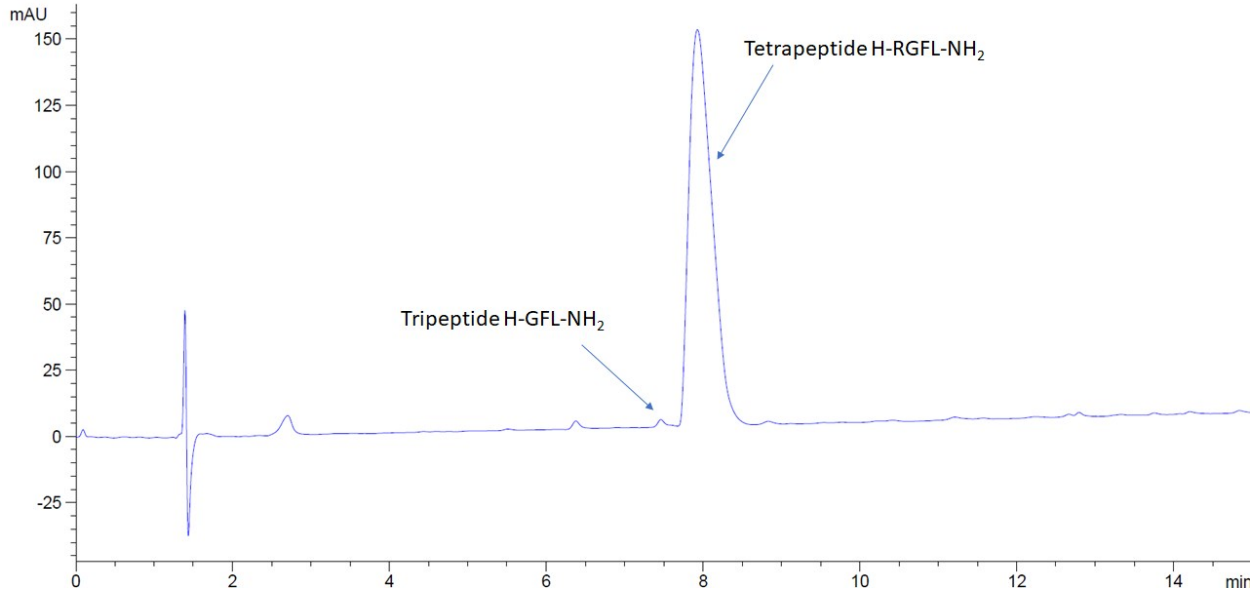


Coupling quantification

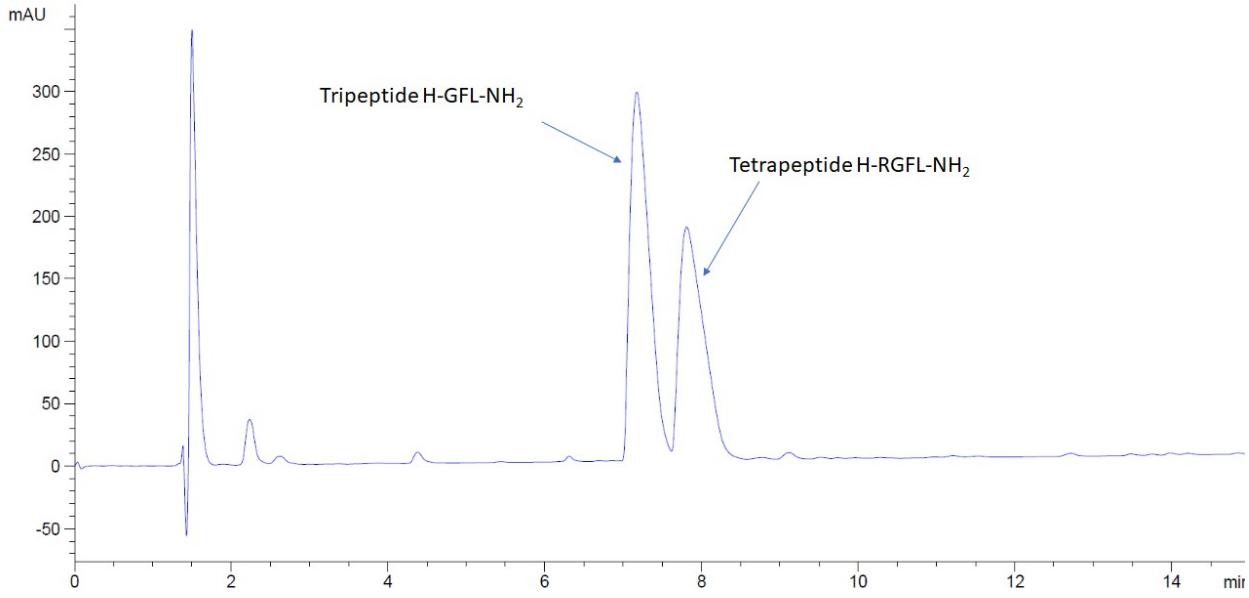
HPLC-S16. Coupling quantification of Tripeptide (H-GFL-NH_2) *vs* Tetrapeptide (H-RGFL-NH_2), Fmoc-Arg(Pbf)-OH : DIC : OxymaPure 1.5 eq. x [1:1:1], at RT, DMF.



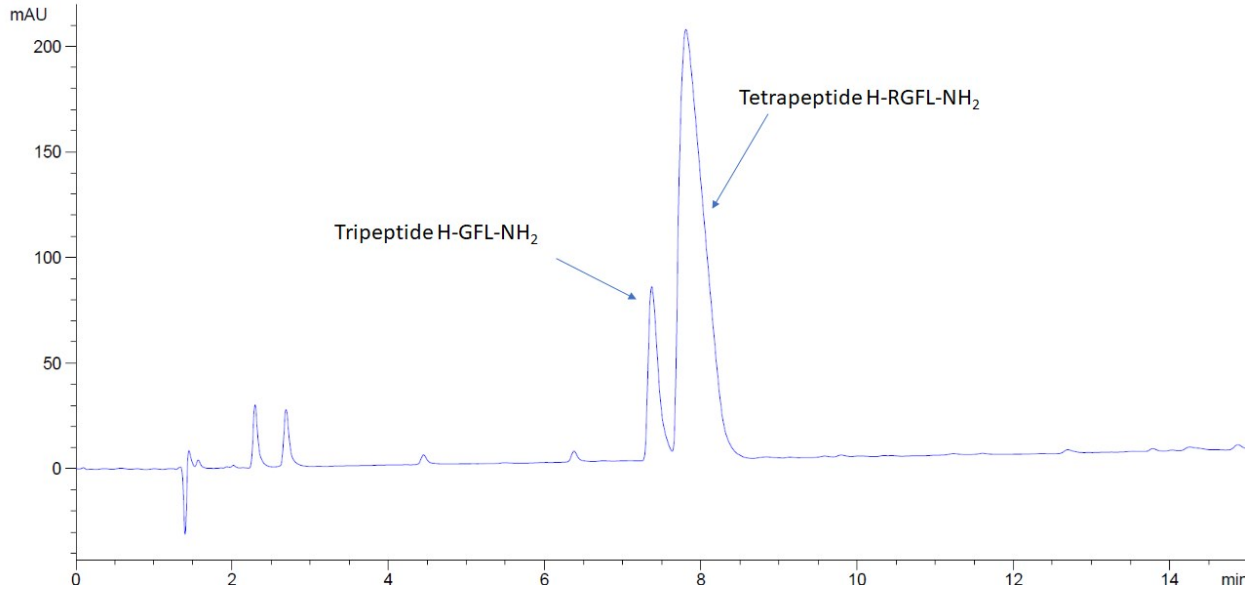
HPLC-S17. Coupling quantification of Tripeptide (H-GFL-NH_2) *vs* Tetrapeptide (H-RGFL-NH_2), Fmoc-Arg(Pbf)-OH : DIC : OxymaPure 1.5 eq. x [1:1:1], 45 ºC, DMF.



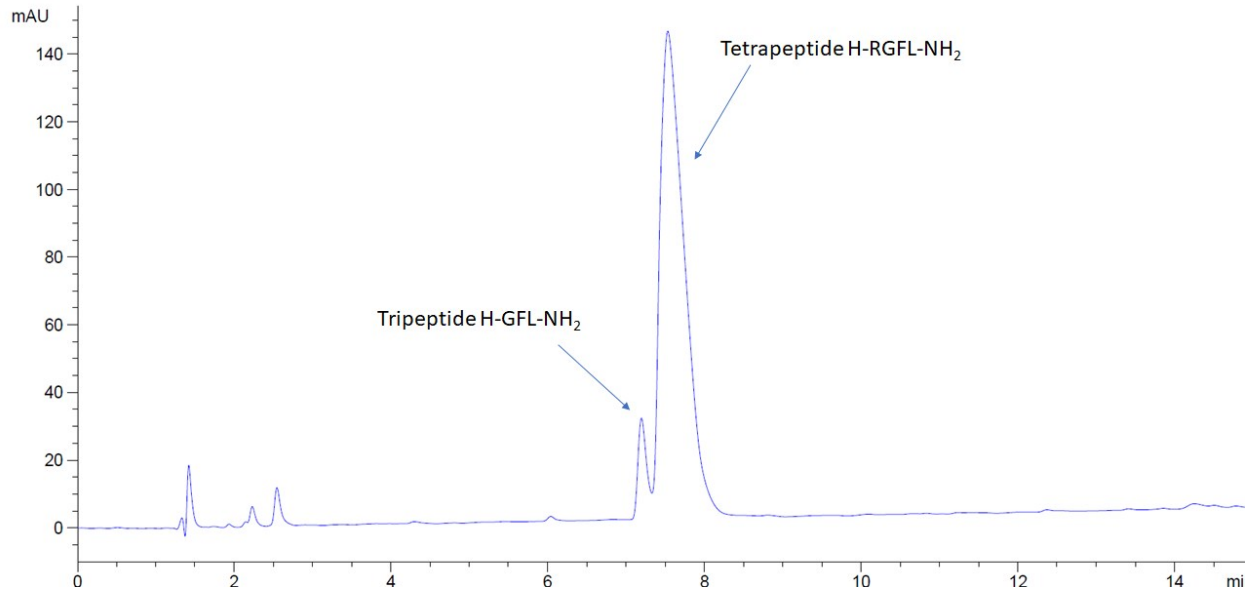
HPLC-S18. Coupling quantification of Tripeptide (H-GFL-NH_2) *vs* Tetrapeptide (H-RGFL-NH_2), Fmoc-Arg(Pbf)-OH : DIC : OxymaPure 1.5 eq. x [1:1:1], RT, NBP.



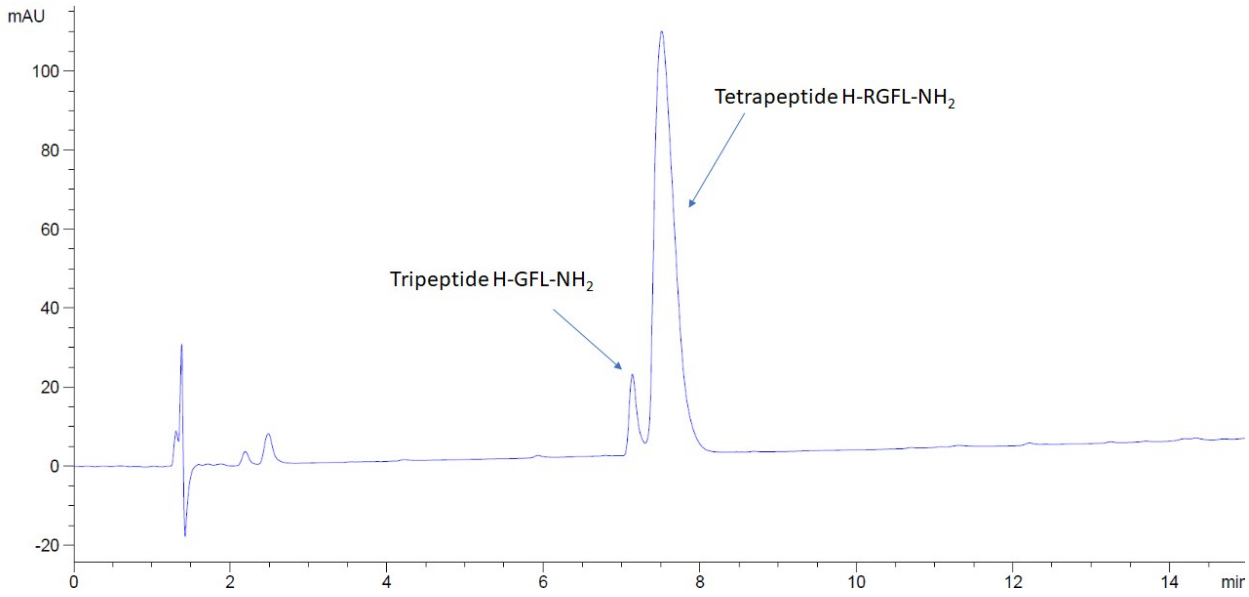
S19. Coupling quantification of Tripeptide (H-GFL-NH_2) *vs* Tetrapeptide (H-RGFL-NH_2), Fmoc-Arg(Pbf)-OH : DIC : OxymaPure 1.5 eq. x [1:1:1], 45 ºC, NBP.



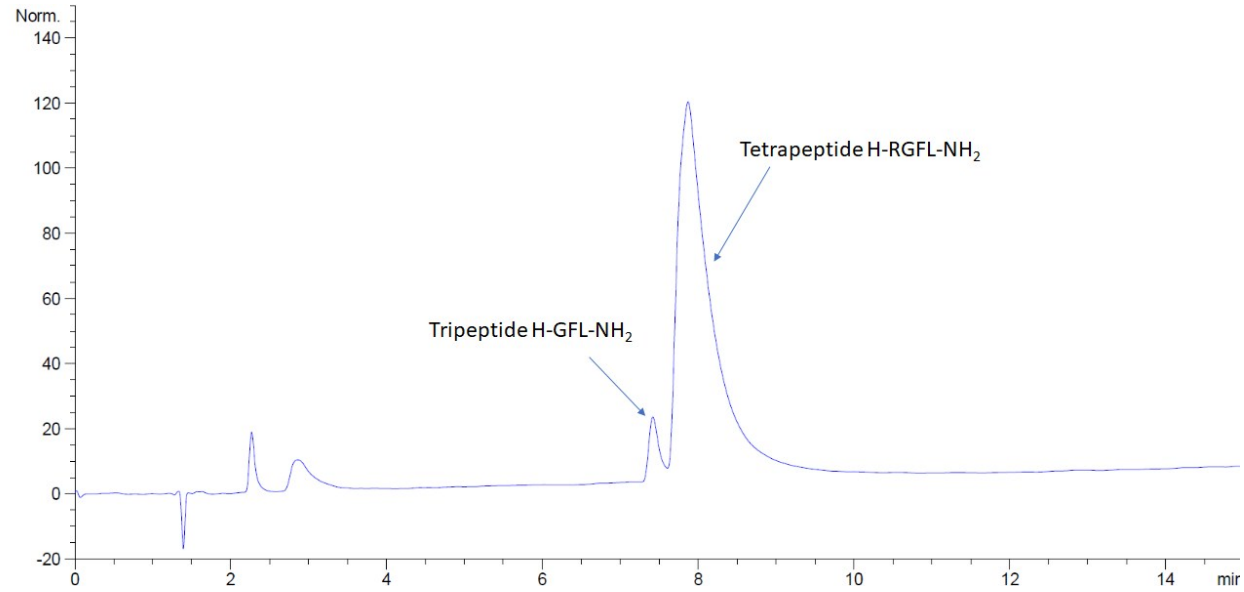
HPLC-S20. Coupling quantification of Tripeptide (H-GFL-NH_2) *vs* Tetrapeptide (H-RGFL-NH_2), Fmoc-Arg(Pbf)-OH : DIC : OxymaPure 1.5 eq. x [1:1:1], 60 ºC, NBP.



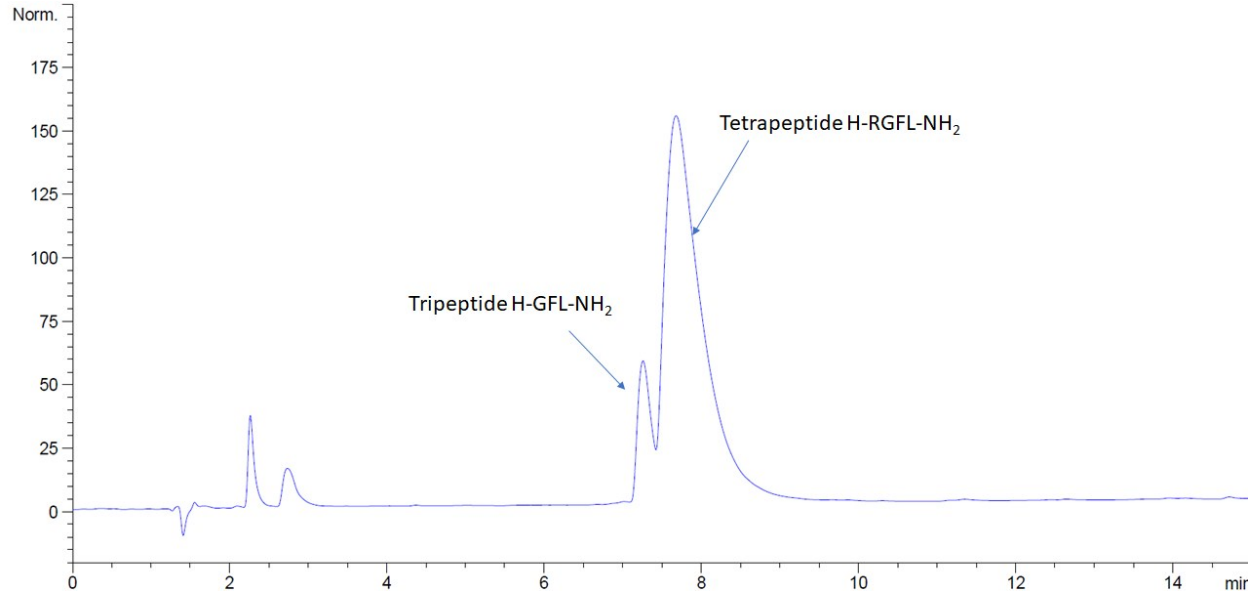
HPLC-S21. Coupling quantification of Tripeptide (H-GFL-NH_2) *vs* Tetrapeptide (H-RGFL-NH_2), Fmoc-Arg(Pbf)-OH : DIC : OxymaPure 1.5 eq. x [1:1:1], 45 ºC, NBP, Pre-conditioning strategy.



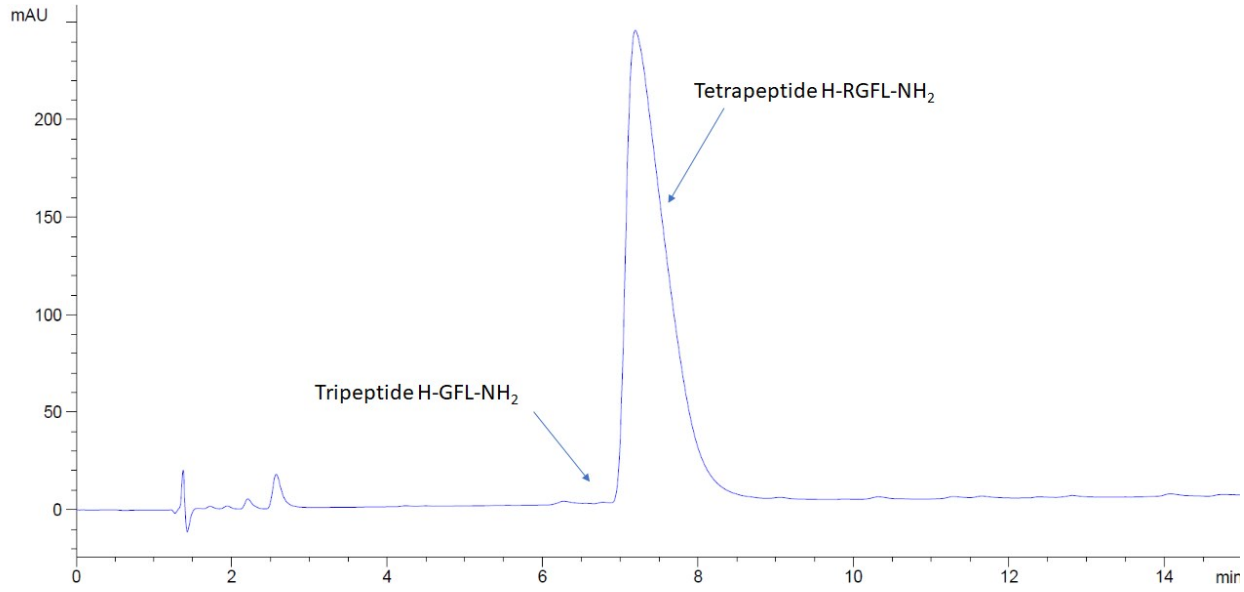
HPLC-S22. Coupling quantification of Tripeptide (H-GFL-NH_2) *vs* Tetrapeptide (H-RGFL-NH_2), Fmoc-Arg(Pbf)-OH : DIC : OxymaPure 1.5 eq. x [1:1:1], 45 ºC, NBP, In situ Activation, 0.15M.



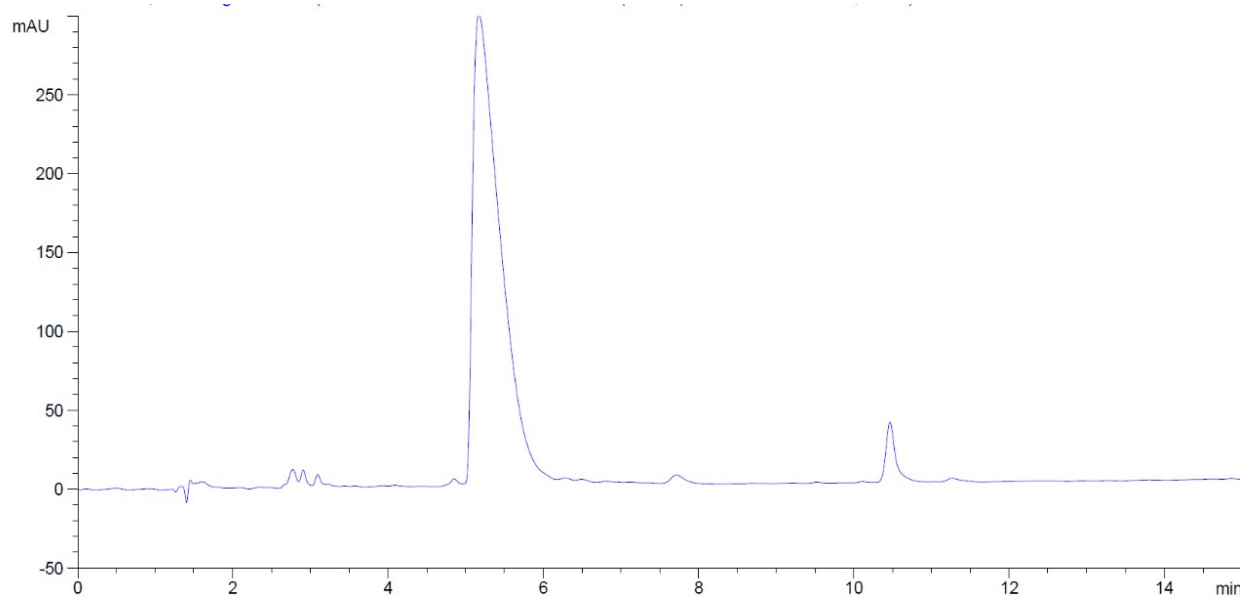
HPLC-S23. Coupling quantification of Tripeptide (H-GFL-NH_2) *vs* Tetrapeptide (H-RGFL-NH_2), Fmoc-Arg(Pbf)-OH : DIC : OxymaPure 1.5 eq. x [1:1:1], 45 ºC, NBP, In situ Activation, 0.075M.



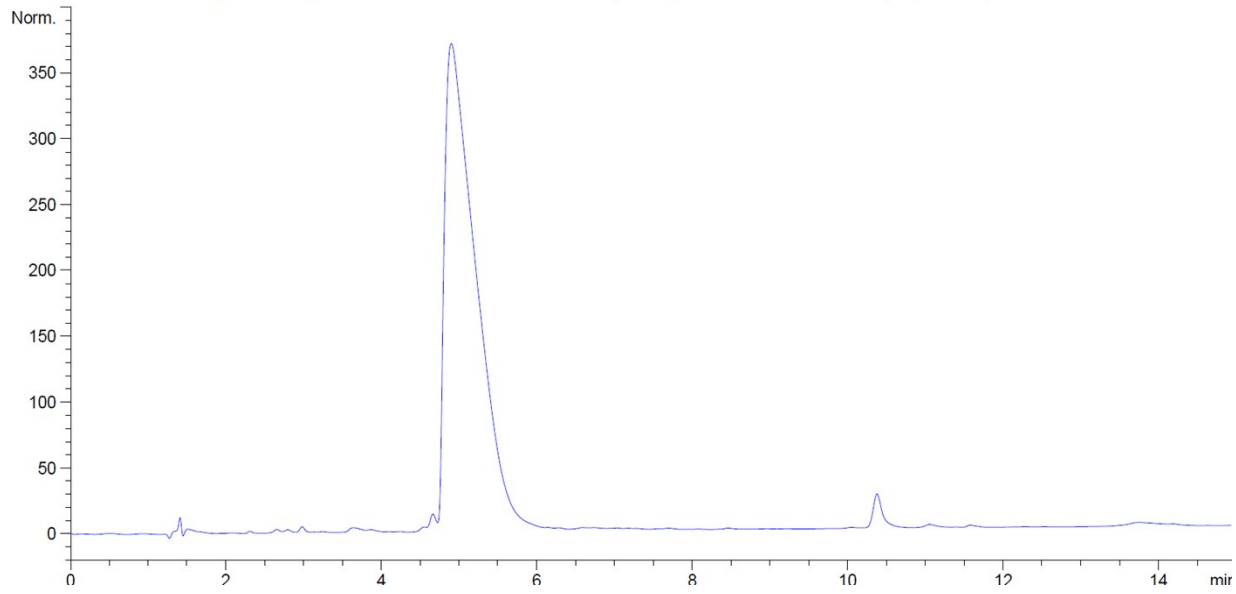
HPLC-S24. Coupling quantification of Tripeptide (H-GFL-NH_2) *vs* Tetrapeptide (H-RGFL-NH_2), Fmoc-Arg(Pbf)-OH : DIC : OxymaPure 1.5 eq. x [1:1.2:1], 45 ºC, NBP, In situ Activation, 0.075M, half DIC at 0 min + half DIC and 0.25 eq Fmoc-Arf(Pbf)-OH at 30 min.



HPLC-S25. SPPS of Linear precursor of RGD Peptide (H-DfKRG-OH) in DMF



HPLC-S26. SPPS of Linear precursor of RGD Peptide (H-DfKRG-OH) in NBP



HPLC-S27. Des-Arg Peptide (H-DfKG-OH) in DMF