Eco-Friendly SPPS: overcoming challenges in cosmetic Argireline production

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Introduction

The growing demand of peptide based active ingredients has highlighted the need to the reduce the environmental impact of cosmetic products. TFA which is currently used in SPPS to both detach the peptide form the resin and remove the protecting groups present on the amino acids side chain, is a highly volatile and corrosive ultra-short-chain per- and polyfluoroalkyl substance (PFAS), a class of persistent "forever chemicals". PFAS are characterized by exceptionally stable C–F bonds and increasing environmental and health concerns, therefore, the development of fluorine-free and more sustainable alternatives to TFA represents an important challenge for the future production of cosmetic-grade peptides.

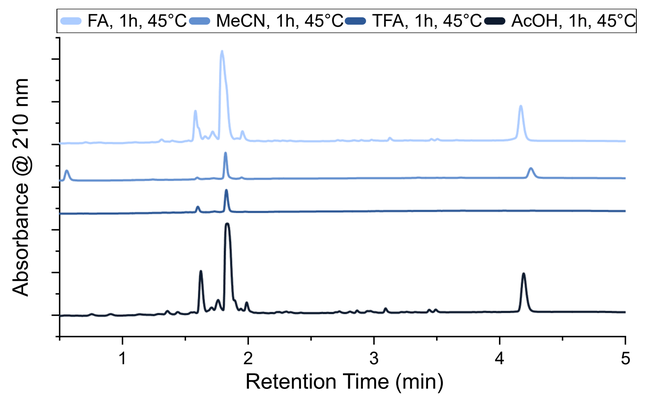
In this work, greener SPPS strategies are investigated using as proof-of-concept a commercially relevant cosmetic peptide Argireline (**Ac-EEMQRR-NH_2**). Ultimately, the study aims to develop scalable, environmentally compatible SPPS protocols that reduce PFAS-related impacts while maintaining the quality and performance required for cosmetic applications.

The rapid rise in demand for peptides for "well-being" is facing a critical gap: Europe lacks an integrated and organized network for the entire peptide life cycle from design and green synthesis to characterization, biological evaluation, and sustainable manufacturing. EUniWell institutions hold complementary, world-class technologies, but their potential remains underutilized without a structured framework to share an European "infrastructure without walls" with specialized scientific know-how and skills. Overcoming this fragmentation will contribute to support the development of the next generation of scientists, equipping them with the advanced skills including entrepreneurial drive needed to successfully translate lab discoveries into market-ready peptide innovation for wellness.

Results: green cleavage "cocktail"

The influence of solvent

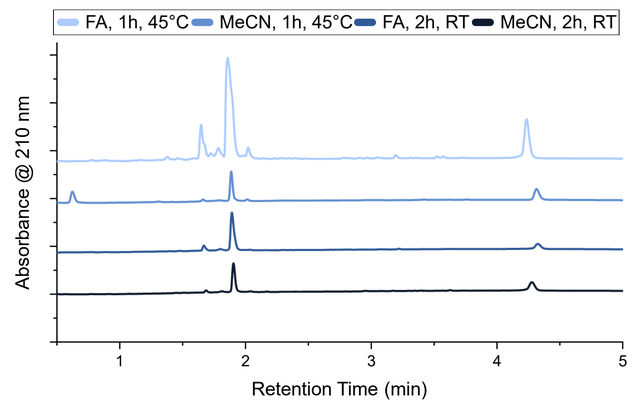
Sample	Conditions	Time; T °C	Argireline t_R= 1.8 min HPLC crude Purity (%)	t-Butylated Argireline HPLC crude Purity (%)	Glutarimide HPLC crude Purity (%)
A	30% MSA, 4% HCl (37%), 10% Thioanisole, 2.5% TIS, 53.5% Formic Acid.	1h; 45°C	72.30	13.73	3.59
B	30% MSA, 4% HCl (37%), 10% Thioanisole, 2.5% TIS, 53.5% Acetonitrile.	1h; 45°C	74.97	6.21	5.54
C	95% TFA, 2.5% TIS, 2.5% H_2O.	1h; 45°C	72.48	18.21	1.14
D	30% MSA, 4% HCl (37%), 10% Thioanisole, 2.5% TIS, 53.5% Acetic Acid.	1h; 45°C	65.81	12.44	2.78



RP-UHPLC/MS analysis carried out on H Class Acquity UPLC from Waters, with an Acquity PDA detector and a Acquity QDa MS detector using Accura Triart C18 (50x2.1 mm, 1.9 µm, 12 nm) column; temperature 40 °C; flow, 0.5 mL/min; eluent, 0.1% (v/v) TFA in H_2O (A) and 0.1% (v/v) TFA in CH3CN (B); λ, 210 nm, gradient, 00–70% B in 5 min.

The influence of time and temperature

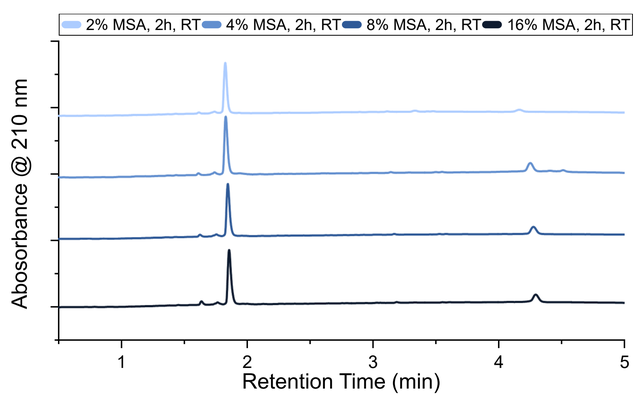
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A	30% MSA, 4% HCl (37%), 10% Thioanisole, 2.5% TIS, 53.5% Formic Acid.	1h; 45°C	72.30	13.73	3.59
B	30% MSA, 4% HCl (37%), 10% Thioanisole, 2.5% TIS, 53.5% Acetonitrile.	1h; 45°C	74.97	6.21	5.54
E	30% MSA, 4% HCl (37%), 10% Thioanisole, 2.5% TIS, 53.5% Formic Acid.	2h; r.t.	78.83	10.30	1.44
F	30% MSA, 4% HCl (37%), 10% Thioanisole, 2.5% TIS, 53.5% Acetonitrile.	2h; r.t.	77.59	5.25	3.43



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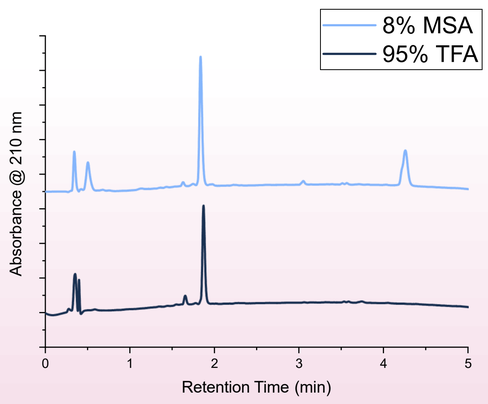
The influence of Methanesulfonic acid (MSA) quantity

Sample	Conditions	Time; T	Argireline t_R= 1.8 min HPLC crude Purity (%)	t-Butylated Argireline HPLC crude Purity (%)	Glutarimide HPLC crude Purity (%)
G	2% MSA, 4% HCl (37%), 10% Thioanisole, 2.5% TIS, 81.5% Formic Acid.	2h; r.t.	86.54	1.77	-
H	4% MSA, 4% HCl (37%), 10% Thioanisole, 2.5% TIS, 79.5% Formic Acid.	2h; r.t.	85.10	2.13	-
I	8% MSA, 4% HCl (37%), 10% Thioanisole, 2.5% TIS, 75.5% Formic Acid.	2h; r.t.	87.72	3.03	-
J	16% MSA, 4% HCl (37%), 10% Thioanisole, 2.5% TIS, 67.5% Formic Acid.	2h; r.t.	83.37	5.19	1.46



RP-UHPLC/MS analysis carried out on H Class Acquity UPLC from Waters, with an Acquity PDA detector and a Acquity QDa MS detector using Accura Triart C18 (50x2.1 mm, 1.9 µm, 12 nm) column; temperature 40 °C; flow, 0.5 mL/min; eluent, 0.1% (v/v) TFA in H_2O (A) and 0.1% (v/v) TFA in CH3CN (B); λ, 210 nm, gradient, 00–70% B in 5 min.

Results: full green synthesis and green cleavage



Instrument: PurePep® Chorus. Scale: 50 µmol. Synthesis: 3 eq. of AAs + 3 eq. of Oxyma dissolved in BuOAc/DMSO (7:3) at 0.5 M concentration; 4 eq. of TBEC in BuOAc at 1.33 M concentration. Washing Solvent: EtOAc/DMSO (8:2). Capping Mixture: Ac_2O/NMM/EtOAc (25:25:50). Deprotection: 20% 4-Methylpiperidine in EtOAc/DMSO (4:6).

Sample	Conditions	Time; T	HPLC crude Purity (%)	Crude Yields (%)
L	8% MSA, 4% HCl (37%), 10% Thioanisole, 2.5% TIS, 75.5% Formic Acid	2h; r.t.	88.30	84.70
M	95% TFA, 2.5% TIS, 2.5 % H_2O	2h; r.t.	85.70	48.50

RP-UHPLC/MS analysis carried out on H Class Acquity UPLC from Waters, with an Acquity PDA detector and a Acquity QDa MS detector using Accura Triart C18 (50x2.1 mm, 1.9 µm, 12 nm) column; temperature 40 °C; flow, 0.5 mL/min; eluent, 0.1% (v/v) TFA in H_2O (A) and 0.1% (v/v) TFA in CH3CN (B); λ, 210 nm, gradient, 00–70% B in 5 min.

Conclusion

- A greener SPPS strategy for Argireline has been successfully developed using alternative solvents and reagents.
- MSA-based cleavage proved to be a promising PFAS-free alternative to conventional TFA, providing high crude purity while minimizing side products.
- These results demonstrate the feasibility of more sustainable SPPS approaches for cosmetic-grade peptides that can be translated into market-ready peptide for wellness.

Acknowledgments

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References

[1] G. Vivenzio, S. Noki, A. Chakraborty, J. Lopez, B.G. De La Torre, F. Albericio, ChemSusChem 18 (2025) e202402752.
[2] L. Pacini, M.K. Muthyala, R. Zitterbart, O. Marder, P. Rovero, A.M. Papini, Int. J. Pept. Res. Ther. 31 (2025) 43.
[3] L. Pacini, M. Muthyala, L. Aguiar, R. Zitterbart, P. Rovero, A.M. Papini, J. Pept. Sci. 30 (2024) e3605.
[4] F. Fidha, A. Kumar, M. Leko, O. Marder, S. Burov, A. Sharma, B.G. De La Torre, F. Albericio, Green Chem. 27 (2025) 14911–14918.

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