

Rapid Process Development of Peptide Production via Microwave-Enhanced SPPS: A Case Study

Dewey Sutton | AmbioPharm, Inc.

Target Sequence:

Ac-(AA)¹-(AA)²-EGKASA8TTATVT14KGS-(AA)¹⁸-(AA)¹⁹-NH₂

- 19 AA Residue Sequence
- N-terminal acetylation, C-terminal amide, ca. 1700 Da
- Difficult hydrophobic stretch between residues A8-T14

Client Criteria

- Required Quantity: 500 mg
- Required Purity ≥ 99%, Acetate salt
- Timeline: 2 weeks

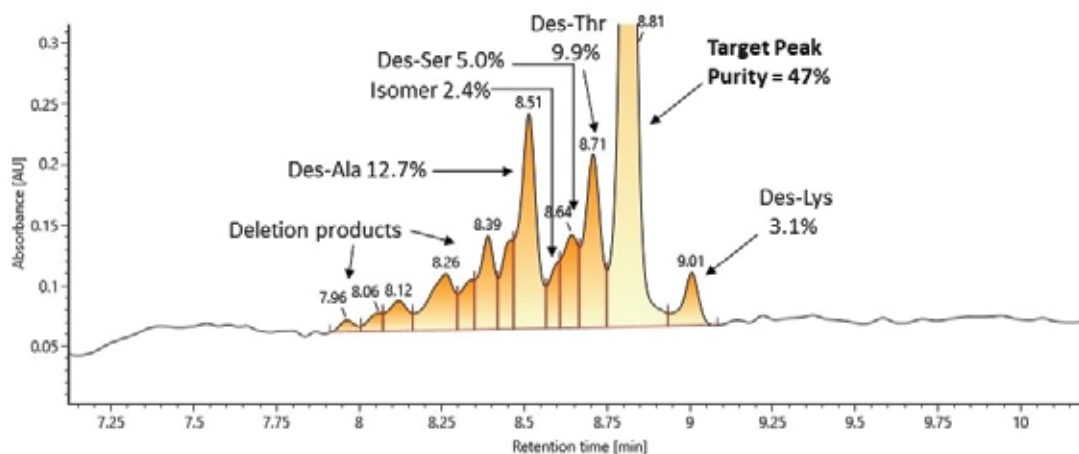


CEM Liberty Blue MW Synthesizer with HT12 Resin Loader*

Conventional Synthesis

(AA)¹-(AA)²-EGKASATTATVTKGS-(AA)¹⁸-(AA)¹⁹-NH₂

1 mmol on Tentagel Rink Amide LL Conventional Synthesis (3 h couplings) 5-5-5 eq. AA-DIC-Oxyma



Key Takeaways

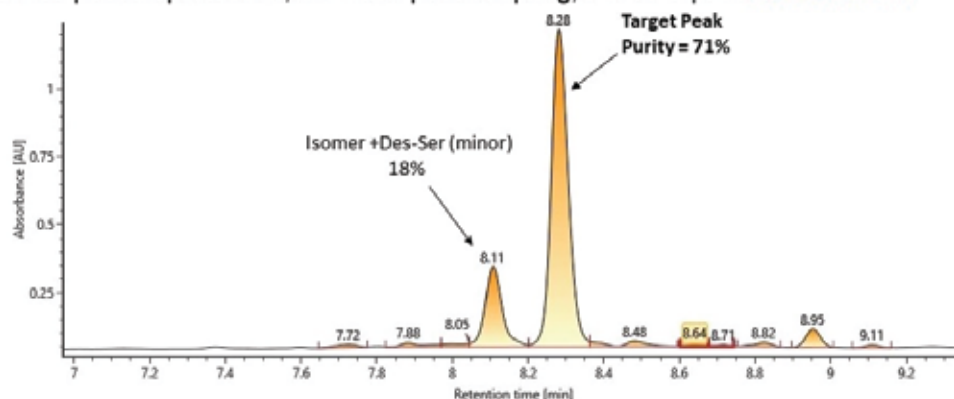
- Low overall purity with conventional synthesis
- Deletion Impurities Predominant
- 60 h run time

*Photo courtesy of CEM Corporation. https://cem.com/media/wysiwyg/liberty_series/liberty_blue_ht12_product_image.png

MW-Enhanced Synthesis with Tentagel Resin and 50°C Coupling



1 mmol on **Tentagel**-Rink Amide LL, MW Synthesis: 1 x 10 min 50°C Deprotection, 1 x 20 min **50°C Coupling**, 4 x DMF washes post-Deprotection, 1 x wash post-coupling, 5-5-10 eq. AA-**HATU-DIPEA**



Key Takeaways

- Lower overall purity with 20 min 50°C couplings compared to 12 min 75°C couplings
- Endo Impurities were markedly decreased
- More deletion impurities present with lower temperature couplings
- Reducing coupling temperature did not reduce isomer content in HATU synthesis product
- (up to 40 L scale)

Conclusions

	Time (h)	Crude Purity (%)
Conventional	60	47
MW Enhanced	6	80

- High Throughput afforded by microwave-enhanced synthesis allowed 16 synthetic conditions to be evaluated in less than two weeks
- MW heating helps disrupts aggregation and affords access to otherwise intractable sequences
- Results with CEM Liberty Blue are directly scalable to CEM Liberty Pro synthesizer (up to 40 L scale)