Peptide Production from mg to kg with Automation and Microwave Assisted Heating

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Abstract

Peptide therapeutics are an attractive alternative to their small molecule drug counterparts [1]. With several high revenue peptide drugs on the market and a pipeline full of potential candidates [2], the demand for highly robust and efficient synthetic methods is of great importance. Microwave assisted SPPS has established itself as the primary chemical method to produce high quality peptides while drastically reducing synthesis time and waste [3]. This poster will highlight new R&D and technology which encompasses GMP peptide production from mg to kg scale.

On the R&D scale, our research team has developed a standardized methodology for synthesis of varied peptides, providing a powerful tool that simplifies peptide therapeutic research. To validate this comprehensive strategy that utilizes microwave (MW) heating and rapid automation, a variety peptides from current literature were linearly synthesized. Key features of this methodology include:

- Improved carbodiimide coupling which increases purity and suppresses side reactions such as epimerization
- One-pot coupling and deprotection process for increased efficiency
- Reduction of up to 95% generated waste compared to conventional methods

Rapid scale-up for clinical trials and peptide production has been accomplished using similar technology with a focus on elevated temperatures. Crude purity from R&D to production scale is preserved if not improved and unwanted side reactions such as epimerization and aspartimide formation are easily controlled. The result, easier purification and reduced labor cost. Cycle times at the production scale range from 10 – 60 min with the capability to produce up to 1 kg crude peptide in a single batch. Several examples, including process development, will be presented.

Performance: Liberty PRIME™

- Automated, sequential SPPS
- Patents SPPS methodology
  - Suppressed epimerization
  - One-pot deprotection-coupling
  - 95% reduction in waste

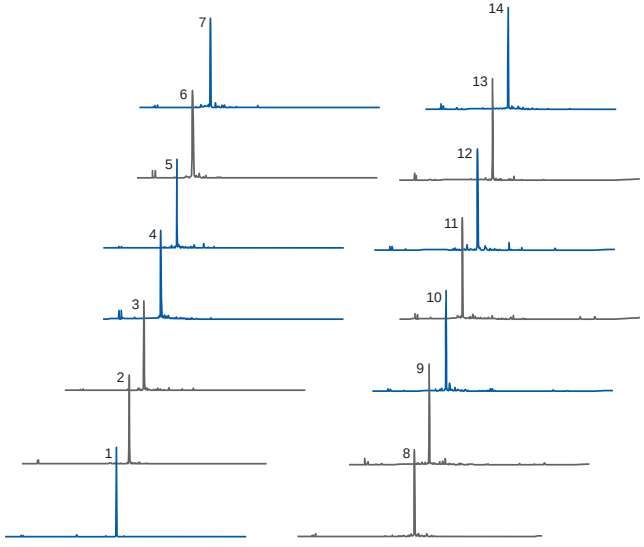
Discussion

- The validation of the protocol was tested on several peptides from a Phase I trial by Terasaki and co-workers [4].
- The peptides were run at 0.4 mmol (0.40 mmol/g Rink amide AM PS resin) scale in sequential fashion using the PRIME™ methodology.

#	Peptide	Sequences	Purity (UPLC %)
1	EGFR-800	DYVREHKDNI	100
2	EZH2-735	KYVGIEREM	97
3	Lck-208	HYTNASDGL	88
4	Lck-486	TFDYLRSVL	90
5	Lck-488	DYLRSVLEDF	85
6	MRP3-503	LYAWEPSFL	92
7	MRP3-1293	NYSVRYRPGL	81
8	PAP-213	LYCESVHNF	89
9	PSA-248	HYRKWIKDTI	85
10	PSMA-624	TYSVSFDSL	80
11	PTH-rP-102	RYLTQETNKV	86
12	SART2-93	DYSARWNEI	88
13	SART2-161	AYDFLYNYL	90
14	SART3-109	VYDYNCHVDL	89

- **Total time = 12 hr, 15 min**
- **Total waste = 3.08 L**

UPLC Analysis of a Variety of Crude Peptides for Personalized Vaccination

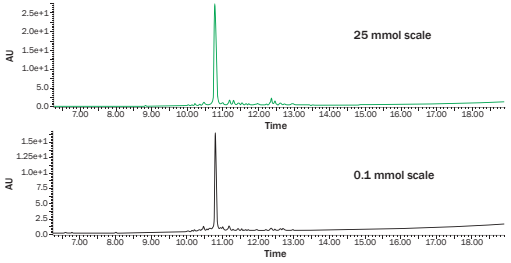


Peptide Production: Liberty Pro

- Automated, up to 15 sequential couplings
- Remarkable scalability from R&D scale to production
- 3 L, 8 L, 15 L reactor size – up to 1 kg of crude peptide in single batch
- cGMP production of APIs

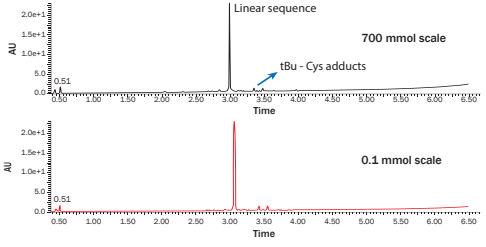
Case Study 2

- 25 mmol production (12 hours)
- >30 amino acids
- 0.35 mmol/g Wang PS
- Reagents: 4.0 fold excess



Case Study 3

- 9mer
- 700 mmol production (9 hours)
- 0.75 mmol/g Rink amide AM PS resin
- Reagents: 2.0 fold excess



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[3] J. M. Collins, K. A. Porter, S. K. Singh, G. S. Vanier, *Org. Lett.* **2014**, *16*(**3**), 940-943
[4] R. Takahashi et al. *Breast Cancer Res.* **2014**, *16*(**4**), R70