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

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Abstract

Peptides are ideal drug candidates due to their inherent high potency, low toxicity, and their ability to effect a broad range of targets [1]. With several high revenue peptide drugs on the market and a full pipeline of potential candidates [2], the demand for a highly robust and effective synthetic method is of great importance [3]. Currently, peptide synthesis research and production both face similar challenges – a sluggish and wasteful workflow in desperate need for optimization. The typical conventional optimization steps usually take a shotgun approach: screen resins, screen different reagent excesses, screen activators, etc. This synthetic process necessitates tens or hundreds of reactions, all of which can often take weeks or months to complete while requiring a great deal of time, money, and resources. To address the needs of the market, new cGMP methodology has been developed utilizing automation and microwave assisted heating. This work details mechanistic-based, innovative improvements to the chemical methodology of solid phase peptide synthesis, application of these improvements to high-throughput SPPS for personalized medicine via peptide vaccines [4,5] and large scale peptide production with cGMP considerations.

Discussion

- ♦ Enhanced carbodiimide coupling method
  - Low levels of epimerization
  - Less base induced side-reactions
  - High temperature stability
  - High crude puirty
- ♦ Chemical entities to reduce side reactions
  - Fmoc-Asp(OMpe) suppresses aspartmimide formation
  - Fmoc-His(boc)-OH vastly out preforms His(trt) in Fmoc SPPS
- ♦ Automation and methodology compatible with cGMP standards
  - 21 CFR 11
  - Risk analysis
  - Temperature validation

High-Throughput Peptide Production

- 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.
- Long difficult sequence chosen to demonstrate versatility and robustness.
- Automated sequencial SPPS
- 95% reduction in total waste
- High-throughput: 24 peptides in 24 hours

Performance: Liberty PRIME™

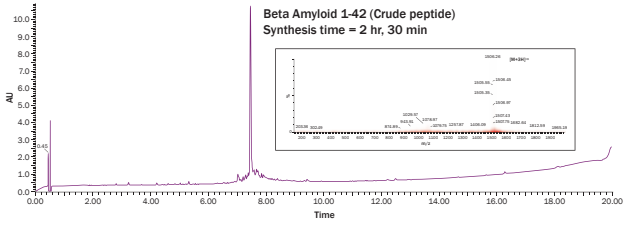
Liberty PRIME™ Peptide Synthesizer

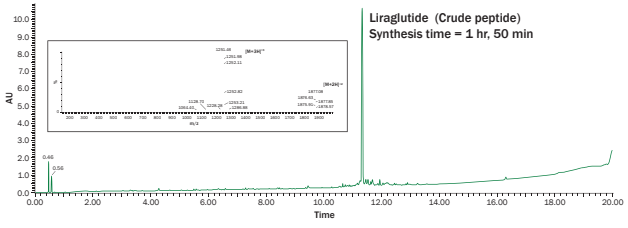
Peptides for Personalized Vaccination

#	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**

Synthesis of Difficult Sequences (UPLC-MS)



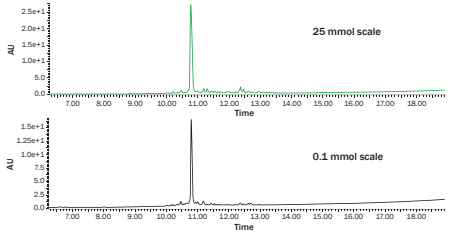


Peptide Production: Liberty PRO™

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

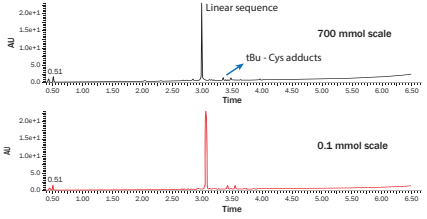
Case Study 1

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



Case Study 2

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



[1] D. J. Craik, D. P. Fairlie, S. Liras, D. Price. *Chem Biol Drug Des*, **2013**, *81*, 136; [2] K. Fosgerau, T. Hoffman. *Drug Discov Today*, **2015**, *20*, 122; [3] J. M. Collins, K. A. Porter, S. K. Singh, G. S. Vanier. *Org. Lett.* **2014**, *16*, 940; [4] P. A. Ott et al. *Nature*, **2017**, *547*, 217; [5] R. Takahashi et al. *Breast Cancer Research*, **2014**,16, R70.