Reduced Cycle Times & Solvent Consumption of $^{65\text{-}74}$ACP on *Symphony® X*

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INTRODUCTION

New and optimized methods for rapid peptide synthesis, combined with instruments capable of parallel synthesis enable the production of peptide libraries with high throughput.[1]

Optimization of synthesis protocols is explored, using the *Symphony X* parallel synthesis platform, to reduce cycle times and decrease solvent consumption and waste generation by removing DMF washes after coupling steps.

METHODS & ANALYSIS

The $^{65\text{-}74}$ACP peptides were synthesized on the *Symphony X* peptide synthesizer at 20 µmol scale using Fmoc-Gly-Wang resin (loading 0.32 mmol/g). Deprotection was performed twice with 20% piperidine in DMF for 2.5 min at RT. Washes: DMF 6 x 30 sec. Couplings were performed at a final concentration of 100 mM AA (10 eq.), 100 mM HCTU (10 eq.) and 200 mM NMM (20 eq.) for 2 x 5 min at RT. Cleavage cocktail used was TFA/Anisole/H_2O/EDT and the reaction was performed for 2 h at RT. Triplicates were performed for each peptide.

The resulting crude peptide was dissolved in water and analyzed on a Varian ProStar HPLC using a C18, 180 Å, 5 um, 250 x 4.6 mm column (Agilent Polaris), over 60 minutes with a flow rate of 1 mL/min, and using a gradient of 5-95% B, where Buffer A is 0.1% TFA in water, and Buffer B is 0.1% TFA in acetonitrile. Detection was at 214 nm. Mass analysis was performed on a Shimadzu LCMS-2020 Single-Quad mass spectrometer, equipped with a C18, 100 Å, 2.6 um, 50 x 2.1 mm column (Phenomenex Kinetex), over 7 min with a flow rate of 1 mL/min and using a gradient of 5-50% B where Buffer A is 0.1% formic acid in water and Buffer B is 0.1% formic acid in acetonitrile

REFERENCES

Chan, W., White, P. Eds.; Fmoc Solid Phase Synthesis – A Practical Approach. Oxford University Press: New York, NY, 2000.

RESULTS

The synthesis of $^{65\text{-}74}$ACP was optimized by reducing the number of washes after coupling. Eliminating post-coupling washes reduced cycle times from 2.8 to 2.3 minutes and solvent consumption from 55 to 41 mL without significantly affecting the purity of the final peptides (Table 1).

Number of Washes	Solvent Consumption	Effective Cycle Time	Purity
	55 mL	2.8 min	84.9%
	50 mL	2.7 min	86.8%
	46 mL	2.5 min	85.1%
	41 mL	2.3 min	84.8%

Table 1. Effect of wash protocol on peptide purity, cycle times and solvent consumption. Peptide Sequence: VQAAIDYING

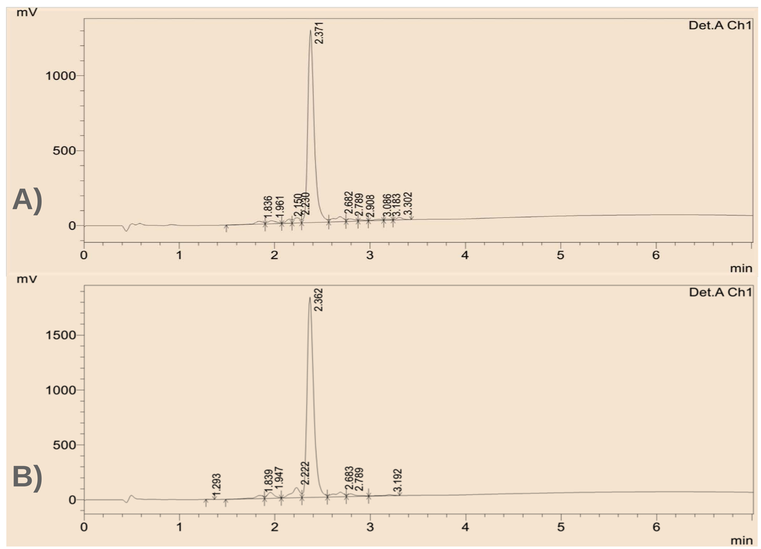

Figure 1. HPLC traces for $^{65\text{-}74}$ACP with:
(A) No DMF washes after coupling steps
(B) 3 DMF washes after coupling steps

CONCLUSION

Eliminating post-coupling washes provides:

- 20% Reduction of overall cycle times
- 25% Reduction in overall solvent consumption
- Minimal change in purity
- Overall improvement in time and cost of peptide synthesis

SYMPHONY X

- 24 Reaction vessels
- Preactivation chemistry
- IR heating
- UV monitoring
- Single Shot™ additions