Preparative Scale Purification of Bradykinin by Concentration Overload

Application Note

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Introduction

Manufacture of synthetic peptides ranges from mg to multi-kg amounts. Where a peptide is a biopharmaceutical API candidate the amount required will increase as it moves through clinical trials to product. When developing purification methods the required "product" API, quantity and purity, should be considered.

Bradykinin, a physiologically and pharmacologically active peptide of the kinin family is used in the development of antagonists and therapies for hereditary angioedema. The amino acid sequence of bradykinin is: Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg.

In this report, preparative scale purification of a sample of synthetic bradykinin using a concentration overload regime is described. Details of the initial method development steps on an analytical HPLC column are also included as this was required to develop the separation. For comparison, a second sample of bradykinin was purified using volume overload, details of which are included in a second application note (5990-7741EN).

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Materials and Reagents

Sample Preparation

A StratoSpheres PL-Rink resin was used for the solid phase synthesis of a crude quantity of bradykinin. 1 mg/mL solutions of the crude peptide were then used for the initial screening work.

Mobile Phase Preparation:

Eluent A:	0.1% TFA in 1% ACN:99% water
Eluent B:	0.1% TFA in 99% ACN:1% water
Flow Rate:	1 mL/min
Detection:	UV at 220 nm

Method Development

Screening of the crude bradykinin was carried out using a high performance PLRP-S 100Å 5 µm 250 x 4.6 mm column and resolution optimized using a linear gradient. The purification costs could be decreased by using a larger particle size media so the screening was repeated using 8 µm and 10 µm columns. Figure 1 shows the separation of the bradykinin on the three columns.

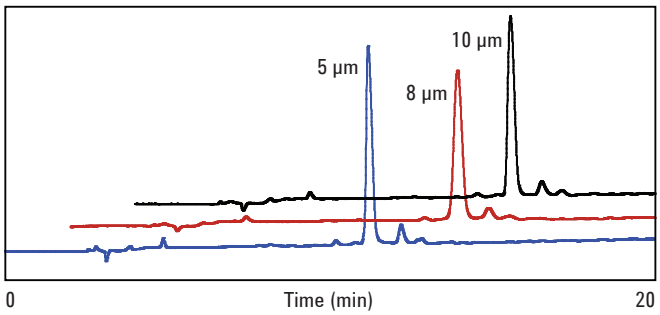

Figure 1. Separation of crude bradykinin, 20 µL of 1 mg/mL solution, on the PLRP-S 100Å, 5 µm, 8 µm and 10 µm 250 x 4.6 mm ID columns. Eluent A: 0.1% TFA in 99:1 water:ACN; eluent B: 0.1% TFA in 1:99 water:ACN; gradient: 10-50% B in 20 minutes at a flow rate of 1.0 mL/min (360 cm/hr).

From this separation it was evident that the 10 µm material could be used for the purification. Bradykinin is a nine amino acid peptide, Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg, and interacts strongly with the PLRP-S material. The impurity peaks elute close to the product peak and therefore it may be possible to use isocratic elution. To assess this option, a plot of ACN content vs retention was produced (Figure 2).

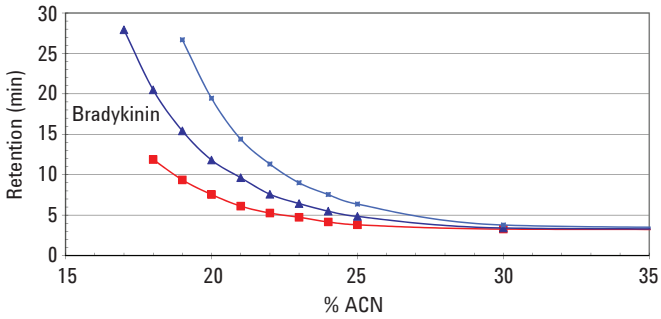

Figure 2. Plot of acetonitrile content vs retention time for bradykinin peptide and the pre- and post- impurities.

From this plot, a concentration of 21% ACN was identified as being the optimum for purification. As before, 5 µm, 8 µm, and 10 µm PLRP-S 100Å columns were all screened to ensure that sufficient resolution remained between bradykinin and its related impurities on the larger particle size material (Figure 3).

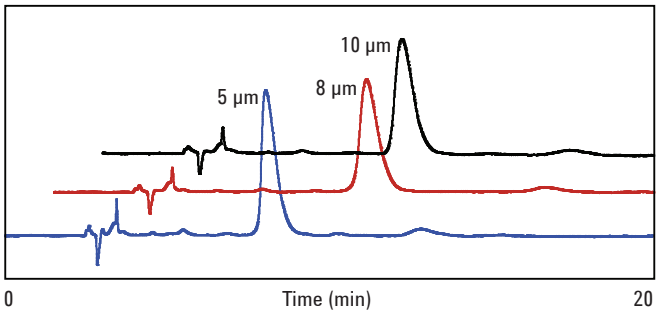

Figure 3. Separation of crude bradykinin, 20 µL of 1 mg/mL solution, on PLRP-S 100Å 5 µm, 8 µm and 10 µm 250 x 4.6 mm ID columns. Isocratic separation using 0.1% TFA in 21% ACN: 79% water at a flow rate of 1 mL/min (360 cm/hr).

These chromatograms indicate that PLRP-S 100Å 10 µm with isocratic elution is suitable for the purification.

Loading Study

Having determined the conditions and media particle size using an analytical column the next stage was a loading study using the PLRP-S 100Å 10 µm 250 x 4.6 mm column. For a 50 mg purification of crude bradykinin a 1 in. dynamic axial compression (DAC/SAC) column was used as it scales to full production. The linear scale up factor from a 4.6 mm ID to a 1 in. (actual ID is 27 mm) Load&Lock (L&L) column is 34 when the length is kept constant at 250 mm. To check the loading capacity of the PLRP-S 100Å 10µm media, a 1.5 mg loading in a 30 µL injection volume (equivalent to 50 mg/mL on a preparative scale, i.e. x 34) was made, as shown in Figure 4.

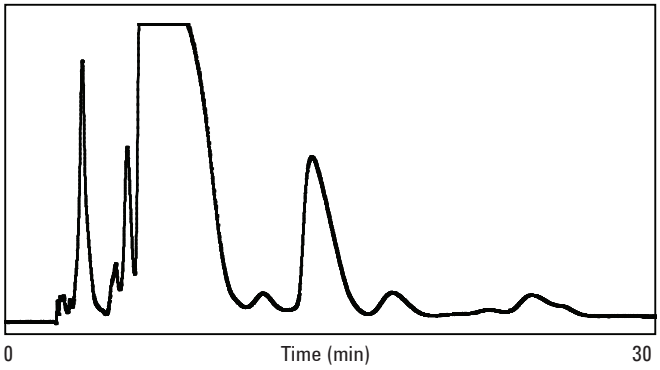

Figure 4. Crude bradykinin prep load, 30 µL containing 1.5 mg on the PLRP-S 100Å 10 µm 250 x 4.6 mm ID column. Isocratic separation using 0.1% TFA in 21% ACN:79% water at a flow rate of 1 mL/min (360 cm/hr).

Sufficient resolution for the purification was achieved with this on-column load.

Packing of a 1 in. L&L Column

For a 250 mm x 1 in. L&L column, 40 g of dry PLRP-S 100Å 10 µm was required. This was dispersed in 175 mL of the packing solvent, 80:20 v/v acetonitrile/water, to give a final slurry concentration of approximately 0.23 g dry PLRP-S per mL of packing solvent.

After mixing on a bottle roller for 30 minutes, the slurry was poured into the assembled column and the piston pressure set to 650 psi (NB: hydraulic pressure set to 260 psi as the compression ratio for a 1 in. L&L is 1:2.5). After packing, the column plunger was locked in the compressed position so that the column could be operated in static axial compression (SAC) mode, the optimum for PLRP-S.

For this application, the efficiency achieved on the 1 in. L&L column, 39,000 ppm and symmetry 1.21, was equivalent to the analytical column, 41,000 ppm and symmetry 1.19. Therefore, when using the same linear velocity a comparable separation would be expected.

Results

As bradykinin is readily soluble in the eluent, a 50 mg load in a 1 mL injection volume can be made to purify the peptides via concentration overload. Figure 5 shows the concentration overload chromatogram.

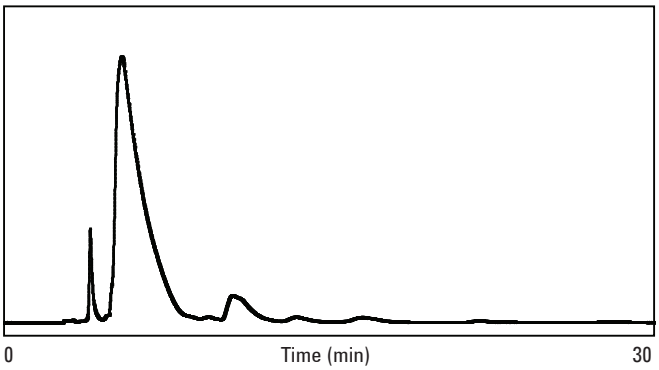

Figure 5. Crude bradykinin separation of 50 mg on-column load. PLRP-S 100Å 10 µm 250 x 27mm ID L&L column. Isocratic separation using 0.1% TFA in 21% ACN: 79% water at a linear velocity 360 cm/hr.

The purification using the concentration overload regime was carried out using fraction collection based on detector response. The analysis of the fractions showed that the peak collection was triggered too late to catch the earlier eluting impurity. Figure 6 shows the results of the fraction analysis.

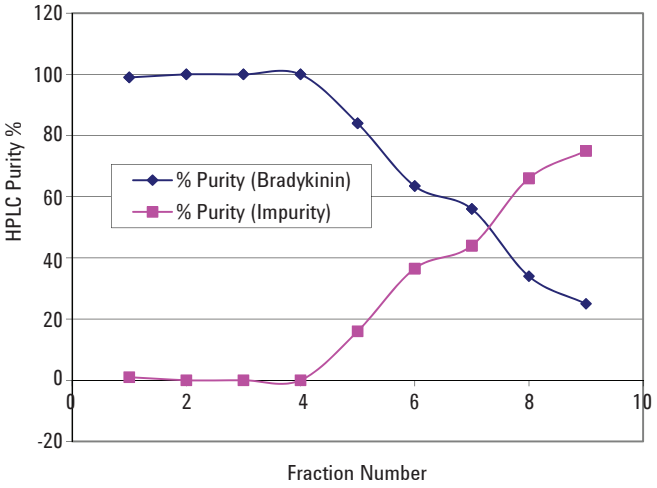

Figure 6. Fraction analysis - the concentration overload purification.

Combining fractions for purity and yield analysis showed that using the concentration overload regime a 100% purity was achieved with a 97% recovery (purity of the crude was 91.7%). For a 100% recovery of bradykinin the purity went down to 99.3%.

Conclusion

Initial screening for a peptide purification can be done using a small particle version of the media which has the same selectivity.

Analytical sized columns packed with the purification material can be used for the loading study.

Bradykinin can be purified using concentration overload with isocratic elution.

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Published in UK, March 21, 2011
5990-7736EN

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