Preparative Scale Purification of Bradykinin by Volume Overload

Application Note

Authors

Stephen Ball, Keeley Mapp and Linda Lloyd
Agilent Technologies, Inc.

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 volume overload regime is described. The initial method development steps on an analytical HPLC column, required to develop the separation, are described in a second application note (5990-7736EN), preparative scale purification of bradykinin by concentration overload.

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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

With volume overload, the bradykinin is purified by loading the crude peptide at a low ACN content, causing concentration of the peptide at the column inlet, followed by a step gradient to the isocratic elution eluent to give the separation.

Firstly, a plot of acetonitrile concentration vs retention time was generated to determine the concentration of ACN required to retain the peptide at the head of the column, see Figure 1.

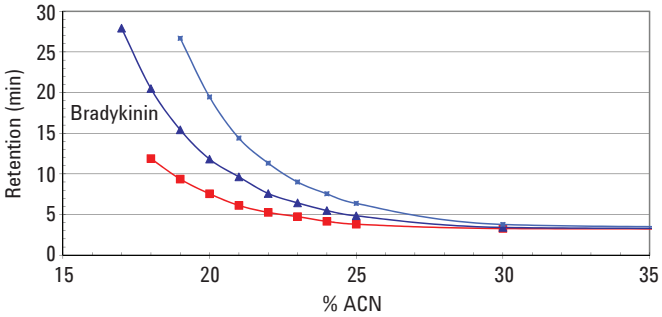

Figure 1. Plot of acetonitrile content vs retention time for bradykinin peptide and the pre- and post- impurities.

From this plot, it is clear that low %ACN concentrations give long retention times for bradykinin.

Packing of 1 in. L&L Column

For a 250 mm x 1in. Load&Lock (L&L) column, 40 g of dry PLRP-S 100Å 10µm 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

For solutes that have limited solubility, purification can be carried out by volume overload. To achieve the required oncolumn load a larger volume of a more dilute solution is used.

To carry out the prep purification by volume overload, the 250 x 1 in. L&L PLRP-S 100Å 10 µm column needed to be preconditioned with 0.1% TFA in 7% ACN: 93% water. Next, 480 mL of a solution containing 50 mg of the crude bradykinin was pumped on to the column, causing concentration of the peptide at the column inlet, followed by a subsequent step gradient to the isocratic elution eluent containing 21% ACN to give the separation. Figure 2 shows the chromatogram obtained.

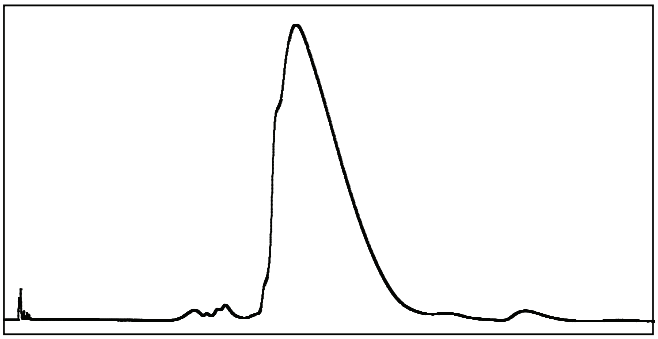

Figure 2. Separation of 50 mg on-column load (480 mL sample volume) of crude bradykinin. PLRP-S 100Å 10 µm 250 x 27 mm ID L&L column. Isocratic separation using 0.1% TFA in 21% ACN:79% water at a linear velocity of 360 cm/hr.

With volume overload there is some broadening of the peak and hence the fractions are more dilute. Fractions were collected on a time base. Figure 3 shows the HPLC chromatograms of three of the fractions and the purity quantitation of all the fractions.

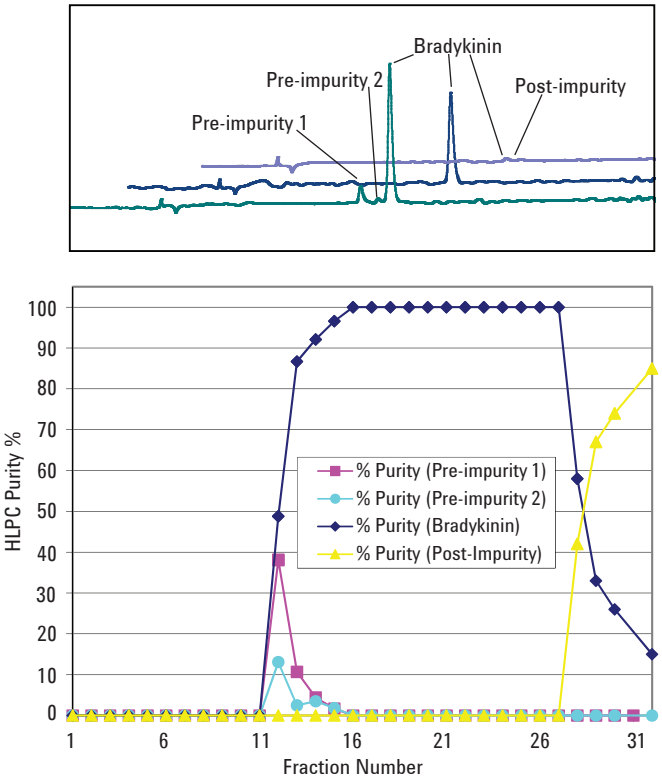

Figure 3. Analysis of fractions 13, 21 and 28 and the purity quantitation for all the fractions.

Combining fractions for purity and yield analysis showed that with volume overload a 100% purity was achieved with a 77% recovery (purity of the crude was 84.6%). For a 100% recovery of bradykinin the purity went down to 77%.

Conclusion

Prep-scale purification of crude peptides can be carried out by volume overload, particularly if solutes have limited solubility.

Volume overload requires isocratic sample concentration oncolumn at low % organic followed by a step gradient to elute the peptide if an isocratic purification method is being used.

The fractions obtained from the volume overload purification are more dilute.

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