

Waters Lab Highlights

LAH 0244 5/85
AN/LS/MD/PR/ST

SEPARATION OF PROTEINS AND PEPTIDES ON μ BONDAPAKTM C₁₈

PART III

This Lab Highlight is the third in a three-part series on protein and peptide separations using a μ BONDAPAKTM C₁₈ column. (For related information see LAH 0242 and LAH 0243). Shown below are several more chromatograms illustrating that even very complex and closely related proteins and peptides can be easily separated using a μ BONDAPAKTM C₁₈ column.

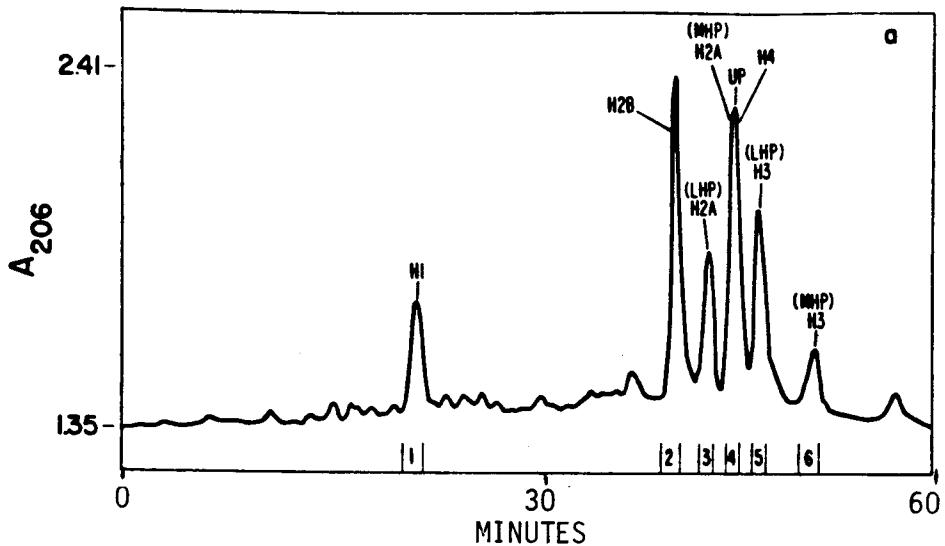


FIGURE 1. Histones separation on a μ BONDAPAKTM C₁₈ column (3.9 X 300 mm) (Ref.1). Chromatographic Conditions: Mobile Phase: 0.3% TFA/aq (A); 0.3% TFA/ACN (B) Linear gradient to 60% B over 60 min. Flow = 1.0 ml/min. Detection at 206 nm. Sample Loading: 140 μ l of histone solution containing 400 - 500 μ g of protein. Peaks are identified as to their class of histone protein.

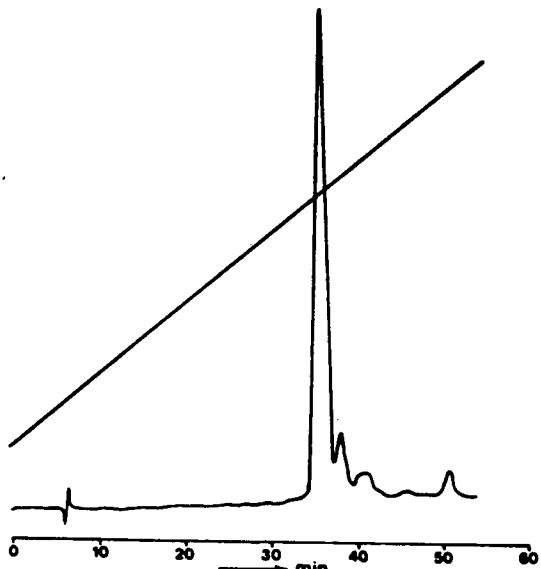


FIGURE 2. Separation of Bovine Insulin on a μ BONDAPAK™ C₁₈ column (3.9 X 300 mm) (Ref. 2). Mobile phase: 0.05 M tetramethylammonium phosphate, pH = 3.0 (TMAP) (A). B is 100% CH₃OH. Gradient: 20% to 80% CH₃OH, over 60 min. Flow = 1.0 ml/min. Temperature: 20°C. Detection: UV at 210 or 280 nm.

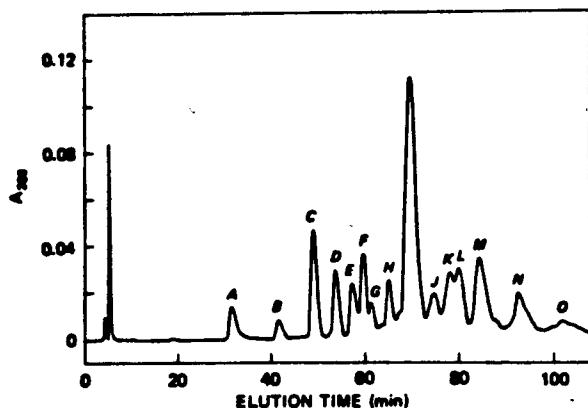


FIGURE 3. Separation of S. Ribosomal Proteins from E. coli on μ BONDAPAK™ C₁₈ (3.9 X 300 mm) (Ref. 3). Mobile Phase: 0.1% TFA/aq (A); 100% ACN (B). Gradient from 20% to 50% (B) in 120 minutes at 0.7 ml/min. Detection at 280 nm. Confirmation of the peaks was also determined by gel electrophoresis.

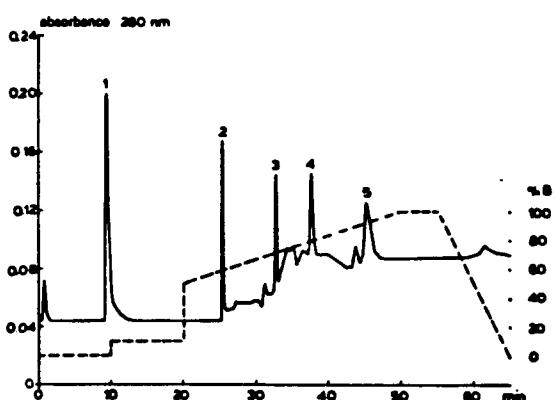


FIGURE 4. HPLC of Kappa Elastin Peptides (Ref. 4). Mobile Phase: 0.01 M Ammonium Acetate pH = 7.8 (A). 80% n-propanol (B). Gradient shown on chromatogram. Flow Rate: 0.5 ml/min. Detection at 280 nm.

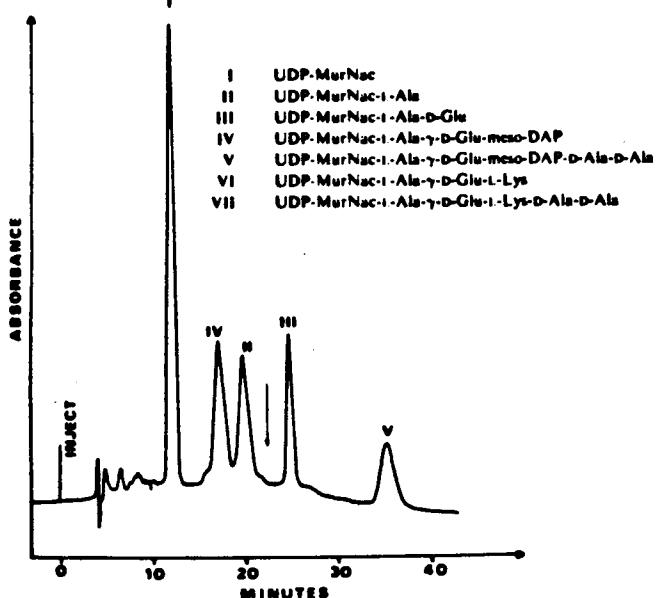


FIGURE 5. Separation of five UDP-MurNac peptide precursors on a μ BONDAPAK™ C₁₈ column (3.9 X 300 mm) (Ref. 5). Mobile Phase: Isocratic elution at room temperature with 0.05 M ammonium phosphate, pH 3.65; amounts ca. 1 nmol of each compound; Detection at 262 nm, 0.02 AUFS; flow rate was changed from 0.5 ml/min to 1.5 ml/min at the time indicated (+).

1. Gurley, L. R., et al., *J. Chromatogr.* 266 (1983) 609-627.
2. Biemond, M. E. F., Sipman, W. A. and Loivie, J., *J. Liquid Chromatogr.* 2 (1979) 1407.
3. Kahan, L., et al., *Anal. Biochem.* 123 (1982) 342-348.
4. Tuy, B. P. D. and Moczar, E., *J. Chromatogr.* 291 (1984) 445-448.
5. Flouret, B., et al., *Anal. Biochem.* 114 (1981) 59-63.