Waters
Lab Highlights

An Internal Communication
of Applications and Techniques

LAH 0243 5/85
AN/LS/MD/PR/ST

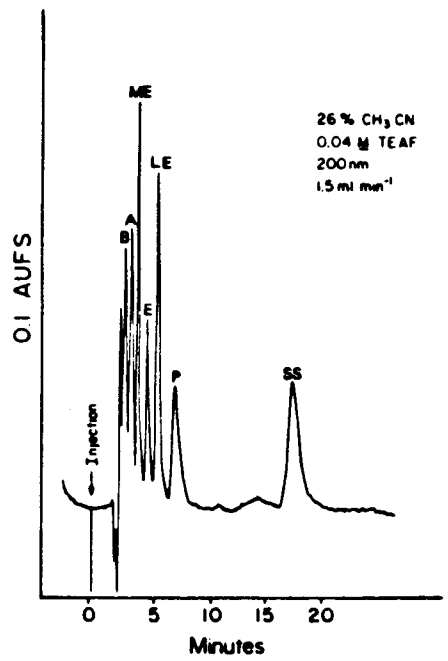
SEPARATION OF PROTEINS AND PEPTIDES ON μBONDAPAK™ C_{18}

PART II

The μBONDAPAK™ C_{18} column is widely recognized as having versatile selectivity and excellent batch-to-batch reproducibility. It is also used extensively in reversed-phase protein and peptide separations, most likely because it has a proportion of wide pores and has the correct "chemistry" of a bonded phase (1). μBONDAPAK™ C_{18} column packing is still the most used packing material as evidenced by the large number of literature citations (2). In order to support these claims, a number of Lab Highlights have been assembled (see LAH 0242 and LAH 0244) to illustrate the use of μBONDAPAK™ C_{18} columns for protein separations.

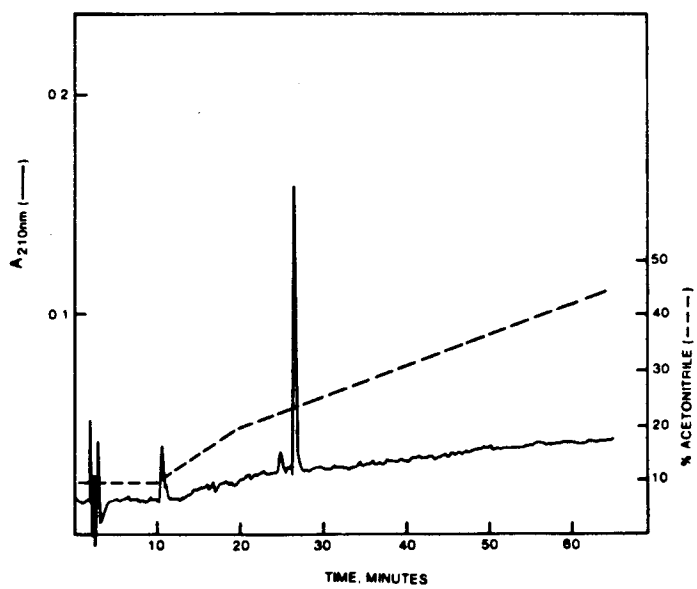
A few of the most impressive separations along with chromatographic conditions are shown below.

FIGURE 1: Oligopeptides on μBONDAPAK™ C_{18} (Ref.3).



HPLC separation of mixture of seven oligopeptides with TEAF buffer: 500 ng each of bradykinin (B), angiotensin II (A), methionine-enkephalin (ME), eledoisin-related peptide (E) and leucine-enkephalin (LE); 1 μg each of substance P (P) and somatostatin (SS). 26% CH_3CN; 74% TEAF; 200 nm; 0.1 AUFS; 1.5 ml min^{-1}; 1200 psi. TEAF is prepared by titrating 0.04 M formic acid with triethylamine to pH = 3.15

FIGURE 2: HPLC of a thymic peptide (used in the maintenance of immune balance and also growth, development and function of the lymphoid system.) (Ref.4).



HPLC analysis of synthetic thymosin α_1 (bovine) in a 30 × 0.39-cm μBondapak C_{18} column (10 μm, Waters Assoc.) at 35°C. The solvents used were: 0.05% TFA in Reservoir A and acetonitrile containing 0.05% TFA in Reservoir B. The peptides were eluted with 10% B for 10 min, followed by a linear gradient from 10 to 20% in 10 min and second gradient from 20 to 45% B in 45 min. The flow-rate was set at 1.5 ml/min, with a chart speed of 0.3 cm/min. The elution was monitored by UV absorbance at 210 nm (——). The solvent gradient is indicated by a dashed line (- - - - -).

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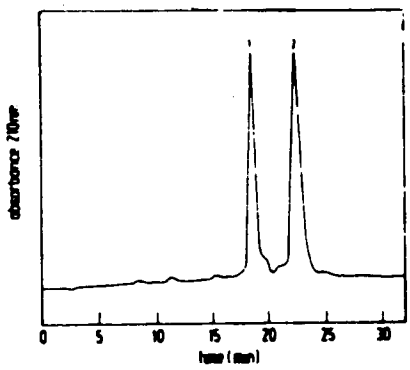
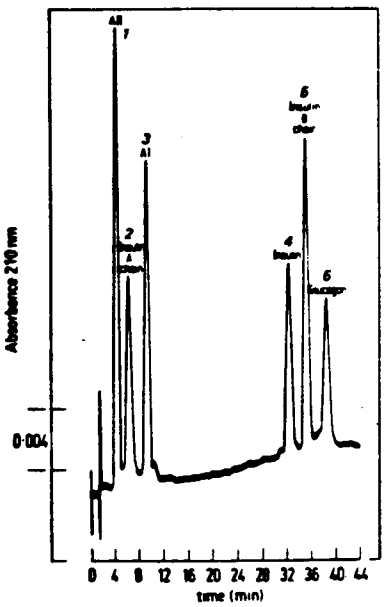
FIGURE 3: Separation of Diastereoisomeric peptides bombesin from [D-Met$_{14}$] bombesin (Ref. 5).



Separation of bombesin (1) from [D-Met$_{14}$]-bombesin (2) by HPLC. Conditions: μ-Bondapak C_{18} column (120 × 0.7 cm); flow rate 6 ml/min; isocratic elution using 30% acetonitrile–10 mM ammonium acetate, pH 4.2.

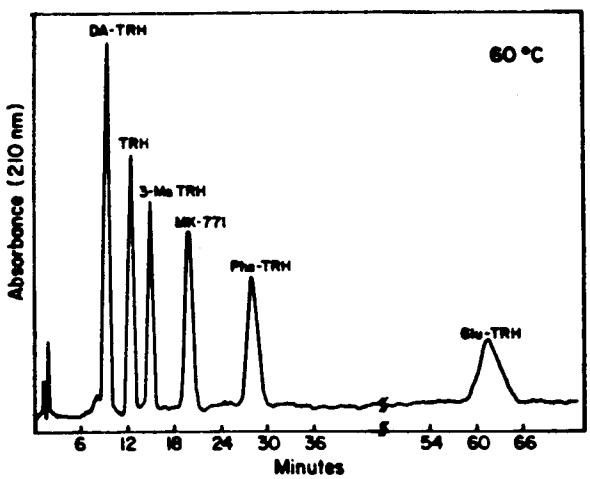
FIGURE 4: Separation of Polypeptides (Ref. 6).



Gradient elution seperation of the polypeptides angiotensin II 1, bovine insulin A chain 2, angiotensin I 3, bovine insulin 4, bovine insulin B chain 5, and porcine glucagon 6.

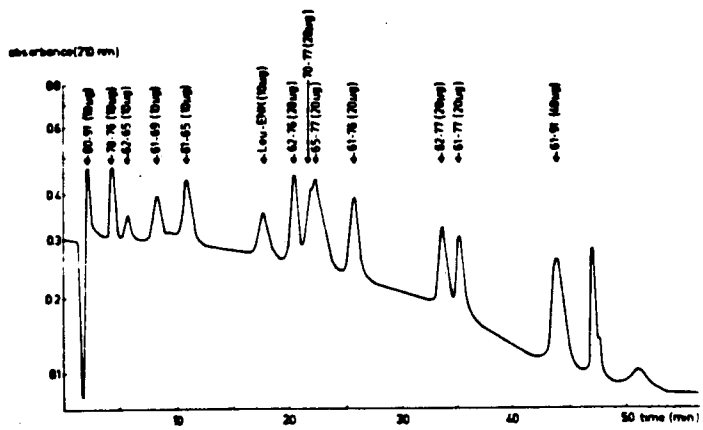
Chromatographic conditions: column, μBondapak C_{18} (10 μm, 30 x 0.4 cm i.d.); flow rate: 2.5 ml/min; temperature: 18°, mobile phase 90-min linear gradient from 15% acetonitrile–water-15mM orthophosphoric acid to 50% acetonitrile-50% water-15mM orthophosphoric acid, sample loading 2 μg each polypeptide.

FIGURE 5: Thyrotropin-releasing hormone analogs. Mobile phase: 0.2 N AcOH, 0.1% C_7SO_3Na. Flow Rate = 2.0 ml/min; Temperature = 60°C (Ref. 7).



DA-TRH = Pyr-His-Pro, Phe-TRH = Pyr-Phe-Pro-NH_2, Glu-TRH = Glu-His-Pro-NH_2

FIGURE 6: Endorphin Fragments of Rat Pituitary Glands. Mobile phase: 10 mM NH_4Ac, pH = 4.15, 30% CH_3OH. Gradient: 30% to 75% MeOH over 45 min at 2.0 ml/min. Temperature at ambient. (Ref. 8).



Extracts of rat pituitary glands were chromatographed. Samples isolated by evaporation of methanol at 60° using a Buchler vortex evaporator and remaining material lyophilised. Sample volumes up to 1 ml did not effect resolution. Samples were found to be active in subsequent radioimmunoassays.

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