Waters
Lab Highlights
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LITERATURE CORNER

PEPTIDE SEPARATIONS ON μBONDAPAK™ C_{18} USING VOLATILE BUFFERS

The ideal mobile phase for peptide separations should be simple, non-UV absorbing, and completely volatile. Although no system at present fulfills all these requirements, the use of low percentage (≤ 1% v/v) trifluoroacetic acid (TFA) in conjunction with acetonitrile of 2-propanol is rapidly gaining favor with many protein biochemists. TFA buffers are volatile, easy to prepare and result in excellent peak shape with high recovery. The weak absorbance at 210 nm does not significantly affect detection.

Clinically significant peptide hormones such as ACTH have been purified to homogeneity using only HPLC with volatile perfluorinated carboxylic acid buffer - acetonitrile gradients on μBONDAPAK™ C_{18} columns (1). Following an initial cleanup of serum on tissue on a C_{18} SEP-PAK® cartridge, the mixture was fractionated using a TFA system. A favorable change in selectivity was obtained by rechromatographing the pooled hormone (ACTH from rat pituitaries) fractions in a buffer system using 0.13% heptafluorobutyric acid instead of TFA (Figures 1 and 2).

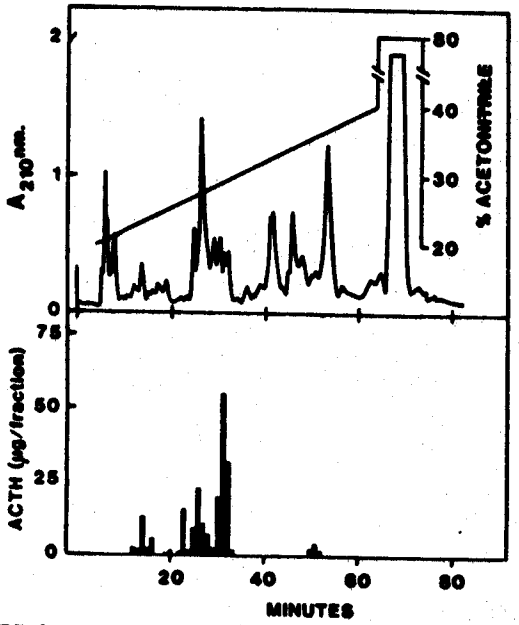

FIGURE 1: Reversed-phase HPLC of SEP-PAK® extract of rat pituitaries. The μBONDAPAK™ C_{18} column was eluted with a linear gradient from 0.1% TFA in H_2O to 80% acetonitrile containing 0.1% TFA. The upper panel shows UV absorbance at 210 nm, while the lower trace shows the immunoreactive ACTH in μg/fraction.

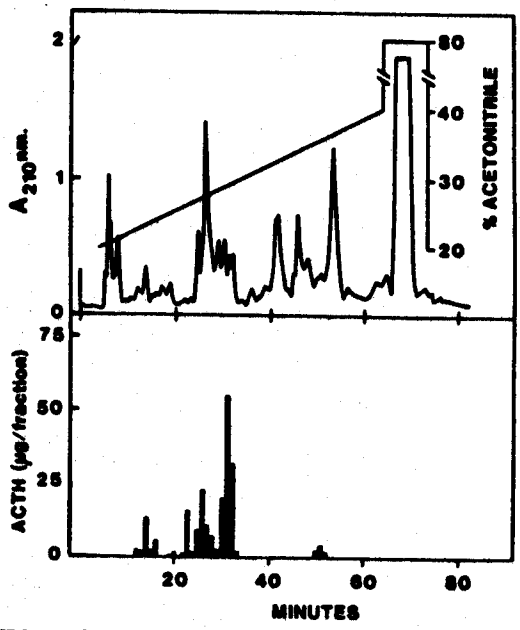

FIGURE 2: Reversed-phase HPLC of fractions 20-32 in Figure 1. The peptides were eluted with a linear gradient from 0.13% HFBA in H_2O to 80% acetonitrile with 0.13% HFBA. Two immunoreactive peptides were resolved from each other, as well as from other UV-absorbing material

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1. H. P. J. Bennett, C. A. Browne and S. Solomon, Biochemistry, 20 (1981) 4530-38.