Waters
Lab Highlights
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Codes

Characterization of Peptide Aromatic Amino Acid Content via Photo Diode Array Detection II. Use of Second Derivative Spectra

Recent work in the Waters Application Development Labs has used the Waters 990+ Photo Diode Array Detector, already a powerful tool for detection and characterization of peptides, to specifically identify the presence and amounts of aromatic amino acids in peptides separated by LC. These developments allow the protein/peptide chemist to quickly identify those peptides in a tryptic digest or other complex sample which contain tryptophan (Trp), tyrosine (Tyr) or both and to determine their relative amounts without the need for hydrolysis and amino acid analysis.

The procedure is based on the characteristic spectra recorded by the 990+ when used as a detector for peptide separations. While the absorbance spectra of aromatic amino acid containing peptides do not present many distinctive features, the presence of Trp is indicated in the second derivative spectra of the same profiles by characteristic, strong double minima with an intervening local maximum in the wavelength range 278 nm to 291 nm (Figure 1).

Inspection of second derivative spectra is usually sufficient to determine the presence of Trp. In order to ascertain the relative amounts of Trp and Tyr it is necessary to perform a mathematical calculation using the absorbance at the exact wavelengths of the minima and maximum to generate a numerical value (R"). Detailed instructions for generating the second derivative spectra and calculating R" are contained in a supplement to this Lab Highlight which can be obtained from the editor. In order to demonstrate that a relationship exists between aromatic amino acid content and R" a calibration curve was generated using mixtures of N-acetyl Tryptophanamide and N-acetyl Tyrosinamide* in ratios varying from 1:0 to 1:4. These spectra were generated using the 990 PDA in a flow injection analysis (FIA) mode (without a column in place).

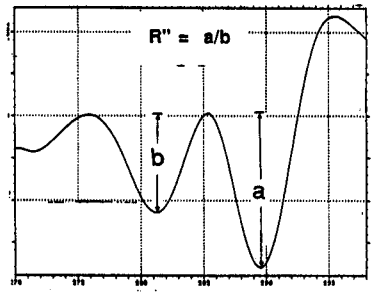

Figure 1. 2nd Derivative Spectrum of N-Acetyl Trptophanamide

* Spectra of these compounds more closely resemble the spectra of Trp and Tyr found in peptides than do those of the free amino acids.

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Following the generation of the calibration curve a mixture of three adrenocorticotrophic hormone (ACTH) peptide fragments was separated (Figure 2, see the supplement for details of the chromatography conditions); R" values for each fragment and the intact peptide were determined. The calculated values were then plotted on the calibration curve (Figure 3).

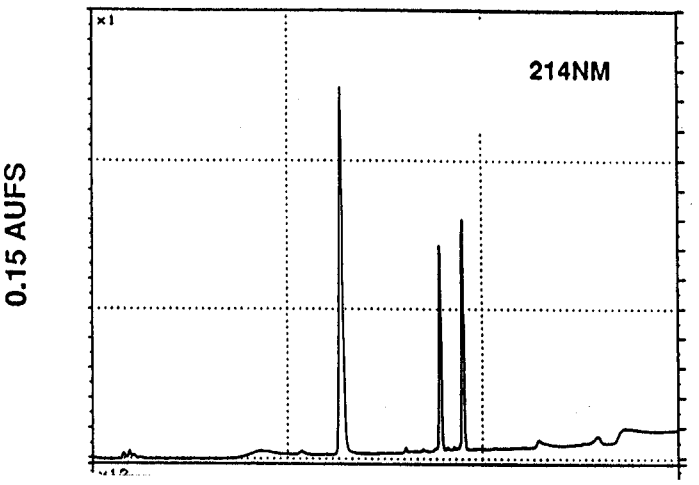

Figure 2. Separation of ACTH Peptides

No value of R" is calculated for the ACTH fragment composed of residues 11-24 as it contains a Tyr but no Trp. The intact peptide contains 1 Trp and 2 Tyr and its R" value of 0.4 falls between the ratios 1:2 and 2:3. The fragment containing residues 1-10 (1 Trp and 1 Tyr) has an R" value of 0.7 which is very close to the ratio 1:1 value. The fragment containing residues 4-10 (1 Trp and no Tyr) has a value of 1.76 which is nearly the same as the ratio 1:0 value. The apparent ambiguity regarding the relative composition of the intact peptide may be due to spectral shifts caused by the internal positions of Trp and Tyr within the peptide.

A number of other model compounds were run and the R" values for each were calculated . The results for the peptides of known composition are in good agreement with the values determined using individual amino acids and R" can be determined from as little as 100 picomoles of sample.

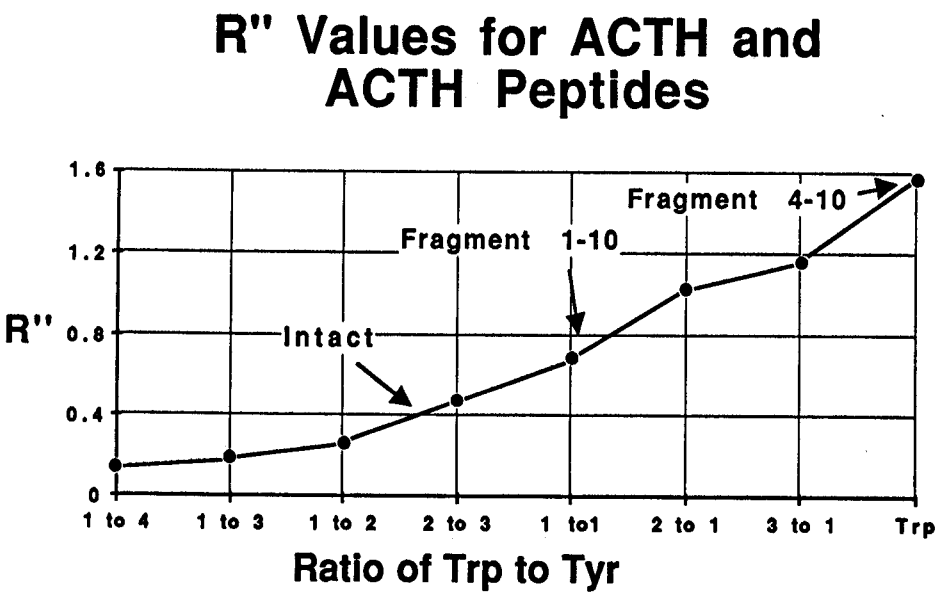

Figure 3. R" Values for ACTH and ACTH Peptides.

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