Waters
Lab Highlights
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Characterization of Peptide Aromatic Amino Acid Content via Photodiode Array Detection: I. Methods for Tryptophan Analysis

Liquid Chromatography adds a new dimension to peptide and protein separations through the use of photodiode array detection to acquire full spectral data of individual components in complex mixtures. This information (e.g. Figure 1) can be used to determine aspects of peptide aromatic amino acid content without further analysis. This series of Lab Highlights will describe on-line chromatographic techniques as non-destructive alternatives to more common procedures for tryptophan analysis.

Identification of DNA sequences which code for tryptophan (Trp) can be a tremendous aid to the biochemist studying mutational change or in the design of oligonucleotide probes used for gene isolation, as this amino acid is specified by a unique trinucleotide sequence (known as a codon), the location of which can serve as a marker in DNA purification. However, identification of Trp-containing peptides is made difficult due to the instability of Trp under the normal hydrolysis conditions for amino acid analysis. Alternate hydrolysis procedures have been developed using special reagents for acidic or basic hydrolysis. One of these using methanesulfonic acid was discussed in LAH#265 and has been successfully employed in conjunction with the Pico•Tag® method.

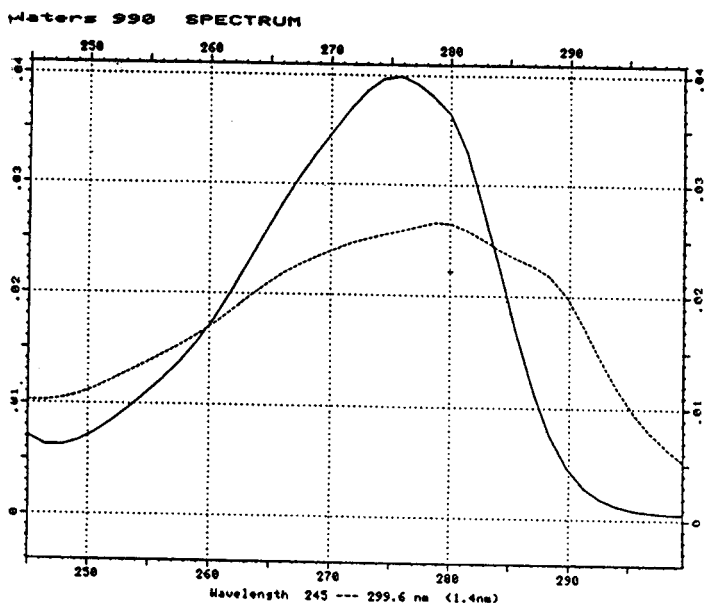

Figure 1
Absorbance Spectra of Model Compounds
Trp-----
Tyr——

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There have also been a variety of non-hydrolytic techniques developed for Trp quantitation. Tagging reagents such as Ehrlich's reagent (p-dimethylamino benzaldehyde) and 2-hydroxy-5-nitrobenzylbromide selectively yield colored reaction products with Trp in intact polypeptides. However, these procedures tend to be too insensitive for the typical amount of sample available in current biochemical studies.

Another approach has been the use of spectrophotometric techniques, commonly performed with pure samples in solution. The methods have ranged from the relatively common UV absorbance and derivative spectroscopy and fluorescence spectroscopy to exotic techniques such as magnetic circular dichroism. Unfortunately, these methods also require large amounts of material.

The introduction of Waters M990+ Photodiode Array Detector has led to the development of spectroscopic techniques which, in conjunction with high resolution chromatographic separations, allow characterization of peptide aromatic amino acid content with picomole sample levels, even in complex mixtures such as tryptic digests. While zero order aromatic residue spectra are broad and overlapping (Figure 1), second-derivative spectroscopy can be an effective tool in resolving these overlapping bands. These differences can be seen in absorbance and derivative spectra of the model amino acid derivatives N-acetyl tyrosinamide and N-acetyl tryptophanamide (Figure 2). In subsequent LAH articles in this series, we will discuss several powerful methods that rely on the second derivative which provide a useful alternative to methanesulfonic acid hydrolysis for the analysis of Trp-containing peptides.

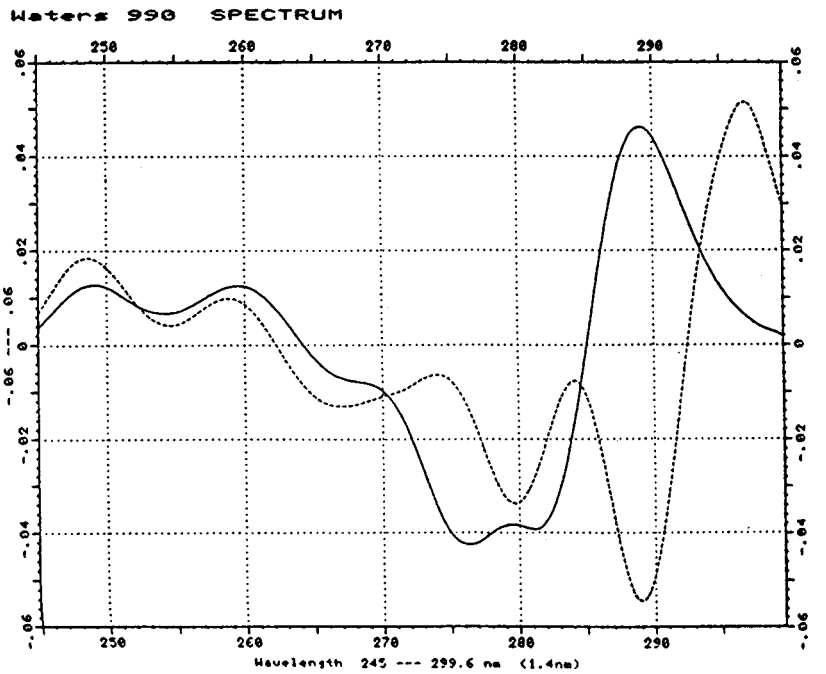

Figure 2
Second Derivative Spectra
Trp-----
Tyr_____

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