Waters Application Notebook

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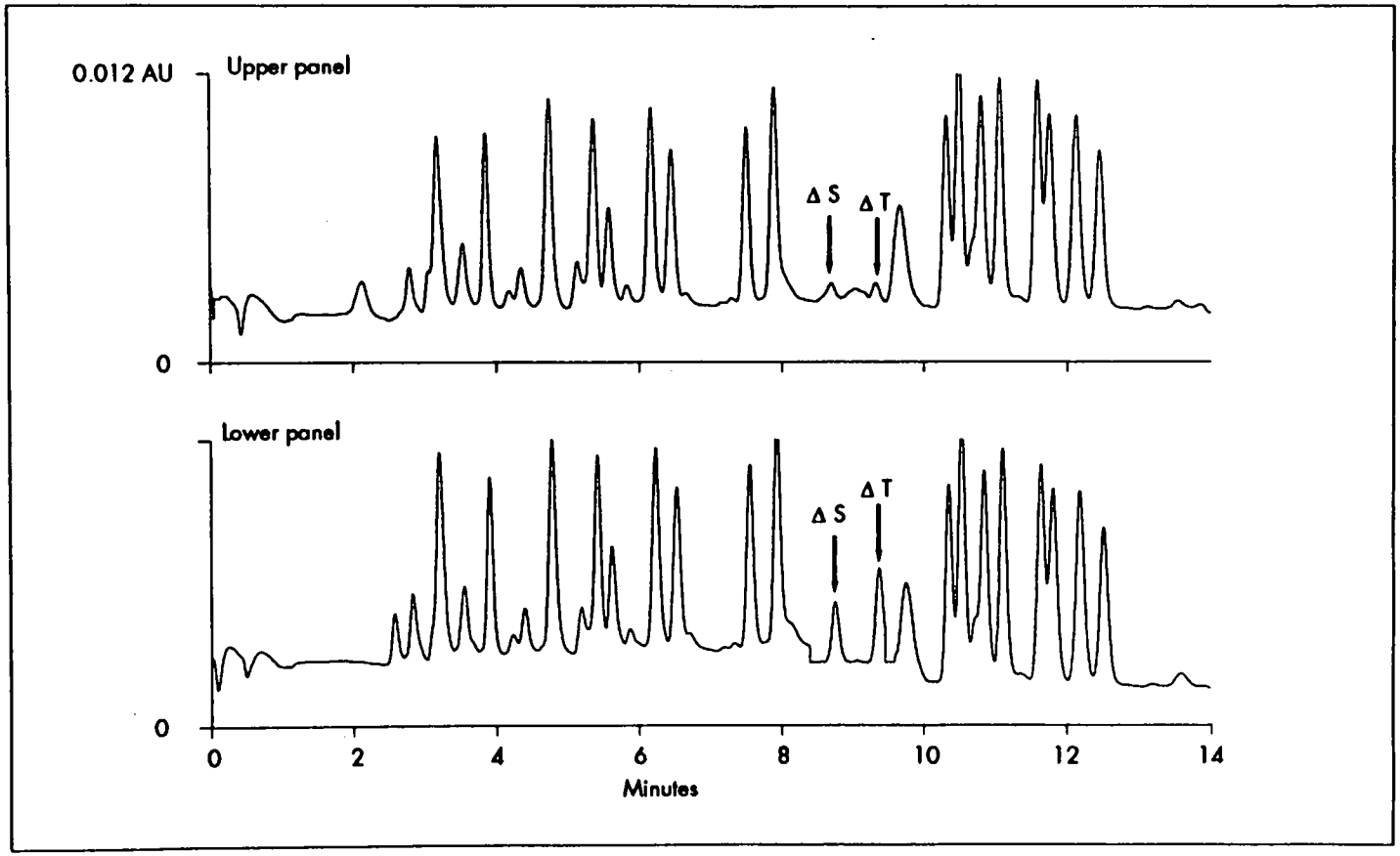
023

# Wavelength Switching for Enhanced Detection of Dehydro Serine and Threonine Phenylthiohydantoins




The upper panel shows a 40 pmol Pth separation monitored at 269 nm during the separation. The lower panel shows the effect of switching the wavelength from 269 nm to 313 nm and then back to 269 nm. A three to five fold increase in signal is obtained for Δ Ser and Δ Thr.

## Conditions:

Sample: A Pth standard was left at 23°C for 12 hours to promote the formation of dehydro (Δ) serine and threonine.

Column: SequeTag™ (3.9 x 300 mm)

Eluent A: 35 mM ammonium acetate, pH 5.00
Eluent B: 100% acetonitrile

Flow Rate: 0.7 ml/min. with standard supplied gradient table.

Detection: A 484 detector employed at either 269 nm or 269 nm and 313 nm during the same run.

Injection: 100 µL (40 pmol)

## Objective:

To improve the ability of protein sequencers to identify the dehydro products of Pth-serine and Pth-threonine.

## Details:

The majority of Pth-Ser and Pth-Thr is converted to the dehydro products during the Edman chemistry process. This makes the identification of authentic Pth-Ser and Pth-Thr problematic Identification of the dehydro Ser and Thr products increases the confidence level in the assignment of these residues during a sequencing experiment.

## System:

The system used consisted of a 600E Multi Solvent Delivery System (100 µl heads), a 484 Tunable UV/Visible Absorbance Detector, and a Pth Maxima™ Chromatography Data Workstation

## References:

1. Coull, J.M., Bonner, A.G., & Dixon, J.D. (1991) Edman protocols for high yield covalent and adsorptive sequence analysis on the ProSequencer™. The Protein Society Fifth Symposium Abstracts. Poster T-63.