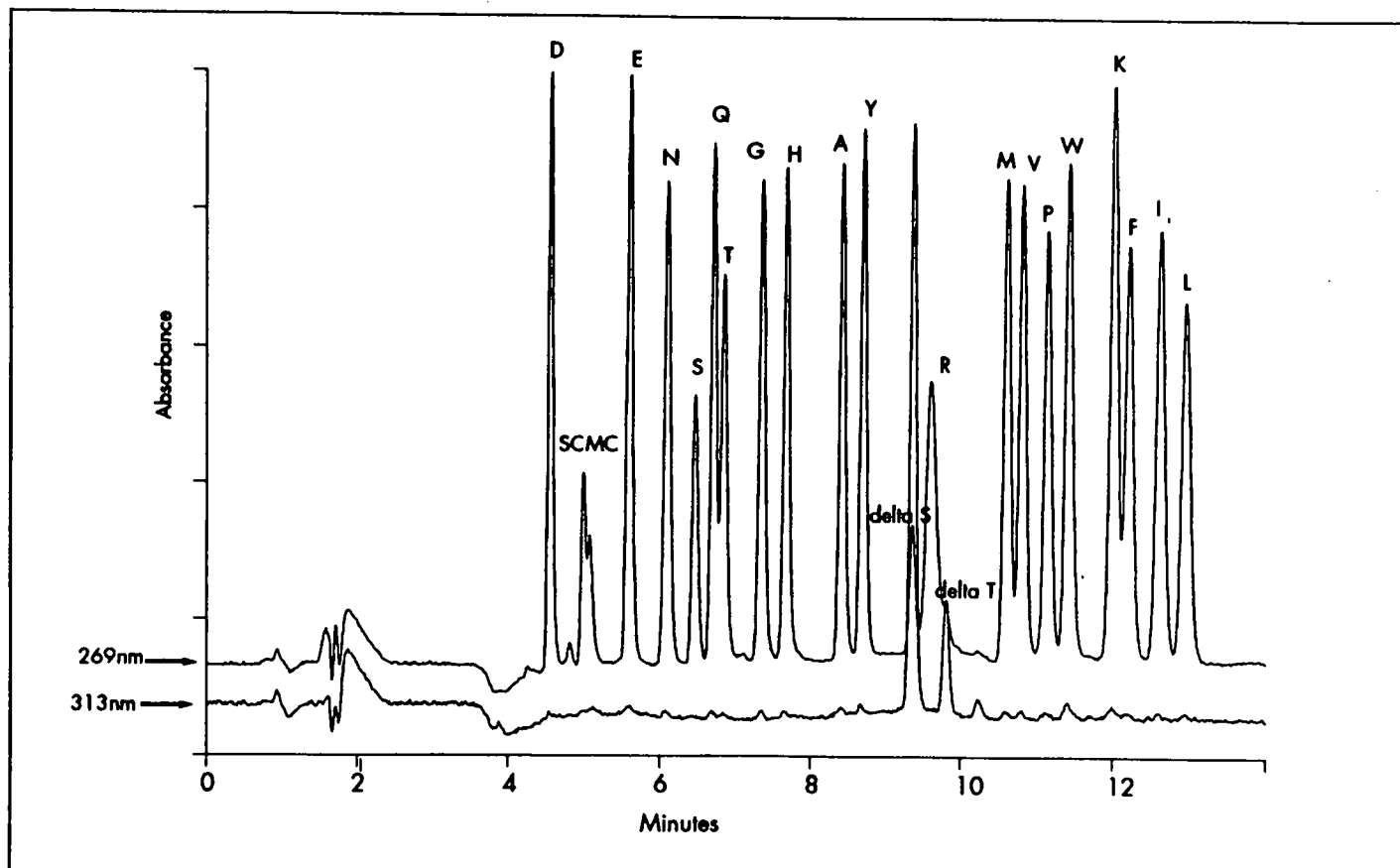


## Waters Application Notebook

David Andrews  
014**PTH-Amino Acid Separation on the Waters SequeTag™ Column.**

The Millipore 6600 and 6625 ProSequencers provide accurate and reliable sequence determination of proteins due, in part, to the exceptional resolution of the Waters SequeTag column.

**Conditions:**

Sample: Pierce PTH-AA standard;  
60pm/150µl

Column: SequeTag™, (3.9 X  
300 mm)

**Mobile phases:**

A: 35mm ammonium acetate,  
pH 4.9.  
B: acetonitrile.

**Gradient:**

Time	%A	%B	Curve
INIT	95	5	*
0.7	75	25	6
1.4	73	27	6
2.8	73	27	6
5.7	55	45	6
7.4	55	45	6
8.1	52	48	6
12	20	80	11
21	95	5	11

Flow rate: 0.7µl/min.

Detection: Waters 490 detector,  
simultaneous monitoring at  
269nm and 313nm.

**Objective:**

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Successful protein sequencing is dependent upon an accurate, sensitive, and reproducible separation of the 20 PTH-amino acids. Contaminants resulting from the sequencing chemistry represent an additional complication. Since the late 1970s, this difficult resolution has been accomplished via HPLC. The best available HPLC separation is illustrated here:

**Details:**

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**System:**

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Protein sequence was determined using a Millipore 6600 ProSequencer that incorporates a low-dispersion 600 pump, 600E controller, 3.9mm X 300mm SequeTag column™, and 490 detector, monitoring at both 269nm and 313nm.

**References:**

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