Waters
Lab Highlights
AMINO ACIDS
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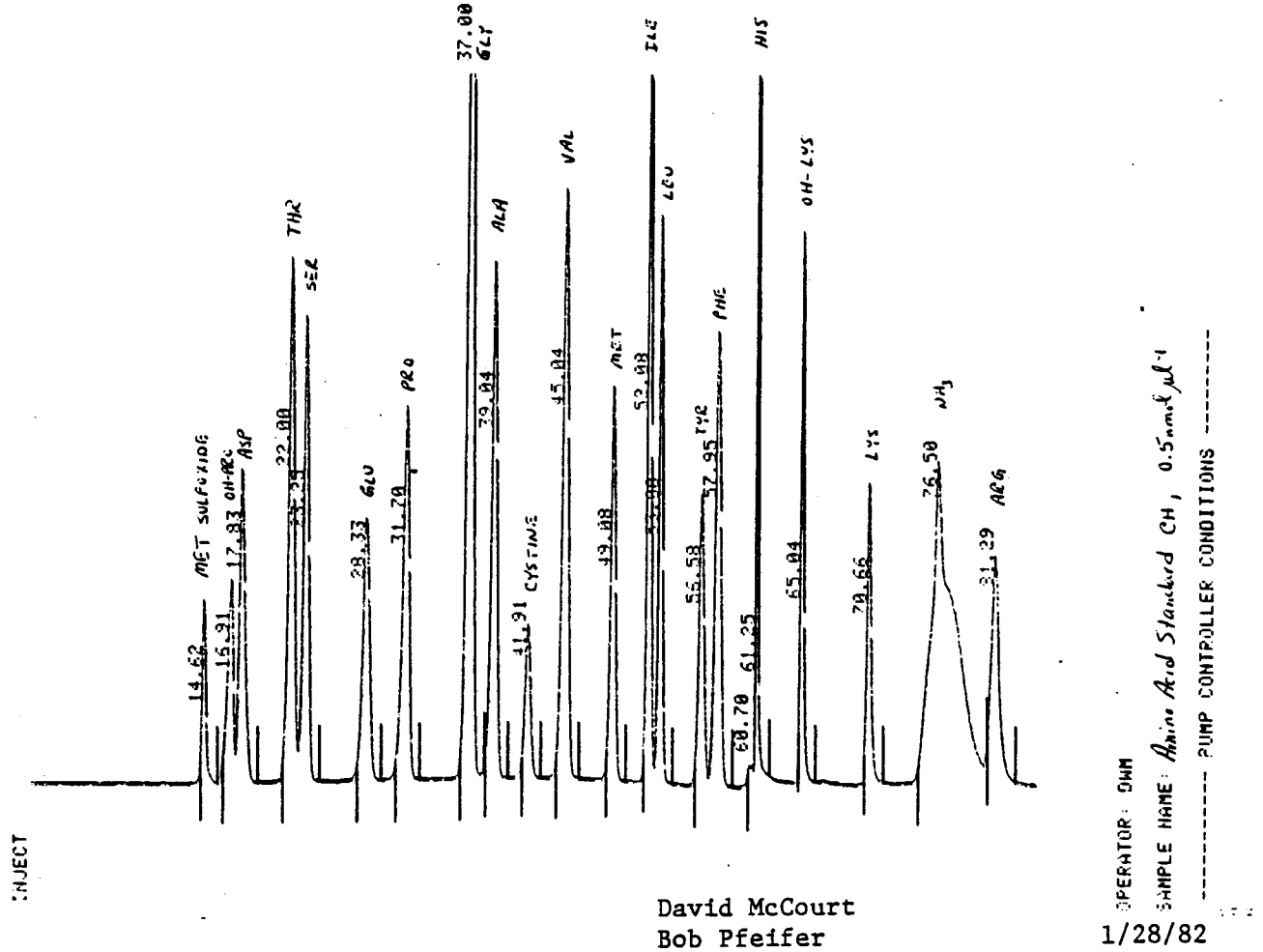
An Internal Communication
of Applications and Techniques

WATERS AMINO ACID ANALYSIS SYSTEM

The analysis of amino acids is important to a variety of markets, some of the most important being bioresearch, pharmaceuticals, food and agriculture. Historically, this analysis has been carried out using a dedicated amino acid analyzer.

Recently, the formation of o-phthalaldehyde derivatives of amino acids and subsequent analysis by reverse phase chromatography has gained considerable interest. However, one limitation of this method is the inability to analyze the secondary amino acids proline and hydroxyproline.

Shown below is an ion exchange separation of a collagen hydrolyzate run* on the Waters LC with amino acid analysis capability. A linear pH gradient was employed and post-column hypochlorite/OPA used for the detection of both primary and secondary amino acids.



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* Reproducibility for 10 consecutive runs is shown on reverse side.

The reproducibility of the ion exchange analysis using the pH gradient was also studied. This was accomplished by repeated injections of an amino acid standard on the Amino Acid Analysis System. Coefficient of variation of retention time and peak area was calculated for ten consecutive runs. The results are shown below.

Column: Waters Amino Acid Analysis Column

Buffer A: 0.2 M Na citrate, pH 3.1

Buffer B: 0.2 M Na borate, pH 9.6

0 to 100% B over 50 min at 0.4 ml/min

COEFFICIENT OF VARIATION FOR TEN CONSECUTIVE RUNS OF ONE NANOMOLE

	RETENTION TIME %	PEAK AREA %
ASPARTIC ACID	0.52	1.78
THREONINE	0.42	1.40
SERINE	0.44	0.96
GLUTAMIC ACID	0.37	1.27
GLYCINE	0.15	1.71
ALANINE	0.11	1.29
VALINE	0.10	2.78
METHIONINE	0.16	2.65
ISOLEUCINE	0.11	1.26
LEUCINE	0.11	1.14
TYROSINE	0.13	0.99
PHENYLALANINE	0.13	1.17
HISTIDINE	0.10	5.82
TRYPTOPHAN	0.24	0.76
LYSINE	0.27	1.92
ARGININE	0.56	2.01
AVERAGE	0.24	1.81