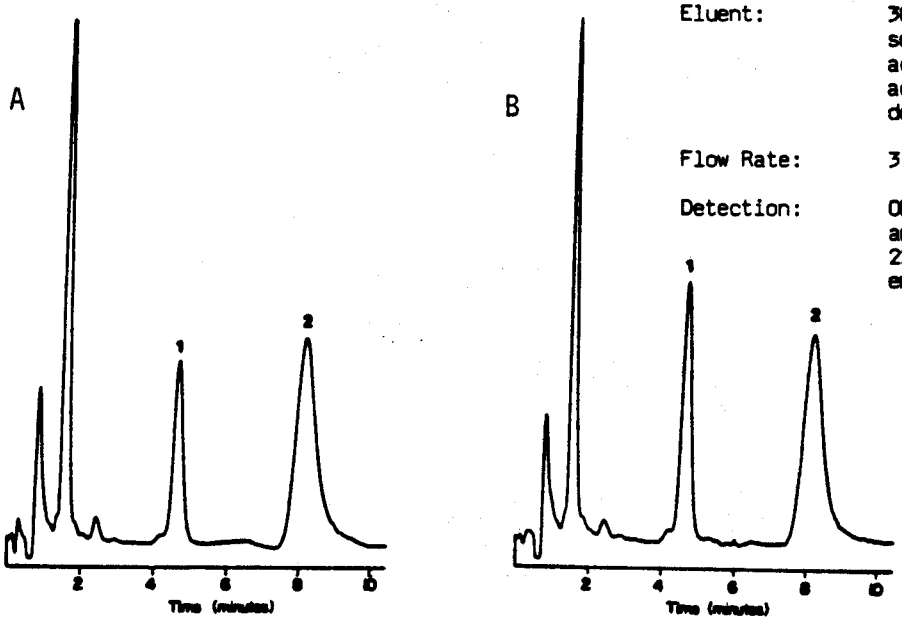
Waters
Lab Highlights
An Internal Communication of Applications and Techniques
LAH 0189 8/84
PD/LS/TD/AA/DV

RADIAL COMPRESSION RESULTS IN FAST DETERMINATION OF TRIMETHYLLYSINE IN PHYSIOLOGICAL SAMPLES

The amino acid 6-N-trimethyllysine is a naturally occurring product in several important proteins, including histones and myosin. It is formed by methylation of selected lysines in these proteins and is metabolized via the carnitine pathway (1). In the past, trimethyllysine was determined by one of several cumbersome ion-exchange amino acid analysis procedures, but these lacked the necessary sensitivity for tissue and serum analysis. Recently, Davis et al (2) reported on a new method using a reverse-phase separation of free trimethyllysine and post-column derivatization with orthophthalaldehyde (OPA) that provides the low detection limits that the metabolic studies required.

The RESOLVE™ cartridge allowed for a very fast analysis (Figure 1), and combined with good sample cleanup and specific detection gave very clean chromatograms.

FIGURE 1: Chromatogram of derivatized rat plasma (A) containing 2 nmol/ml free trimethyllysine and rat muscle (B) containing 16.3 nmol/g trimethyllysine. Peak 1 = trimethyllysine, peak 2 = triethyllysine (internal standard).



Column:	10 micron RESOLVE™ C_{18} Cartridge, 10 x 0.8 cm
Eluent:	30% acetonitrile, 70% 50 mM sodium dihydrogen phosphate, pH adjusted to 2.75 with sulfuric acid, containing 50 mM sodium dodecyl sulfate.
Flow Rate:	3.0 ml/min.
Detection:	OPA post-column derivatization and fluorescence detection, 229 nm excitation, 418 nm emission full scale set to 1 μA.

Waters
Division of MILLIPORE 34 Maple St. / Milford, MA 01757 / 617-478-2000

Steve Cohen

Cleanup of serum and tissue samples was a key element in simplifying the chromatographic analysis. Tissues were homogenized in perchloric acid, and serum proteins were precipitated with the same denaturant. After neutralization with potassium bicarbonate, the samples were applied to a short mixed-bed, ion-exchange column consisting of Dowex 50W-X8 and Dowex 1-X8. The trimethyllysine was eluted with 1M ammonium hydroxide and concentrated. Usually the analog triethyllysine was added as an internal standard along with the perchloric acid.

The separation was performed on an RCM-100^{R} Radial Compression Module using a 10 micron Radial-PAK^{TM} RESOLVE^{TM} C_{18} cartridge along with a Model M6000A pump and U6K injector. The post-column reagent was 300 mg of OPA and 500 microliters of 2-mercaptoethanol dissolved in 10 ml of 3% (v/v) Brij-35 in 95% ethanol and mixed with 500 ml of 0.5 M sodium borate pH 10.4. Detection was accomplished with a fluorometric detector (Kratos SF970) using 229 nm excitation and a 418 nm cutoff filter for emission.

Tissue and serum samples were obtained from rats fed a trimethyllysine limiting diet. The table below shows that rapid reverse-phase chromatography was successful in detecting very low levels of trimethyllysine. The reported detection limit was 25 pmol per injection.

TABLE I: Samples were obtained from the five sacrificed rats. Values are expressed as mean ± the standard deviation.

TISSUE	FREE TRIMETHYLLYSINE
Plasma	1.9 ± 0.1 (nmol/ml)
Liver	3.2 ± 0.2 (nmol/g)
Kidney	2.7 ± 0.1 (nmol/g)
Muscle	19.3 ± 1.2 (nmol/g)

1. R. A. Cox and C. L. Hoppel, Biochem.J., 136 (1973) 1083.
2. A. T. Davis, S. T. Ingalls and C. L. Hoppel, J. Chromatogr. 306, (1984) 79.