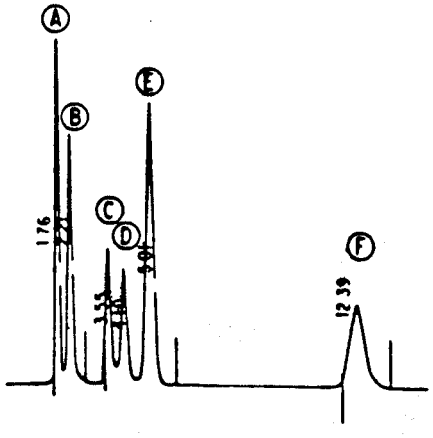
Waters
Lab Highlights
An Internal Communication of Applications and Techniques
LAH 0200 10/84
AN/LS/MD/NA/NT

NUCLEOTIDE ANALYSIS OF CANINE CARDIAC MUSCLE USING A RADIAL-PAK™ RESOLVE™ C_{18} COLUMN

Many investigators (1,2) have used the depletion of nucleotides and subsequent formation of degradation products associated with ischemia (suppression of blood flow to organ or tissue) to study changes in cardiac cell metabolism, structure and function. A recent method (3) provides excellent resolution of nucleotides and their major degradation products from totally ischemic canine cardiac muscle with minimal analysis time using an isocratic elution system.

The standard nucleotides used in this study were ATP, ADP, AMP, inosine, hypoxanthine and xanthine. They were dissolved in high purity water at appropriate concentrations. The separation was accomplished on a Radial-PAK™ RESOLVE™ C_{18} cartridge (10µ, 8 mm i.d. X 100 mm) held in an RCM-100® Radial Compression Module. Figure 1 shows a separation of six standard nucleotides accomplished in ≈13 minutes. Figure 2 is a separation of a control canine cardiac tissue after a perchloric acid/potassium carbonate-potassium hydroxide extraction. Figure 3 is a sample of canine cardiac tissue after 60 minutes of total ischemia. Note the increase or decrease in the concentration of the nucleotides after this procedure.

Column:	Radial-PAK™ RESOLVE™ C_{18} cartridge (10 µm, 8 mm i.d. x 100 mm)
Mobile Phase:	0.1 M ammonium dihydrogen phosphate buffer, pH = 5.5 with 3M ammonium hydroxide.
Flow Rate:	4.0 ml/min
Temperature:	Ambient
UV Detection at	254 nm.



A. ATP
B. ADP
C. Hypoxanthine
D. Xanthine
E. AMP
F. Inosine

FIGURE 1. Chromatogram showing the resolution of standard nucleotides.

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

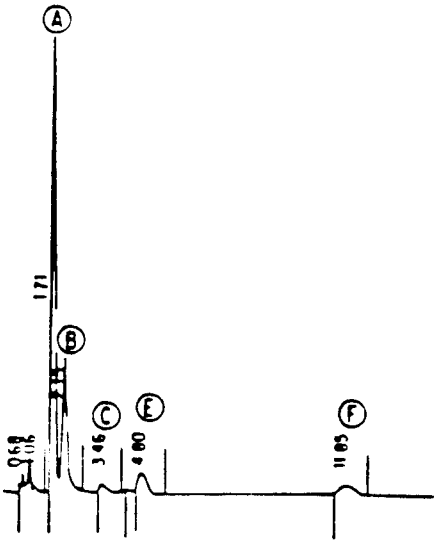


FIGURE 2. Control canine cardiac tissue. Peaks and chromatographic conditions as given in Figure 1.

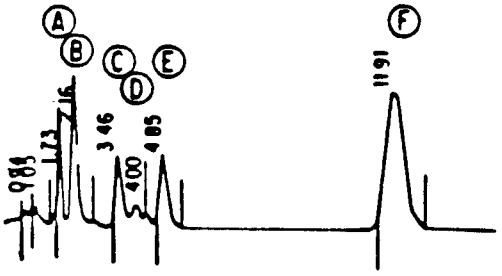


FIGURE 3. Canine cardiac tissue after 60 min. of total ischemia. Peaks and chromatographic conditions as given in Figure 1.

The ability to monitor nucleotides and their major metabolites is essential for delaying or reversing myocardial ischemic cell injury or death. The described method utilizes an inexpensive, easy to prepare, low viscosity and large buffering capacity mobile phase along with a Radial Compression Separation System. These two factors will assist investigators in easing the enormous volume of work needed in metabolic studies because they provide efficient, precise and economical results.

1. J. E. Lowe, R. B. Jennings and K. A. Reimer, J. Mol. Cell Cardiol., 11 (1979) 1017.
2. J. E. Lowe, H. K. Hawkins and R. B. Jennings, Surg. Forum, 29 (1978) 247.
3. J. E. Lowe, R. G. Cummings and E. A. Hull-Ryde, J. Chromatogr., 275 (1983) 411.