

**HPLC LACTATE AND VFA**  
**Updated September 2013**

**I. References:**

Ehrlich, G. G., D. F. Goerlitz, J.H. Bourell, G. V. Eisen, and E. M. Godsy. 1981. Liquid chromatographic procedure for fermentation product analysis in the identification of anaerobic bacteria. Appl. Environ. Microbiol. 33:955.

**II. Personal Protective Equipment:**

- A. Lab coat
- B. Safety glasses/goggles
- C. Latex gloves

**III. Reagent:**

- A. 0.013 N Sulfuric Acid ( $H_2SO_4$ )
  - 1. Add .36 ml of concentrated  $H_2SO_4$  to 500 ml of dd $H_2O$
  - 2. q.s to 1 L

**III. Procedure:**

- A. Weigh 5.0 g of sample into 125 ml Erlenmeyer flask
- B. Add 15 ml of 0.013 N  $H_2SO_4$
- C. Mix well and refrigerate overnight
- D. Filter mixture through #40 Whatman filter paper using gravity filtration
- E. Filter filtrate through 0.45  $\mu m$  disposable filters into HPLC vials for analysis

**IV. Standards:**

- A. Add the following approximate amounts to a 1 liter volumetric flask. Write down the exact weights for calculation of actual concentrations.

VFA	MW	Approximate g/L	Approximate Working Conc., mM
Acetate ( $C_2H_4O_2$ )	60.0	0.18	3.00
Propionate ( $C_3H_6O_2$ )	74.0	0.06	0.75
Butyrate ( $C_4H_8O_2$ )	88.1	0.02	0.25
Lactate ( $C_3H_6O_3$ )	90.1	0.43	4.75

- B. q.s. to 1 liter using 0.013 N  $H_2SO_4$
- C. Filter standards through 0.45  $\mu m$  disposable filters into 4 HPLC vials

**V. HPLC Conditions:**

Column: Bio-Rad HP x -87 caution-exchange  
Column Temp: 30°C  
Eluent: .013 N  $H_2SO_4$  filtered through .2  $\mu m$  filter paper and degassed  
Flow rate: 0.7 ml/min