HPLC LACTATE AND VFA
Updated September 2013

I. References:


II. Personal Protective Equipment:
A. Lab coat
B. Safety glasses/goggles
C. Latex gloves

III. Reagent:
A. 0.013 N Sulfuric Acid (H₂SO₄)
   1. Add .36 ml of concentrated H₂SO₄ to 500 ml of ddH₂O
   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 H₂SO₄
C. Mix well and refrigerate overnight
D. Filter mixture through #40 Whatman filter paper using gravity filtration
E. Filter filtrate through 0.45 µm 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.

<table>
<thead>
<tr>
<th>VFA</th>
<th>MW</th>
<th>Approximate g/L</th>
<th>Approximate Working Conc., mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate (C₂H₄O₂)</td>
<td>60.0</td>
<td>0.18</td>
<td>3.00</td>
</tr>
<tr>
<td>Propionate (C₃H₆O₂)</td>
<td>74.0</td>
<td>0.06</td>
<td>0.75</td>
</tr>
<tr>
<td>Butyrate (C₄H₈O₂)</td>
<td>88.1</td>
<td>0.02</td>
<td>0.25</td>
</tr>
<tr>
<td>Lactate (C₃H₆O₃)</td>
<td>90.1</td>
<td>0.43</td>
<td>4.75</td>
</tr>
</tbody>
</table>

B. q.s. to 1 liter using 0.013 N H₂SO₄
C. Filter standards through 0.45 µm disposable filters into 4 HPLC vials

V. HPLC Conditions:
Column: Bio-Rad HP x –87 caution-exchange
Column Temp: 30°C
Eluent: .013 N H₂SO₄ filtered through .2 µm filter paper and degassed
Flow rate: 0.7 ml/min