Question: What suggestion can you offer for analysis of metabolites such as glucose, pyruvate, taurocholic acid, gamma glutamylcysteine, glycochenodeoxycholate, glycerol-3-phosphate, spermidine, taurine, and s-adenosylmethionine?

Suggestion: We would recommend starting by using the Cogent Diamond Hydride™ HPLC column for this analysis. Many of these compounds are quite polar and would be very difficult to separate by conventional Reversed Phase methods and The Diamond Hydride™ column can retain compounds based on **Aqueous Normal Phase** ANP chromatography. There are a variety of functional groups present in these analytes so it is difficult to say which mobile phase a**dditive** would be most effective overall.

Start with 10mM ammonium acetate in solvent A and solvent B and next try 0.1% formic acid. You may even want to consider ammonium acetate in one solvent and formic acid in the other if you find that you need a pH Gradient.

Additives to consider are ammonium formate and acetic acid. Use of either isopropanol or methanol as 50% of the A solvent for complex matrices is recommended. This is because these solvents help to wash strongly adsorbed matrix components from the column and extend column life.

An example Gradient might be:

A: 50:50 DI water / methanol / 0.1% formic acid

B: acetonitrile / 0.1% formic acid

Time *(minutes)*	%B
0	95
2	95
10	0
14	0
15	95

You would start at high organic (B solvent) and end at high water (in this case 50% water due to the methanol in the A solvent) and keep a hold time to help wash some strongly retained matrix components off the column. You can adjust the gradient steepness if you need more separation.

MicroSolv Technology Corporation
9158 Industrial Blvd. NE, Leland, NC 28451
tel. (732) 380-8900, fax (910) 769-9435
Email: customers@mtc-usa.com
Website: www.mtc-usa.com