

HPLC Laboratory  
C433 Spring 2007

Methods:

The mobile phase consists of 10 mM ammonium acetate, pH 3, with 7% (v/v) methanol added.

Protocols are as follows for the HPLC experiments and sample preparation:

Flow rate: 1.2 ml/min

Detector 254 nm

Injection volume 20ul

Run time 10 min

Using a stock solution of 1.0 mg/ml 3-acetamidophenol (provided) prepare solutions for a four-point calibration curve (1, 4, 10, 40 ug/ml). The internal standard of 3-acetamidophenol will be added at a concentration of 10 ug/ml to each sample.

Urine samples need to be diluted 1:4 (v/v) with mobile phase.

All standards and samples need to be made up in the sample vials for a total volume of 1.5 ml.

At least three samples will be run per section, one undoped urine, one spiked urine, and one urine from a person who took 3-acetamidophenol.

Writeup:

In the background section, summarize how reversed phase HPLC can be used to analyze for this compound in urine. In the methods section, describe the experimental procedure used for analysis. In the results and discussion, discuss the results that you obtained for the samples. For calibration curves, you will need to obtain the peak area for each of the standard concentrations from both classes. Use these data points to calculate your concentrations in the urine samples.