Colorado State University

CHEM 431
Instrumental Analysis Laboratory

Notes for
High Performance Liquid Chromatography
Principles and Applications

The following is a set of short notes to outline the experiment in question and to provide helpful guidance to those executing the experiment.

A. A. High Performance Liquid Chromatography (HPLC) is a powerful technique for separating complex mixtures and quantitating their components. HPLC equipment can be as simple as a specialized pump, a means of permitting sample injection, a specialized column and a suitable detector or as complicated as an integrated system with highly engineered equivalent hardware and exceedingly sophisticated and capable software.

B. B. In this experiment the basic setup, that is, preparation of solvents and preparation of suitable analytic samples, and operation, that is, software control, of a modern, integrated and powerful HPLC system will be demonstrated and used.

C. C. For convenience, expediency and simplicity, an **isocratic elut**ion solvent will be used to separate a set of pharmocologically active molecules and quantitate the individual molecules. A set of calibration samples will be prepared to establish the optimal elution solvent for separation then a series of solutions containing varying concentrations of each analyte studied to permit construction of suitable calibration curves and quantitation of them in representative commercial preparations.

D. Prepare several "stock" solutions (25 mL each, 10 mg/mL each, in methanol). Use acetyl salicylic acid, salicylic acid, caffeine, and 4-acetoamidophenol. Store these in polyprop bottles (not volumetric flasks, of course).

E. Collect UV absorption spectra of each sample. The absorbances of each may be so high that modest (small yet sufficient) volumes of individual diluted solutions (always in straight methanol) will need to be prepared. Dispose of these diluted solutions appropriately and immediately.

F. Prepare HPLC samples (1 mL each, 1 mg/mL each, in methanol, in HPLC vials). Make five samples, one for each of the molecules (that's four) and one with all four molecules (1 mg/mL each) in it (that's the fifth).

G. Look in the literature for the close-to optimum chromatographic elution solvent blend. Choose several wavelengths for detection. Explain to teaching staff the basis for your choices before using them. Use the multi-component sample to test and possibly tune the solvent. Later, use the individual component samples to assign the absorption signals.