University of Wisconsin
Chemistry 524
Separations Instrumentation*

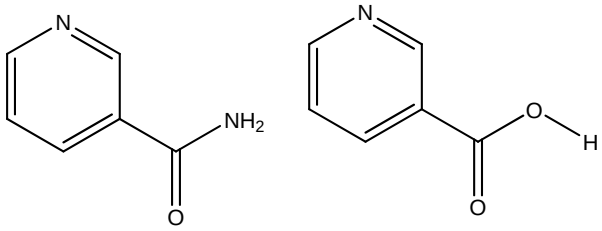
Gas Chromatography In this application you are going to use gas chromatography with a mass spectrometer detector (GCMS) to attempt to identify some of the components in gasoline samples. You will also compare the relative amount of these components in two popular Madison brands of gasoline: the more expensive Shell gasoline and the cheaper U-pump gasoline. Detailed instructions for operating the instrument will be provided in lab. **In your report sheet you should include**:

- the chromatograms of each gasoline sample with integrated peaks
- the instrumental conditions used to obtain the chromatogram
- mass Spectra of three major components of the gasoline samples
- an educated guess of the identity of the three components
- an educated guess of the structure of the main M.S fragments for each
- the approximate relative amounts of the three components in each sample

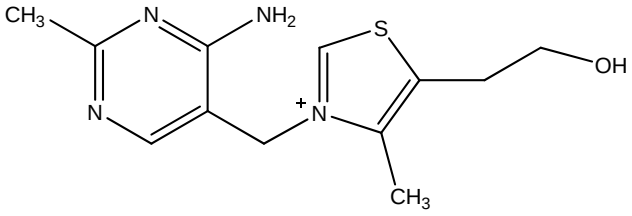
Liquid Chromatography In this experiment, you are going to use a reverse phase C18 HPLC column to separate and quantify the amount of nitrate and nitrite in a water sample. Ordinary reverse phase liquid chromatography separations require the analytes to have some affinity for the stationary phase and you would not expect reverse phase LC to work for the separation of ionic species. However, if an amphoteric molecule is added to the system, the amphoteric molecule can interact with both the ionic analyte and the non polar stationary phase causing a partitioning of the analyte between the phases. When the amphoteric molecule and the analyte are ionic, the technique is referred to as *ion pairing*. The ion pairing agent used in this experiment is octylamine, which is positively charged at pH=7. Detailed instructions for operating the instrument will be provided in lab. **In your report sheet you should include**:

- the chromatogram of the lake water identifying the nitrate and nitrite peaks
- table of integrated areas for sample and standards
- concentrations of nitrate and nitrite in sample

Capillary Electrophoresis In this experiment you are going to set up and condition a capillary for use in a capillary electrophoresis (CE) instrument. You will then use the CE to separate a mixture of 3 vitamins. These vitamins were selected because at a neutral pH, one is charged negative, one is neutral, and one is charged positive. The structures of the vitamins are:



niacinamide nicotinic acid



thiamine

Detailed instructions for operating the instrument will be provided in lab. **In your report sheet you should include**:

- the electropherogram with peak identities.
- calculations of electrophretic mobilities

LCMS (Liquid Chromatography Mass Spectrometry): In the demonstration you will see a peptide mixture in a vial in an autosampler be injected into the flow of an HPLC. The peptide mixture eluting from the high performance liquid chromatograph will flow into a photodiode array and then into an electrospray ionization needle that generates ions for a single quadrupole mass spectrometer to analyze. Instrumental Details: Column: C18 reverse phase, 150 x 2.1 mm, 5 µm, 300 angstrom pores; Mobile phase A = 0.1% formic acid/water; Mobile phase B = 0.1% formic acid/acetonitrile; flow rate = 200 µL/min; photodiode array detector (PDA): 200-400 nm; mass spec scanning 300-2000 Da. **In your report sheet you should include**:

- a structure for you peptide,
- a copy of the LCMS printout
- a list of the masses observed for each HPLC peak.

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[*] GCMS,CE,and HPLC Activities were developed R. McClain, March 2005. LCMS demonstration developed by Martha Vestling from the Chemical Instrument Center (CIC) in Chemistry at the University of Wisconsin-Madison, 2009.