EXPERIMENT 12. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY DETERMINATION OF CAFFEINE IN COFFEE, COKE, OR TEA

The purpose of this experiment is to identify the caffeine which is present in an unknown mixture (drink) and to determine the concentration of caffeine in this mixture.

Instrument Start Up

1. Turn on valves at nitrogen and helium cylinders.
2. Turn on all modules of HPLC instrument.
3. Load **ChemStation** software package. Open *CAFFEIN2* method.

In this laboratory experiment you are going to run the quantitative assay for caffeine in a drink

Procedure

Make a standard 0.4 mg/mL solution of caffeine in water (dissolve 50 mg of caffeine in 100 mL of water in a volumetric flask or dilute the standard solution provided). Make 0.2, 0.1 , and 0.05 mg/mL solutions.

Make tea or coffee the way you like. Do not add sugar or milk! You may also use another caffeinated drink for this experiment.

Filter the resulting sample (around 2 mL volume) in a vail. In order to run the chromatographic determination, set the injection volume as 5 microL of the sample. Use aqueous acetonitrile as an eluent with the flow of 1.0-0.8 mL/min. Inject the sample and obtain the chromatogram.

Inject your standard caffeine solution using exactly the same parameters as before.

Find which peak is corresponding to caffeine.

Using area of the caffeine peaks in your unknown I_x and in your standard solution

$I_{standard}$, determine the concentration of the caffeine in your beverage using the simple proportion:

$$m_x = m_{\text{standard}} \times \frac{I_x}{I_{\text{standard}}}$$

Repeat these calculations for other standards you have measured. Build a calibration curve and receive the concentration of caffeine using the calibration.