Objective
The primary objective of your work in this experiment is to determine whether or not the concentration of the antihistamine doxylamine in an over-the-counter cough syrup is consistent with the value reported by the manufacturer on the label.

Materials and Guidance
You will be provided with (nominally) pure doxylamine (Sigma Aldrich), HPLC grade methanol, HPLC grade water, and a sample of over-the-counter cough syrup.

To quantitate the doxylamine in the cough syrup you ultimately will need to construct a calibration curve. You can use the dosage information on the cough syrup bottle to help make initial estimates for the range of your calibration standards. The number of standards you choose to use is up to you, but keep in mind that this is one factor that affects the precision of your estimate of the doxylamine concentration in the cough syrup.

It is recommended that you first prepare a stock solution of doxylamine in 10/90 (v/v) methanol/water at a concentration of about 1 mg/mL, and then prepare your calibration solutions by diluting this stock solution with water. **Doxylamine is expensive; please do not use more than 10 mg for your entire experiment.** The final calibration solutions should not contain more than 5% methanol (v/v). To ensure that the doxylamine fully dissolves, first weigh out the doxylamine solid, then add the volume of methanol needed, and then add the amount of water needed. Make sure the solid is fully dissolved before moving ahead.

The cough syrup sample must be treated before injecting it into the HPLC for analysis:
1. Filter roughly 1 mL of cough syrup through a 0.2 μm syringe filter
2. Dilute the filtered syrup 1:5 with water (4 parts water, 1 part syrup, by volume)

Finally, the dosage of doxylamine in the cough syrup is specified to two significant figures, so your results ultimately need to be precise to at least two significant figures as well.

Keep in mind that each HPLC analysis will take about 3 minutes to run.

Making the Measurements
1. Work with your instructor to set up the HPLC instrument using the operating parameters listed below. The HPLC column we will use for this work is an Restek DB C8, with dimensions of 50 mm x 3.0 mm i.d.
   - Stop time – 2.5 minutes
Flow rate – 2.5 mL/min.
Temperature – 60 °C
Injection volume – 10 μL
Detection by UV absorbance at 220 nm (make sure the detector is set to collect ‘All’ spectra)
Solvent A – 0.1% (w/w) trifluoroacetic acid in water
Solvent B – acetonitrile

Set up the following program to control the pump; the initial solvent should be set at 2.0% B.

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>%B</th>
</tr>
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<tbody>
<tr>
<td>0.00</td>
<td>2.0</td>
</tr>
<tr>
<td>1.00</td>
<td>2.0</td>
</tr>
<tr>
<td>2.00</td>
<td>100</td>
</tr>
<tr>
<td>2.01</td>
<td>2.0</td>
</tr>
</tbody>
</table>

2. Setup a sequence in the instrument software to perform two ‘dummy’ injections (no sample injected, but the instrument still executes the method), followed by two injections of your most concentrated calibration standard, followed by two injections of the cough syrup sample. Use the retention time of the doxylamine peak in the chromatogram for the standard to identify which peak in the cough syrup chromatogram is doxylamine (consult with your instructor at this point if needed).

3. Work with your instructor to choose a wavelength for quantitation of doxylamine based upon the UV spectrum obtained in your initial runs. Before running the rest of your samples, be sure to change the wavelength in your analysis method.

4. If it looks like the doxylamine peak in the cough syrup will be within your calibration range, then set up a sequence to analyze the calibration samples and the cough syrup sample in duplicate (with two dummy runs at the beginning).

Analyzing the Data
Work with your instructor to integrate the doxylamine peak in each chromatogram and collect the peak areas for all of the samples in an Excel spreadsheet. Use your knowledge about calibration curves and regression to estimate the concentration of doxylamine in the cough syrup, along with an estimate of the uncertainty associated with this number. Finally, use a statistical test to judge whether or not the actual doxylamine concentration is as specified on the label.

Reporting the Results
Prepare a short (less than ten pages of text) report (one report per team) summarizing results; a plot of your calibration data, a residuals plot, and a representative chromatogram for the cough syrup sample with the doxylamine peak clearly indicated should accompany the text on separate pages. The plot of the calibration data should contain symbols ONLY for the raw data, and a line ONLY for the calibration line.
Send a copy of the report and the spreadsheet containing your data and regression analysis results to dstoll@gustavus.edu. The report should be detailed enough that a person that has completed the Quantitative Analysis course, but not necessarily this experiment, could complete the experiment by following the description of your approach outlined in your report. At a minimum, include the following information in your summary:

1. An explanation of your approach to the preparation of calibration solutions.
2. A table summarizing your raw data.
3. Estimates of the slope and intercept of your calibration line, and the standard deviations associated with these estimates; use appropriate numbers of significant figures in reporting these values.
4. An estimate of the concentration of the unknown and an estimate of the uncertainty associated with this estimate. Use the number of significant figures warranted by the quality of your data.
5. Using an appropriate statistical test, make a judgement about whether or not the dosage of doxylamine specified on the label accurately reflects what is in the bottle. If you find that the label is not accurate, you should suggest possible problems with your experiment that may have resulted in inaccurate results.