CH 315: Quantification of Caffeine in Carbonated Beverages by HPLC

By Heather M. Brinson

Unknown Number: 1

Abstract:

High performance liquid chromatography is useful in determining not only what components are in an unknown sample, but, if done properly, also the concentrations of the components. After given an unknown mountain dew-type soda and asked to determine the concentration of the caffeine within it, using HPLC seemed a logical choice. While one typical HPLC chromatogram is not useful in determining concentration, by employing standard addition of a known concentration, using the range of data and creating a graph, the concentration can be calculated. By adding a changing amount of standard to a constant amount of unknown, the changing area can be correlated to concentration. However, before such tests could be conducted, it was necessary to determine what mobile phase results in the best separation between the two major components of the soda – caffeine and benzoate. By testing solutions with changing ratios of water and methanol with a standard solution of mixed 0.1018 mg/mL of caffeine and 0.3014 mg/mL of benzoate, it was determined that the mobile phase of 50% water/50% methanol produced the best separation. Once that was decided, it was necessary to test the above mentioned concentrations of caffeine and benzoate, but separately so that the individual peaks could be identified on the chromatogram. Finally, the unknown could be tested through standard addition, resulting in a concentration of caffeine, with the 95% confidence interval, of 0.07997 ± 7.94% mg/mL.

Results:

As can be calculated from the slope of Graph 1, the concentration of caffeine was found to be $0.07997 \pm 7.94\%$ mg/mL in the tested soda. Full details of the calculations can be found in the following section.



Graph 1: Determining Caffeine Concentration

The following figures show the HPLC graphs gathered during the course of the experiment. The first four show the testing of the best separation by changing the composition of the mobile phase.











Figure 3: 50% Water/50% Methanol MP Standard Test



Figure 4: 25% Water/75% Methanol MP Standard Test

The next two figures show the retention time and subsequent area and height of the standard concentration of caffeine and benzoate, tested separately in the 50/50 mobile phase.



Retention Time	Area	Area %	Height	Height %
0.470	75777	4.59	8363	2.85
0.915	1574894	95.41	284798	97.15

Figure 5:	Pure	Caffeine,	0.10	mg/μL
-----------	------	-----------	------	-------



Figure 6: Pure Benzoate, 0.3 mg/µL

The five following figures are the graphs from testing the unknown in the 50/50 mobile phase using the method of standard addition.



Retention Time	Area	Area %	Height	Height %
0.715	646545	13.93	39652	9.68
1.135	1632822	35.18	280456	68.44
1.582	2361445	50.88	89681	21.88

Figure 7: Soda Test with No Standard



0.607	926060	15.73	56106	11.31
1.020	1967513	33.42	331012	66.72
1.502	2978020	50.58	108215	21.81
2.558	16215	0.28	751	0.15

Figure 8: Soda Test with 1 mL Standard Addition



Figure 9: Soda Test with 2 mL Standard Addition



UV/Vis Results				
Retention Time	Area	Area %	Height	Height %
0.722	789058	13.02	47892	9.00
1.137	2318273	38.24	377751	70.98
1.588	2954348	48.74	106577	20.02





Figure 11: Soda Test with 4 mL Standard Addition

The following four figures show the graph from testing the standard addition method in water, using the 50/50 mobile phase.



Figure 12: 1 mL Standard Addition in Water



UV/Vis Results				
Retention Time	Area	Area %	Height	Height %
0.687	204319	44.90	29930	38.29
1.123	250767	55.10	48237	61.71



Figure 13: 2 mL Standard Addition in Water

Figure 14: 3 mL Standard Addition in Water



Figure 15: 4 mL Standard Addition in Water

Calculations:

In this experiment, there were eight different types of calculations employed. The first is the conversion of the slope of Graph 1's trend line into concentration of mg/mL.

$$m = {}^{I_X}/[X] \rightarrow [X] = {}^{I_X}/m$$

$$m = 5.082 \times 10^9; I_X = 2.093 \times 10^6$$

$$[X] = {(2.093 \times 10^6)}/(5.082 \times 10^9)$$

$$[X] = \frac{4.118 \times 10^{-4} \text{ mol}}{L} \times \frac{194.1906 \text{ g}}{\text{mol}} \times \frac{1000 \text{ mg}}{\text{g}} \times \frac{L}{1000 \text{ mL}}$$

$$[X] = 7.997 \times 10^{-2}$$

The second type of calculation used in this experiment was the calculation of the vertical deviation of the graph's trend line from each point.

$$\begin{aligned} d_{i} &= y_{i} - (mx_{i} + b) \\ y_{i} &= 2.296 \times 10^{6}; \ m = 5.082 \times 10^{9}; \ x_{i} &= 2.621 \times 10^{-5}; \ b = 2.093 \times 10^{6} \\ d_{i} &= (2.296 \times 10^{6}) - \{ [(5.082 \times 10^{9}) \times (2.621 \times 10^{-5})] + (2.093 \times 10^{6}) \} \\ \hline d_{i} &= 7.007 \times 10^{4} \end{aligned}$$

The third type of calculation used in this experiment was to determine the vertical standard deviation.

$$\begin{split} s_y &= \sqrt{\frac{\Sigma(d_i^2)}{n-2}} \\ s_y &= \sqrt{\frac{(-5.164*10^4)^2 + (7.007*10^4)^2 + (1.941*10^4)^2 + (-4.247*10^4)^2 + (4.628*10^3)^2}{5-2}} \\ \hline \\ s_y &= 5.709 \times 10^4 \end{split}$$

The fourth type of calculation required in this experiment was the calculation to find the determinant for Graph 1.

$$D = \begin{vmatrix} \sum(x_i^2) & \sum x_i \\ \sum x_i & n \end{vmatrix}$$

D

$$= \begin{vmatrix} [(2.621E - 5)^2 + (5.242E - 5)^2 + (7.863E - 5)^2 + (1.048E - 5)^2] & [2.621E - 5 + 5.242E - 5 + 7.863E - 5 + 1.048E - 4] \\ & [2.621E - 5 + 5.242E - 5 + 7.863E - 5 + 1.048E - 4] & 5 \end{vmatrix}$$

$$D = 3.435 \times 10^{-8}$$

The fifth type of calculation used was the calculation of the standard deviation of the trend line's slope.

$$s_{\rm m} = \sqrt{\frac{s_y^2 * n}{D}}$$

$$s_{\rm m} = \sqrt{\frac{(5.709 \times 10^4)^2 \times 5}{3.435 \times 10^{-8}}}$$

$$s_{\rm m} = 6.888 \times 10^8$$

The sixth type of calculation employed during the course of this experiment was to determine the standard deviation of the intercept of the Y axis.

$$s_{b} = \frac{s_{y}^{2} * \Sigma(x_{i}^{2})}{D}$$

$$s_{b} = \sqrt{\frac{(5.709E4)^{2} * [(2.621E - 5)^{2} + (5.242E - 5)^{2} + (7.863E - 5)^{2} + (1.048E - 5)^{2}]}{3.435E - 8}}$$

$$s_{b} = 4.422 \times 10^{4}$$

The seventh calculation type used in this experiment was to calculate the horizontal standard deviation.

$$s_{X} = \left(\frac{s_{Y}}{|m|}\right) \times \sqrt{\left(\frac{1}{k}\right) + \left(\frac{1}{n}\right) + \left[\frac{(y - \bar{y})^{2}}{m^{2}(\sum(x_{i} - \bar{x})^{2})}\right]}$$

 s_{Y} = Standard deviation in Y direction = 5.709E4; m = slope of trendline = 5.082E9; k = number of replicant measurements = 1; n = number of data points = 5; y = measured y point; \bar{y} = average y value =2.359E6; x_{i} = measured x values; \bar{x} = average x value =5.242E-5

$s_x = 1.820 \times 10^{-5}$

The final calculation used in this experiment was the propagation of error between the trend line's slope, the y intercept, and the molecular weight of caffeine. Running through the numbers gives a value of 7.94% for the 95% confidence interval.

Discussion:

Concerning the mobile phase runs, it was necessary to experiment to determine the mobile phase with the best separation of caffeine and benzoate, the primary unknowns in the soda considered in this experiment. As can be seen in figures one through four, the only mobile phase with functional peak separation was the 50%water/50%methanol solution. The 75%water/25%methanol and the 25%water/75%methanol mobile phases only produced one peak. Although the 95%water/5%methanol mobile phase produced two peaks, the one of the left is more indicative of sample impurity. If that is the case, then the peak on the right contains both caffeine and benzoate peaks within it and there is no separation between the two.

Figures five and six are the chromatograms of pure caffeine and pure benzoate in the 50%water/50%methanol mobile phase. The purpose of these tests was to be able to differentiate between the caffeine and benzoate peaks when the unknown was tested. As can be seen in the figures, benzoate has a shorter retention time than caffeine, by an average of

0.416 minutes. With this knowledge in hand, it was then possible to identify and manipulate the data retrieved by the remaining experiments.

Figures seven through eleven are the chromatograms from the tests of the unknown with added standard concentrations. The purpose of these graphs was to begin determination of the concentration of caffeine. By subtracting the areas of the caffeine peaks from the added standards (as a result of testing the standard just in water, see figures twelve through fifteen) and drawing Graph one, the slope of the trendline could be used to determine the concentration of the caffeine in the soda alone. The slope of the trendline graphed is $m = I_X/[X]_i$, where I_x is the area from the initial solution, ie, only the soda, and [X]_i is the concentration of caffeine in the unknown solution. By dividing the Y-intercept, which is I_X , it is possible to derive the initial concentration of caffeine in the tested soda. While the R² value is not overly extraordinary, only R^2 = 0.9478, it is certainly within a reasonable proximity to be a basis of calculations. As stated earlier, the concentration was found to be $0.07997 \pm 7.94\%$ mg/mL, including the 95% confidence interval. According to Wikipedia, there is 0.15 mg/mL of caffeine in Mountain Dew, a similar soda to the unknown tested in this experiment. While the experimental value is only 53% of the accepted value in Mountain Dew, it must be remembered that not only is the brand name of the unknown soda not specified. Different brand names will have differing amounts of caffeine in their product, not to mention the inherent variation from bottle to bottle. With these considerations in mind, it is possible that the experimental value is within range of the "true value".