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CHEM 1255
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WAKE UP – Using ATR-FTIR to Determine the Concentration of Caffeine in Caffeine Supplements

I submit this lab report as an original document. I assert that all ideas and discussion of data contained herein is my own work, unless otherwise referenced.

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Introduction

ATR-FTIR spectroscopy is used plentifully in scanning liquids and solids because of its convenience and ease of use. Its practically makes it a good candidate to use for spectroscopy because the overall length of time the procedure takes is significantly lower than other analytical instrumentation. This practically has led it to be used in multiple experiments, such as analyzing anion-caffeine interactions and studying electro-oxidation of methanol through various electrodes. ATR-FTIR can be used for a wide variety of potential studies.

Infrared spectroscopy is a useful form of analysis used in chemistry. IR spectroscopy is not only reliable, but a multitude of things can be determined from the readings, such as identification of an unknown chemical, characterization of a sample, or even determining the amount of a certain specimen present in the sample. However, the usefulness of IR spectroscopy comes with its fair share of downsides. For one, IR spectroscopy tends to require a large amount of sample preparation in order to receive a clean reading from the instrument. Also, IR spectroscopy tends to be extremely delicate, and readings are often difficult to repeat multiple times and get the same data from it.

ATR-FTIR is a special type of infrared spectroscopy, that not only keeps all of the benefits that standard IR spectroscopy holds, but also accounts for all of the downsides and nullifies them. While IR spectroscopy tends to require a significant amount of the sample in order for an IR beam to pass through it, ATR-FTIR does not need a large amount of sample. This is because the IR beam is not passed through the sample but instead reflected off of the sample. ATR-FTIR is also more reliably repeatable than IR, as many outside variables are nullified in FTIR, such as outside light and sample density.

ATR-FTIR is run by placing the sample onto the crystal found on the die of the instrument. This crystal is important because it is the medium in which the IR beam passes through. When the sample is placed on the crystal and the scan is run, the IR beam is sent into the crystal at an angle, where it then reflects multiple times off of the top and bottom of the crystal. The top side, containing the sample, will alter the signal of the IR beam, which is picked up by the detector and evidently shows up on the reading received from the instrument.

For this experiment, ATR-FTIR was used to determine the concentration of caffeine present in samples of different caffeine supplements. In particular the supplements used in this lab were Stay Awake caffeine pills, and Hydroxycut weight loss supplements. The experiment will show which of the two supplements is a greater source of caffeine. ATR-FTIR was used in this experiment for its ease of use and overall convenience. Since caffeine is able to be picked up from the ATR-FTIR spectrometer, it just would not make sense to not take advantage of the instrument.

Data

Table 1 – Masses of unknown samples and additions.

Sample	Mass of Sample (mg)	Mass of Ground Sample (mg)	Mass of Salicylic Acid Added (mg)
Caffeine Pill	582.7	206.1	81.6
Hydroxycut	487.9	218.9	88.1

Table 2 – Masses of Caffeine and Salicylic Acid and calculated actual % w/w.					
Sample	Target w/w Caffeine (%)	Measured Caffeine Mass (mg)	Measured Salicylic Mass (mg)	Actual w/w Caffeine (%)	Average Actual w/w Caffeine (%)
1	10	29.8	270.6	9.92	51.64
2	30	90.2	209.8	30.1	
3	45	135.4	164.7	45.12	
4	60	179.7	120.4	59.88	
5	75	224.6	74.9	74.99	
6	90	269.9	30.5	89.85	

The fifth column in **Table 2** is calculated by using the mass of caffeine and the total mass of the powder mixture. The formula for this calculation is shown below.

$$\%\frac{w}{w} = \frac{m_{Caffeine}}{m_{Caffeine} + m_{Salicylic\ Acid}} \cdot 100\%$$

$$\%\frac{w}{w_1} = \frac{29.8\ \text{mg}}{29.8\ \text{mg} + 270.6\ \text{mg}} \cdot 100\% = 9.92\%$$

The sixth column in **Table 2** is calculated by a simple average calculation.

Table 3 – Peak areas received from the FTIR calculations, and the calculated averages for each sample.					
Sample	Run	Caffeine Peak Area	Average Caffeine Peak Area	Salicylic Acid Peak Area	Average Salicylic Acid Peak Area
6	1	2.60759	2.65977	0.771683	0.79788
	2	2.71194		0.824073	
5	1	2.71553	2.73638	1.24214	1.25903
	2	2.75723		1.27591	
4	1	3.91654	3.88426	2.06344	1.83540
	2	3.85197		1.60736	
3	1	3.33086	3.26602	2.04232	2.07556
	2	3.20117		2.10880	
2	1	2.45835	2.43695	2.12354	2.13690
	2	2.41555		2.15025	
1	1	2.09093	2.05000	2.99973	2.95488
	2	2.00906		2.91002	

The average values in **Table 3** were calculated by a simple average formula.

Discussion

1.

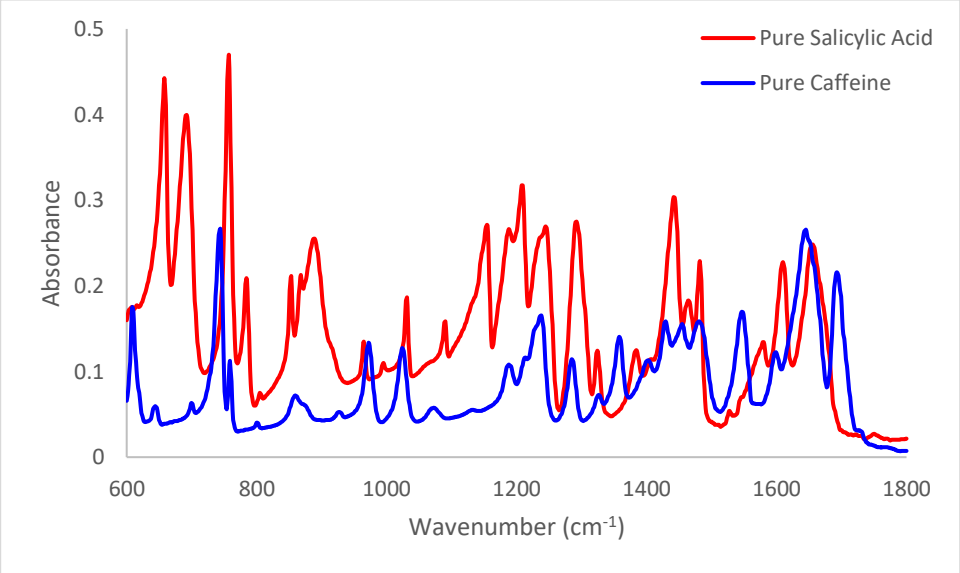

Figure 1 – FTIR readings of pure Salicylic Acid and Caffeine.

The significant peaks of 785 cm^{-1} for salicylic acid and 1700 cm^{-1} for caffeine are determined using this graph. As shown, 785 cm^{-1} shows a lot of absorbance for salicylic acid, and little for caffeine. The opposite goes for 1700 cm^{-1}. Salicylic acid is used specifically for the peak that it causes that is so different compared to the peaks that caffeine presents.

2.

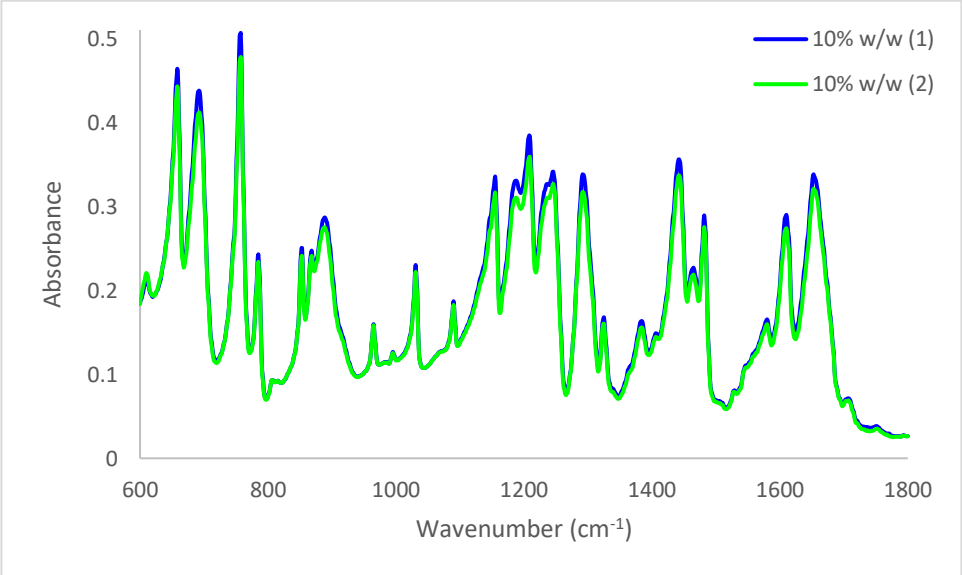

Figure 2 – FTIR reading of both 10% w/w runs.

The FTIR readings show that the absorbance values for the peaks can change with each run. Because of this, it is recommended to test each sample more than once in order to increase the accuracy of the calculations performed later.

3.

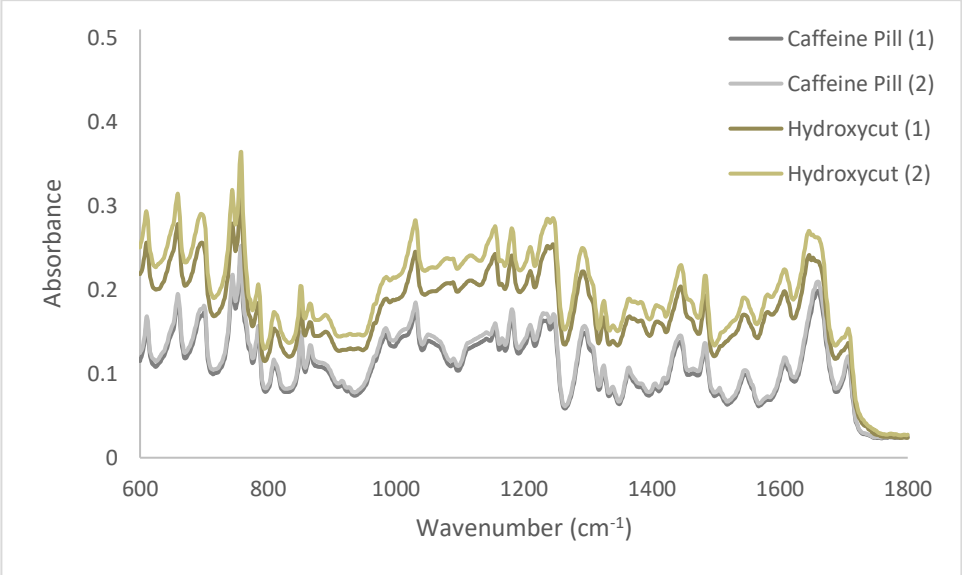

Figure 3 – FTIR readings for both runs of both the caffeine pill and the Hydroxycut powder.

4.

Table 4 – Peak Area Ratio compared to concentration of caffeine.

Sample	Actual w/w Caffeine (%)	Average Caffeine Peak Area	Average Salicylic Acid Peak Area	Peak Area Ratio (Caffeine/Salicylic Acid)
6	9.92	2.65977	0.79788	3.33355
5	30.1	2.73638	1.25903	2.17341
4	45.12	3.88426	1.83540	2.11630
3	59.88	3.26602	2.07556	1.57356
2	74.99	2.43695	2.13690	1.14042
1	89.85	2.05000	2.95488	0.693767

The peak area ratios shown in **Table 4** are found through the following calculation.

$$Peak\ Area\ Ratio = \frac{Peak\ Area_{Caffeine}}{Peak\ Area_{Salicylic\ Acid}}$$

$$Peak\ Area\ Ratio_6 = \frac{2.65977}{0.79788} = 3.33355$$

Using the data received for the % w/w of caffeine and the peak area ratios, a calibration curve between the two data sets can be constructed.

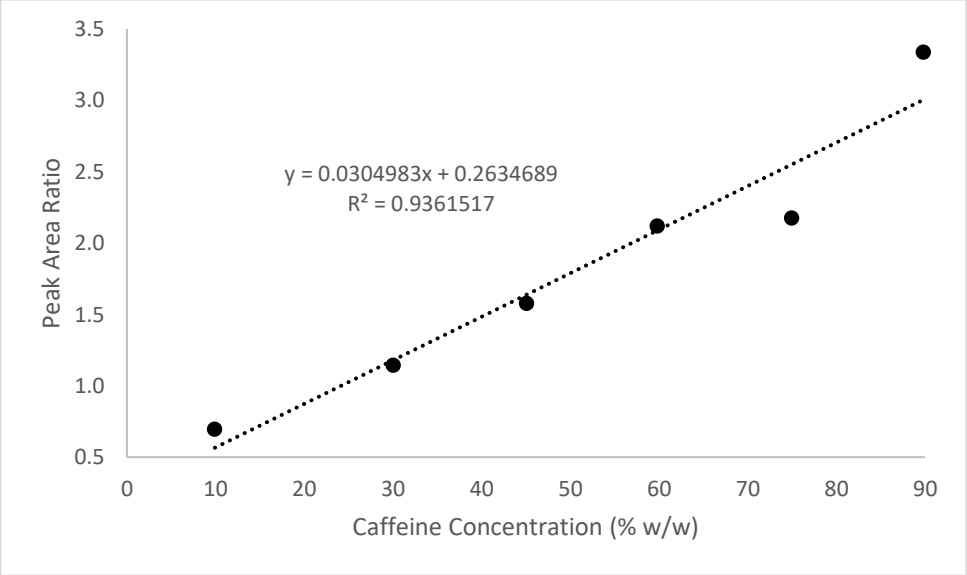

Figure 4 – Calibration curve comparing the concentration of caffeine to the ratio of the average peak areas for salicylic acid and caffeine. The equation for the trendline and R^2 value are also shown.

5.

Table 5 – Peak areas for caffeine and salicylic acid, the calculated peak area ratios, and the determined caffeine concentration for each unknown sample.

Unknown	Average Caffeine Peak Area	Average Salicylic Acid Peak Area	Peak Area Ratio (Caffeine/Salicylic Acid)	Final Caffeine w/w (%)	Standard Deviation of Caffeine w/w (%)
Caffeine Pill	2.78002	2.13227	1.30379	34.1037	7.37
Hydroxycut	3.81326	2.90073	1.31459	34.4579	7.37

The peak area ratios for the unknown samples were found the exact same way as they were found for the standard samples, dividing the caffeine peak area by the salicylic acid peak area. The caffeine concentration was found by using the inverse of the calibration curve and the peak area ratio values for the unknowns. The process is shown below. Keep in mind that b is the y-intercept of the trendline and m is the slope of the trendline.

$$\%\, w/w_{Caffeine} = \frac{Peak\ Area\ Ratio - b}{m}$$

$$\%\, w/w_{Caffeine_{Pill}} = \frac{1.30379 - 0.263905}{0.0304918} = 34.1037\ \%$$

The standard deviation found using another more in-depth calculation

$$s_{\%\ w/w_{Caffeine}} = \frac{s_r}{m}\sqrt{\frac{1}{L}+\frac{1}{n}+\frac{\left(\overline{PAR}_{samp}-\overline{PAR}_{std}\right)^2}{m^2\sum_{i=1}^{n}\left(C_{std_i}-\bar{C}_{std}\right)^2}}$$

$$s_{\%\ w/w_{Caffeine}} = \frac{0.262}{0.0304918}\sqrt{\frac{1}{2}+\frac{1}{6}+\frac{\left(\frac{34.1037\%+34.4579\%}{2}-\frac{3.33355+\cdots+0.693767}{6}\right)^2}{0.0304918^2\cdot[(9.92\%-51.64\%)^2+\cdots+(89.85\%-51.64\%)^2]}}$$
$$= 7.37\%$$

Table 6 – Key for variables used in standard deviation calculation.	
s_r	Standard Deviation of the Regression
m	Slope of Calibration Curve
L	Number of Unknown Samples
n	Number of Standards
$\overline{PAR}_{samp}$	Average Peak Area Ratio of Unknown Samples
$\overline{PAR}_{std}$	Average Peak Area Ratio of Standards
C_{std_i}	Concentration of Standard "i"
$\bar{C}_{std}$	Average Concentration of Standards

s_r is determined by the following function.

$$s_r = \sqrt{\frac{\sum_{i=1}^{n}\left(PAR_i-\widehat{PAR}_i\right)^2}{n-2}}$$

$$s_r = \sqrt{\frac{\left(3.33355-(89.85\%\cdot 0.03050+0.2635)\right)^2+\cdots+\left(0.693767-(9.92\%\cdot 0.03050+0.2635)\right)^2}{6-2}}$$
$$= 0.262$$

PAR_i is the value received from the FTIR readings for the sample "i", and $\widehat{PAR}_i$ is the readings from the trendline using the given concentration for that standard.

It is important to remember that this is not the actual concentration of caffeine in the unknown samples, as an amount of salicylic acid was added in the preparation steps of the samples. To account for this, a proportion must be taken to account for this extra addition. C_1 is the concentration before the salicylic acid was added, m_1 is the mass of the sample before the salicylic acid was added, and C_2 and m_2 are the concentration and mass of the sample after the salicylic acid was added.

$$C_1 = \frac{m_2 C_2}{m_1}$$

$$C_{1_{Pill}} = \frac{(206.1\text{ mg}+81.6\text{ mg})\cdot 34.1037\%}{206.1\text{ mg}} = 47.61\%\ Caffeine$$

This calculation was repeated for both the caffeine pill sample and the Hydroxycut sample. The table below shows the values determined. The masses for the calculations used can be found in **Table 1**.

Table 7 – Determination of initial caffeine concentration in the unknown samples.			
Sample	Final Caffeine w/w (%)	Initial Caffeine w/w (%)	Standard Deviation of Caffeine w/w (%)
Caffeine Pill	34.1037	47.61	7.37
Hydroxycut	34.4579	48.33	7.37

6. One major benefit of using ATR-FTIR compared to regular FTIR is the quick and simple preparations for the samples to be run through the instrument. A sample simply has to be loaded onto the crystal and given pressure through the dial. Likewise, ATR-FTIR is much more useful for highly absorptive liquids and solids.

7. A key component of the ATR-FTIR instrument is the crystal used. The crystal allows for IR light to be passed through the crystal and reflected multiple times against the side of the crystal that the sample is on. The sample will produce changes in the IR signal, which will show up on the final reading.

8. The crystalline sample would result in the sample being less compact and could potentially allow outside light to affect the reading. By grinding the sample, the overall surface area of the sample is reduced, and is able to pack much more firmly together.

9. As explained in the previous question, any outside light could affect the readings. If pressure is put on the sample, the sample packs firmly together, and inherently reduces the light that could possibly reach the crystal. Without the pressure, the powder would have the tendency to spread out and could potentially leave spaces where light could enter.

10. The areas of the peaks that show up on the ATR-FTIR readings correspond to the amount of a specific compound present in the sample. In the case of this experiment, two peaks were looked at in specific, the salicylic acid peak and the caffeine peak, which correspond to the amount of each compound. By taking the ratio of the peak areas, a value corresponded to the concentration of caffeine vs salicylic acid is determined.

    It is also important to realize that the mass of caffeine cannot reliably be found through ATR-FTIR. This is because only a specific amount of the sample covers up the crystal used in the instrument. If the caffeine powder was not used along with salicylic acid, it is most likely true that the amount of caffeine would not fully cover up the crystal, and have light interfere with the readings. The filler helps to block out the outside light.

11. Most of the error likely comes from the lack of precision used in the preparation of the samples. It can be hypothesized that getting more accurate measurements to what is proposed in the procedure of the lab, that the overall results of the lab would result in less error.