DETERMINATION OF CAFFEINE CONCENTRATIONS IN 5 DIFFERENT COFFEE BEANS WITH UV/VIS SPECTROPHOTOMETER

Research Question:

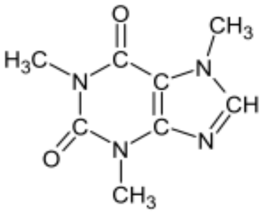
What are the caffeine concentrations (mg/L) of five different coffee beans having different origins that are India, Indonesia, Honduras, El Salvador, and Costa Rica, determined by UV/Vis Spectrophotometry?

Introduction and Background Knowledge:

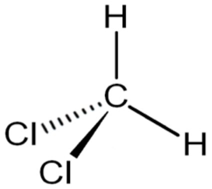
Caffeine is a chemical, a stimulant, found in seeds, nuts, or leaves of many different plants that can be found everywhere in the world, yet it is mostly seen in South America and East Asia. Its natural form is a white, odourless powder (*Caffeine _ C8H10N4O2 - PubChem*). Caffeine's chemical formula is $C_8H_{10}N_4O_2$. The plants that are mostly known which contain caffeine are coffee beans, tea leaves and chocolate beans (Abebe Belay). When this chemical is ingested, it acts on the central nervous system of people, acting as a stimulant (*Caffeine _ C8H10N4O2 - PubChem*). While acting as a stimulant and being active on the central nervous system, caffeine finds a solution to what many people try to achieve: staying awake and active (Nehlig et al.)

Most people, including myself, use coffee, so the effect of caffeine, to stay awake. Also, as told above, too, more caffeine intake induces less need for sleep. To access more caffeine, two most common ways are used: choosing the coffee with more caffeine concentration or just drinking more coffee. Of course, as drinking more coffee is much more expensive compared to drinking less of the highly concentrated coffee, people, again including myself, want to learn which coffee is more caffeinated. As a person who loves the atmosphere of staying awake late at night and working on research papers with the mere company of continually renewed coffee, I was quite curious about which origin's coffee was most caffeinated so that I would buy that one when I need to stay further awake.

This investigation aims to find, among the most consumed coffees' producer locations, which geographical place's coffee beans have the highest caffeine concentration. Therefore, out of the highest coffee producing origins, five of them - India, Indonesia, Honduras, El Salvador and Costa Rica - were chosen and their raw coffee beans were bought. Then, these coffee beans were processed to be powder, and concentrations of these coffee beans with dichloromethane were created to extract the caffeine.



Structure of Caffeine



Structure of Dichloromethane

Dichloromethane is a great solvent for caffeine to be extracted. Dichloromethane, which can be shown as CH_2Cl_2, due to its molecular geometry and dipole-dipole attractions not cancelling out, is a slightly polar hydrocarbon. Also, caffeine, $C_8H_{10}N_4O_2$, is a hydrocarbon that is slightly polar as it has some oxygen bonded in the carbonyl functional group of the molecule. As it is known that "like dissolves like," dichloromethane, by being a polar hydrocarbon, is a very suitable solvent for caffeine, another polar hydrocarbon (Bell-Young) (Kitiş). Then, the solutions were analysed with UV/Vis Spectrophotometric absorbance analysis.

This analysis is based on light absorbance. Basically, every compound has different molar light absorbance, meaning every compound absorbs the highest amount of ultraviolet light energy at different unique wavelengths. Therefore, different compounds can be identified with their unique wavelengths of absorbance. Also, as the particle number, so the concentration of a sample increases, its absorbance increases, too. This is because as more particles are present, more atoms become present to absorb more energy possibly to create a higher absorbance value. The UV/Vis Spectrophotometer shoots rays into the compounds' solution and the mechanism aims to measure the absorbed energy amount. Then, this measure is compared with the compound's pure absorbance values which are demonstrated as a calibration curve. In this experiment, the calculated absorbance values were matched to their concentrations by using the initially created calibration curve of caffeine - a curve which is constructed with known concentrations and absorbance data of pure caffeine - which represents the molar absorbance of pure caffeine.

Variables:

Independent	Different types of raw coffee beans are the independent variables. These coffee bean types differ regarding their originated countries: - Indian originated raw coffee beans - Indonesia originated raw coffee beans - Honduras originated raw coffee beans - El Salvador originated raw coffee beans - Costa Rica originated raw coffee beans The types of coffee beans independent variables are varied as they are taken from different geographical locations - they are bought from a coffee shop, yet they are confirmed regarding that they have different origins.
Dependent	The caffeine concentration of the coffee beans (mg/L) is the dependent variable. It is expected that the caffeine concentration will differ among the coffee beans as they are grown in different environmental conditions. In order to measure caffeine concentrations, solutions of raw coffee beans and dichloromethane solutions are made for caffeine extraction from the coffee beans. Then, these solutions were filtered and samples from the filtered solutions were put in UV/Vis Spectrophotometer. From the graphs recorded by spectrophotometer, the area under the caffeine's specific absorbance wavelength is calculated to find the concentration by using the calibration curve.

Controlled	**Method of Control**	**Possible Effects On The Result**
Brands of raw coffee beans	All of the raw coffee beans were shipped from the same coffee company and were confirmed regarding all having different origins.	If the coffee beans were to be the same origin, then the meaning of the independent variable would be derailed: there would be two same, non-manipulated independent variables - something that is completely opposing to the idea of independent variable.
Size of raw coffee bean powder	All of the coffee bean powders were screened with a 250-micrometre sieve for uniform and homogenous texture.	If the sizes of the powder were different, the dissolution, and so the separation, of caffeine could have been inhibited partially as big particles may not allow for higher dissolution and extraction because of having less surface area for interaction.
Mass of dissolved raw coffee bean powder	All of the raw coffee bean samples placed into the dichloromethane solutions were measured with electronic mass balance to be 0.05 grams.	If the masses of the dissolved raw coffee bean powders were different, then the amount of caffeine dissolved would be different affecting the concentration results, making the concentrations incorrect because for correct comparison of relative concentrations, the same mass is desired in order to see the ratio of caffeine concentration to the same mass of the coffee powder.
Dissolving and spinning time with the temperature applied of raw coffee solutions	All of the raw coffee bean samples' solutions were dissolved for 60 minutes on a magnetic stirrer with 60 degrees Celsius.	If the dissolving and spinning times were different from sample to sample, per time and mass, non-relative caffeine extraction would occur because the temperature, as it varies, affects the dissolving rate of the caffeine in dichloromethane.
Volume of solvent	All of the raw coffee bean powders were separately	If the dichloromethane amount were to be different in every coffee sample solution, then the comparison of the

dichloromethane in raw coffee solutions	dissolved in 50 mL of dichloromethane.	concentrations would not be correct as the volume of dichloromethane for the same mass of the coffee powder would be different, making the concentrations incomparable by altering the absorption data, not because of the coffee type, yet because of the possible different concentration occurring from dichloromethane, not the raw coffee.
Type of used lab equipment such as filter paper	For all of the filtration phases of the coffee solutions, the same filter paper with the same funnel is used.	If the filter papers were different, then the filtration of caffeine-binded dichloromethane could have been affected, either by being more filtered or less filtered, which in return would affect the absorption as there would be either less or more caffeine remaining in the filtered solution.
Brand of the pure caffeine powder	The caffeine powder used for the calibration curve is shipped from a chemistry company, so its purity is ensured.	If the pure caffeine is not pure enough, then the caffeine concentrations of the coffees would be incorrect as the caffeine concentrations of the coffee samples are calculated from the calibration curve.
The length of the cuvette used in the measurement	The length of the cuvette used for the measurements is kept constant as it would alter the molar absorbance values on different concentrations' measurements	As it is dictated in the Beer-Lambert's law, $A = \varepsilon bc$, - A is absorbance, - ε is molar absorptivity, - b is cuvette's width - the distance light travels through the solution, - c is concentration of the solution. So, as can be seen, when the b value – the cuvette's width- is altered, the absorbance changes too. Therefore, the cuvette's width is held constant.

Apparatus and Chemicals:

Apparatus:

1 x Electronic mass balance (± 0.001 g)
5 x 50 mL beaker
5 x Combined magnetic stirrer and heater
5 x Fish
1 x 25 mL graduated cylinder (± 0.5 mL)
2 x 50 mL graduated cylinder (± 1 mL)
1 x UV/Visibile spectrophotometer
5 x Quartz cuvette

1 x Sieve (250 micrometre)
6 x 100 mL Volumetric flask
1 x 25 mL Graduated pipette (± 0.5 mL)
1 x Lab Quest
1 x Computer and Logger Pro program
1 x Excel, Origin and Google Sheets programs
1 x Glass funnel
A5 Size cut-out filter paper

Chemicals:

845 mL dichloromethane
0.025 grams pure caffeine powder

Each different coffee bean powder being 0.05 grams

Risk Assessment:

Hazard	**Control Measure**
Safety: Pure caffeine powder causes damage on body: - Causes skin irritation. - Causes irritation, nausea, vomiting and similar symptoms when inhaled.	When the caffeine powder was used while creating the calibration curve, in order to prevent the skin irritation, plastic latex gloves and a lab coat covering the arms were worn. Also, in order to prevent inhalation of the powder, the experiment was carried out with surgical masks being worn (*Material Safety Data Sheet - Caffeine*).

Safety: Dichloromethane causes damage on body: - Causes skin irritation. - Causes serious eye irritation. - May cause drowsiness. - Suspected of causing cancer.	When dichloromethane was used while creating both the calibration curve and the sample coffee solutions, in order to prevent skin irritation, eye irritation, possible drowsiness and dizziness and possible cancer risk, protective clothing such as a lab coat covering the body fully, goggles, gloves, and surgical mask. On a specific note, as dichloromethane directly melts plastic down, all of the safety equipment that were worn included the minimum plastic material - especially, it is important to note that the gloves were not made out of plastic.
Environmental: Disposal of dichloromethane and caffeine.	Dichloromethane causes eradication and damage on plastic material when in direct contact. Therefore, it should not be disposed of to any drains as it can melt the plastic pipes (*Material Safety Data Sheet - Dichloromethane*). Caffeine causes lethality, oxidative stress, negative effects on energy reserves and metabolic activity, neurotoxic effects, and adverse effects on reproduction and development of terrestrial and aquatic environment species. Therefore, it should not be disposed of any drains as the drain disposal is possibly directly carried to aquatic and terrestrial environments affecting the living negatively (Li et al.) In the experiment, all of the used dichloromethane and caffeine were disposed to specifically labelled "liquid waste" containers which are later on disposed of by the government with necessary precautions.
Ethical:	There were no ethical issues spotted in this experiment as no living organisms were used in or endangered by the actions taken.

Procedure

Methodology For Calibration Curve

The Need For Creating A Calibration Curve: The aim of creating a calibration curve is to see which concentration of caffeine corresponds to which absorbance value. In the particle level, as known, when the concentration, so the molecule amount, increases, the absorbance proportionally increases too. By obtaining such a graph and drawing the best fit line to it, an approximate absorbance to concentration proportionality is obtained – such a proportionality can be deduced as different caffeine concentrations' absorbance values are obtained by the after-mentioned methodology.

Preparing The Pure Caffeine Stock Solution (1 M – grams per litre) - (Talab).

1. Weigh 0.025 grams of pure caffeine powder with an electronic mass balance by the aid of a weight boat.
2. Measure, 250 mL dichloromethane with a 50 mL graduated cylinder - measure 5 times full 50 mL and pour all of them into a 250 mL volumetric flask.
3. Put the 0.025 grams of pure caffeine powder into the 250 mL dichloromethane to make the 100 mg/L (100-ppm) stock solution of caffeine and dichloromethane.

Preparing The Pure Caffeine Standard Solutions - (Talab).

1. By using two separate graduated pipettes to transfer the dichloromethane and caffeine stock solution, create 6 different standard solutions for the calibration curve.
    a. To make 5 mg/L caffeine concentrated dichloromethane solution, take 5 mL stock solution and dilute it to 100 mL in a 100 mL volumetric flask.
    b. To make 10 mg/L caffeine concentrated dichloromethane solution, take 10 mL stock solution and dilute it to 100 mL in a 100 mL volumetric flask.

   c. To make 15 mg/L caffeine concentrated dichloromethane solution, take 15 mL stock solution and dilute it to 100 mL in a 100 mL volumetric flask.
   d. To make 20 mg/L caffeine concentrated dichloromethane solution, take 20 mL stock solution and dilute it to 100 mL in a 100 mL volumetric flask.
   e. To make 25 mg/L caffeine concentrated dichloromethane solution, take 25 mL stock solution and dilute it to 100 mL in a 100 mL volumetric flask.
   f. To make 30 mg/L caffeine concentrated dichloromethane solution, take 30 mL stock solution and dilute it to 100 mL in a 100 mL volumetric flask.

Measuring The Absorbance With UV/Vis Spectrophotometer - (Kitiş).

1. Take samples from all of these standard solutions (the solutions that are mentioned in the part "Preparing The Pure Caffeine Standard Solutions" as bullet point labelled: a, b, c, d, e, f) and put them into separate quartz cuvettes to take the absorbance measurements of them with UV/Vis Spectrophotometer.
2. Record the wavelength - absorbance graphs from the spectrophotometer with Lab Quest.
3. Transfer these recorded graphs to the computer and analyse them in the program Origin.
4. Detect the peaks of caffeine at 274 nm.
   a. The peaks under the 274 nm are chosen for the caffeine's absorbance analysis as they are known to be the highest absorbance occupying peaks. As every compound has its highest absorbance peaks at a different wavelength, it is logical to use its highest absorbance peaks' to differentiate the caffeine from other compounds and analyse its absorbance (Ahmad Bhawani et al.).
5. By using the Origin program, calculate the areas under the 274 nm peaks of caffeine for all of the 6 standard solutions that are measured.
6. With the area's numerical values and their corresponding concentrated solutions, create a calibration curve in which the x-axis is the concentrations of the solutions and the y-axis is the area under the peaks, so the absorbance.

Methodology For Determination of Coffee Beans Caffeine Concentration

Sample Preparation - (Weldegebreal et al.).

1. Screen all of the 5 different raw coffee beans from a 250 micrometre sieve separately.
2. Place the 5 different 0.05 grams of coffee beans into 5 different 50 mL beakers.
3. To all of these 5 beakers, add 25 mL of dichloromethane by measuring them with a 25 mL graduated cylinder.
4. Place a fish into each one of the beakers and place the beakers onto magnetic stirrers.
5. Stir the solutions for 60 minutes while heating them at 60 degrees Celsius for relatively easy removal of caffeine from the solution.
6. After 60 minutes, filter the solutions separately into 5 different test tubes.
   a. For filtration, use a funnel: place the filter paper onto the funnel and pour the solution into the funnel, onto the placed filter paper, and wait for the filtration.
7. Prepare five different coffee beans' caffeine extracted dichloromethane solutions ready to be taken samples.

Caffeine Concentration Measurement - (Weldegebreal et al.).

1. From the 5 different samples, prepare 5 filled quartz cuvettes.
2. Put the cuvettes into the UV/Vis Spectrophotometer one by one and each time, record the absorbance - wavelength graphs.
3. Repeat the first two steps for two more times to get 3 trials per sample.

Analysis of The Gathered Measurements - (Weldegebreal et al.; Kitiş).

1. In order to obtain an absorption peak of the caffeine molecule, the raw absorbance - wavelength curve was decomposed to the four different main peaks. These decomposed peaks are called "simulated" peaks, and the absorbance peak at 274 nm was assigned to the caffeine molecule (Kitiş).

Raw data is composed of these four simulated peaks and an exponentially decaying background curve. All analyses were done by decomposition of this background and simulated peaks.

   a. The other three simulated peaks are caused by other molecules which can be on any coffee bean. In other words, every chemical has its own absorbance wavelength and the samples not only have caffeine, but also other chemicals.

2. The simulation procedure is as follows: The absorption data is generated by excel for corresponding wavelength for each peak according to the Gaussian equation which is commonly used in analysis of absorbtion and/or emission spectra:

$$y = A^{-(\frac{x-x_c}{2w^2})}$$

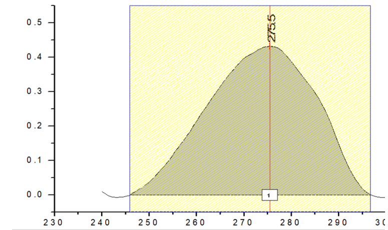
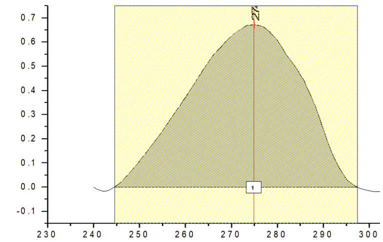
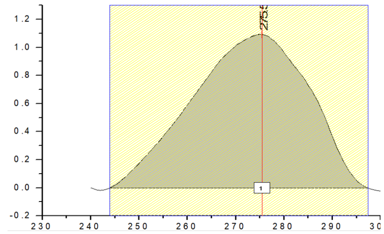
   Where, A is intensity of peak, x is the wavelength, x_c is the centre of the peak, and w is the width of the peak (Belay et al.). The generated, simulated, data curves for each peak and exponential decaying background curve are summed - shown by the red solid line - in order to fit experimental (raw) absorbance peak (black circle) as shown in Graph 9 to Graph 13.
3. Decomposed caffeine peak is analysed by Logger Pro in order to calculate the area under the caffeine peak separately.
4. The above procedure is applied to the other samples, too.
5. The calculated area corresponding sample were drawn in graphs 1 to 5 according to their concentration in mg/L.

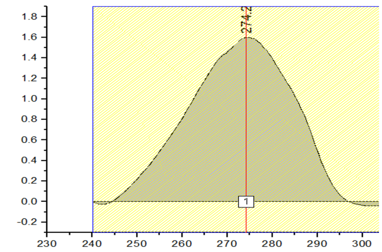
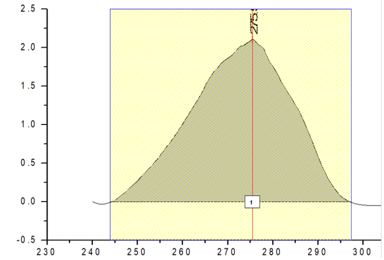
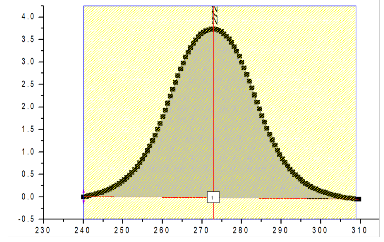
Raw Data: All the full raw data graphs are shown in the "Appendix" section.

Processed Data:

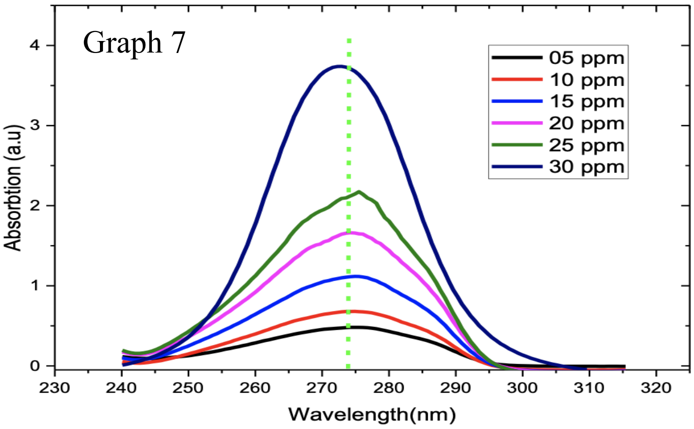
Calibration Curve Data Analysis - Caffeine Peaks With Shaded Areas

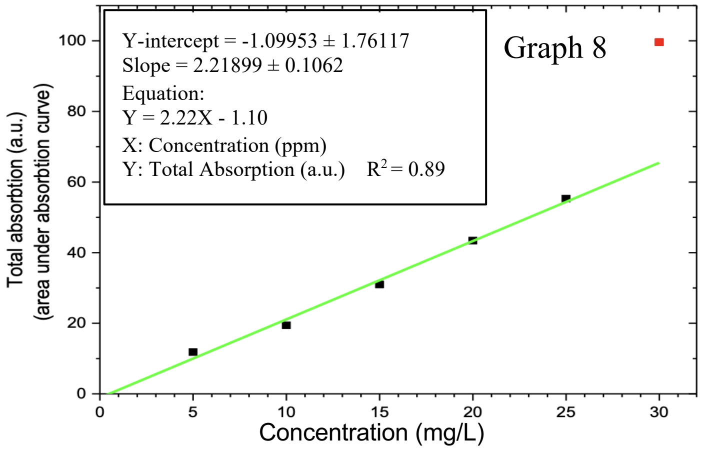
The below graphs belong to different pure caffeine concentrations' "absorbance (a.u.) vs. wavelength (λ)" graphs. Their areas under the 274 nm peaks are interpreted as the absorbance amount and used to construct the calibration curve.

5 ppm - Graph 1	10 ppm - Graph 2	15 ppm - Graph 3
 Area Under Peak: 11.8	 Area Under Peak: 19.4	 Area Under Peak: 31.0

20 ppm - Graph 4	25 ppm - Graph 5	30 ppm - Graph 6
 Area Under Peak: 43.3	 Area Under Peak: 55.3	 Area Under Peak: 99.6

All Concentration Curves For Different mg/L - Resultant Under Area of Corresponding Concentrations (Graph 7) and The Caffeine Calibration Curve (Graph 8)

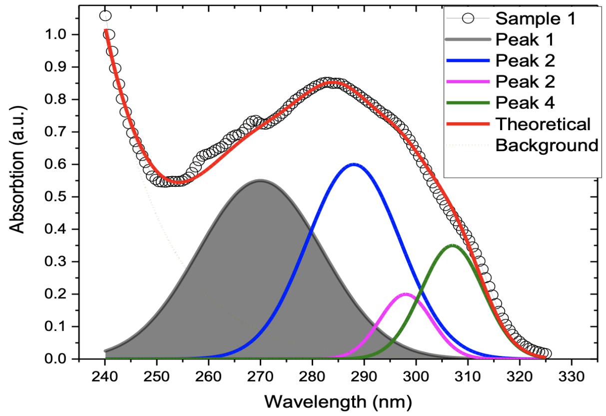




Coffee Sample's Simulated Data:

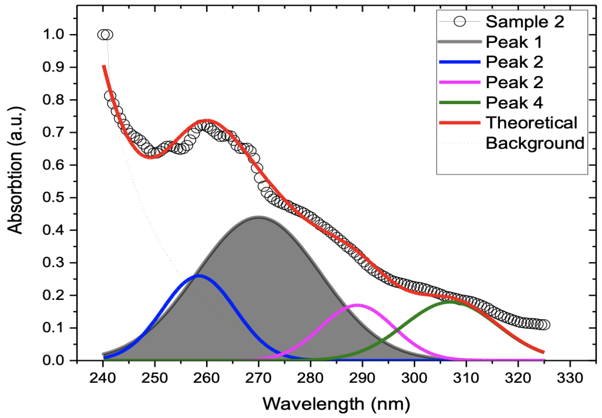
Data obtained from samples were analysed by the above-mentioned Gaussian equation (the equation mentioned in the part "Analysis of The Gathered Measurements" at the methodology section) which is used for UV visible absorption spectrum. The black circles show the sample's average experimental absorption peak. The blue, magenta, green and shaded grey peaks belong to the composite molecules such as tannin, and thiamine. Especially, shaded grey peak, located at 274 nm, belongs to the caffeine of the sample. The summation of these four peaks gives us the theoretical absorption peak shown as the red solid line. The caffeine peak's under-area was calculated and demonstrated below their corresponding graphs.

India Originated - Graph 9



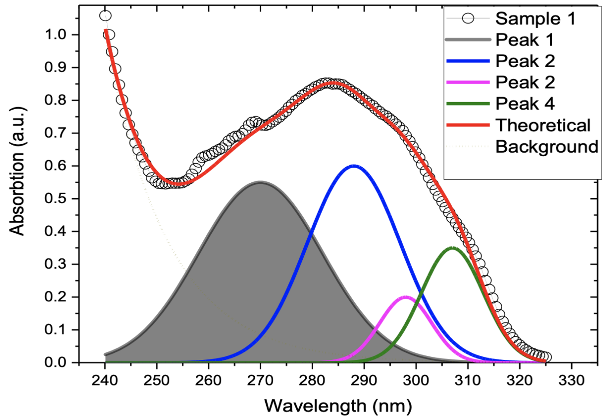
Area Under The 274 nm (grey) Peak: 16.4
Calculated Caffeine: 7.89 mg/L

Indonesia Originated - Graph 10



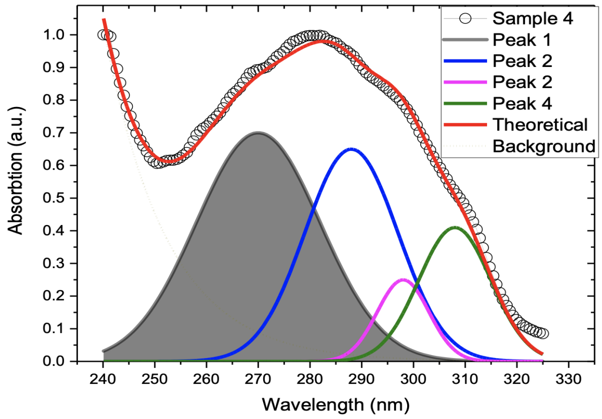
Area Under The 274 nm (grey) Peak: 13.1
Calculated Caffeine: 6.42 mg/L

Honduras Originated - Graph 11

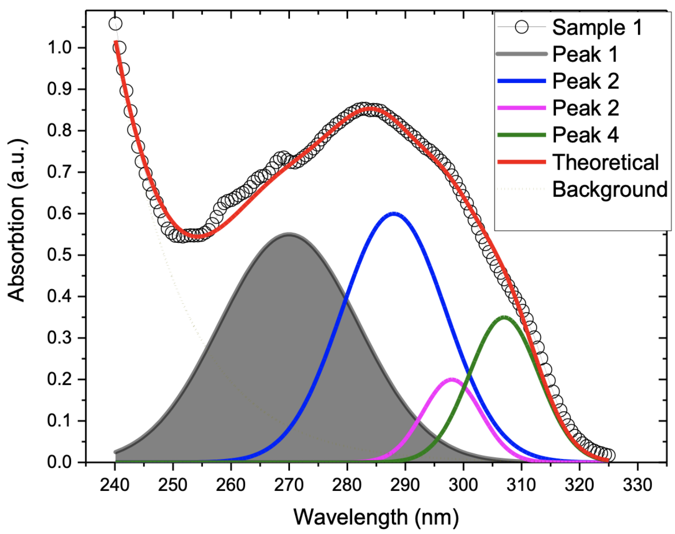


Area Under The 274 nm (grey) Peak: 10.16
Calculated Caffeine: 5.07 mg/L

El Salvador Originated - Graph 12



Area Under The 274 nm (grey) Peak: 20.9
Calculated Caffeine: 9.92 mg/L



Costa Rica Originated - Graph 13

Area Under The 274 nm (grey) Peak: 21.5
Calculated Caffeine: 10.2 mg/L

Sample Calculation:
y = 21.5 a.u. → Plug in this value to the equation of the calibration curve.
"y = 2.2x - 1.1". → 21.5 = 2.2x - 1.1 → This equation is solved in order to find x.
x = 10.2 mg/L → This value represents the Costa Rica originated concentration of caffeine

Above, all of the graphs are the average absorbance graphs of the 3 trials that are carried out which can be found in the "Appendix" section. In each graph, after the area under 274 nm caffeine peak was found, the value was plugged into the calibration curve's linear equation "y = 2.2x - 1.1" as the y value - the area under the curve. Then, the x value was calculated which corresponds to the caffeine concentration in mg/L. A sample calculation is carried out for the "Graph 13"s calculated caffeine. The calculation can be found next to the graph.

Uncertainty Calculation:

Percentage (%) Uncertainty: $\frac{Absolute\ Uncertainty}{Reading}\ x\ 100\ = Percentage\ Uncertainty\ (\%)$

Uncertainty Calculation For Calibration Curve:

- $\frac{0.001\ (Uncertainty\ of\ Electronic\ Mass\ Balanced)}{0.025\ (The\ Used\ Mass\ of\ Pure\ Caffeine)}\ x\ 100 = 4\ \%\ uncertainty$ while measuring the pure caffeine in grams.
- $\frac{1\ (Uncertainty\ of\ Graduated\ Cylinder)}{50\ (The\ Used\ Volume\ of\ Dichloromethane)}\ x\ 100\ =\ 2\ \%\ uncertainty$ while measuring the pure dichloromethane in millilitres.
- $\frac{2\ (Uncertainty\ of\ UV/Vis\ Spectrophotometer's\ Wavelength)}{850-220\ (The\ Range\ Of\ The\ Wavelength)}\ x\ 100 = 0.317\ uncertainty$ while the UV/Vis Spectrophotometer uses the wavelength as a nanometer.
    - Total percentage uncertainty (%) is 4 % + 2 % + 0.317 % = 6.32 %

Uncertainty Calculation For Sample Preparation:

- $\frac{0.001(Uncertainty\ of\ Electronic\ Mass\ Balanced)}{0.05\ (The\ Used\ Mass\ of\ Coffee\ Samples)}\ x\ 100 = 2\ \%\ uncertainty$ while measuring the coffee sample in grams.
- $\frac{0.5\ (Uncertainty\ of\ Graduated\ Cylinder)}{25\ (The\ Used\ Volume\ of\ Dichloromethane)}\ x\ 100 = 2\ \%\ uncertainty$ while measuring the pure dichloromethane in millimetres.
- $\frac{2\ (Uncertainty\ of\ UV/Vis\ Spectrophotometer's\ Wavelength)}{850-220\ (The\ Range\ Of\ The\ Wavelength)}\ x\ 100 = 0.317\ uncertainty$ while the UV/Vis Spectrophotometer uses the wavelength as a nanometer.
    - Total percentage uncertainty (%) is 4 % + 2 % + 0.317 % = 6.32 %

Conclusion

This research aims to determine different caffeine concentrations of different coffee beans. With the conducted research, the question "What are the caffeine concentrations (mg/L) of five different coffee beans having different origins that are India, Indonesia, Honduras, El Salvador, and Costa Rica?" was able to be answered. As can be seen by the above data that is found, the area under the absorption peaks of samples were used to calculate the caffeine concentration by calibration curve: "y = 2.2x - 1.1" where "x" is the caffeine concentration in mg/L and "y" is the area under the caffeine peak at 274 nm. The calculated caffeine concentrations are found as 7.90 mg/L, 6.42 mg/L, 5.07 mg/L, 9.92 mg/L, and 10.2 mg/L for samples Indian originated, Indonesian originated, Honduras originated, El Salvador originated and Costa Rica originated coffees, respectively.

As a result of the caffeine concentration findings:

- Caffeine concentration linearly depends on total UV visible absorption - the area under UV visible absorption peak. Such linear trends are observed in the studies in literature, too (Weldegebreal et al.; Talab; Belay et al.; Ullah; Li et al.; Demissie et al.) This study is parallel with literature, too, as, even though there is an approximate 6 % uncertainty and an outlier which is not included in the best fit line, a linear correlated trend can be found. The outlier mentioned is discussed in the "Evaluation" section.
    - In the found linear trend, a R-squared value of 0.89 is reached. An R-squared value shows how much the "y" values change with the affect of "x" values. So, in this case, this means that the absorbance depends on the increasing concentration of caffeine values. This dependence is 89 %, and it is not more than this because the caffeine that is used may not be completely pure.
- Each of the unroasted green coffee beans have various caffeine content.
    - The tested caffeine brands show that coffee beans include about 5 - 10.2 mg/L caffeine. These results are in harmony with the literature values which are ranging between 1 - 15 mg/L. (Ullah; Demissie et al.; Yu et al.). As the percentage uncertainty is not very high, it did not affect the values making them distorted either higher or lower than the literature values stated.
- The fact that "Dichloromethane is a suitable solver that can be used in order to extract caffeine as it can dissolve caffeine better than many other different liquids such as water" was verified. Even in making decaffeinated coffee, dichloromethane is used to rinse the coffee beans to make their caffeine go away (Bell-Young).
- While pure caffeine has one absorption peak, coffee beans' solutions have more than one peak. These peaks, the ones that are not corresponding to caffeine may correspond to other chemicals such as tannin, thiamin, xanthine, and spermidine. As the coffee beans are not pure caffeine.

Evaluation

Every coffee bean in the world probably has slightly different caffeine concentrations as their chemical ingredients are made up by their development, and their development is carried out by genetic codes which continually vary within individual plants, and so continually vary within plant parts such as coffee beans, too. Yet, the origins from which the coffee beans are taken would differ significantly regarding the coffee beans' caffeine concentrations as different origins have different environments directly affecting the chemical composition of coffee beans just as like the genetic codes. Even though the different caffeine concentrations were found in different coffee beans, in literature, there is no definite concentration value for caffeine with which this experiment's results could be discussed. Yet, caffeine concentration ranges for differently originated coffee beans are present in literature. Thus, the obtained data can be compared with the literature ranges at the best, by inserting the uncertainties.

All of the percentage uncertainties of both sample data collection part and the calibration curve part were around 6.32 %, yet the results found that the caffeine concentrations of green coffee beans were in the commonly seen literature value ranges, demonstrating the validity of the results.

In the calibration curve, there was an outlier. This anomaly was not included in the linear best fit line as it would affect the line's slope, and so the found concentrations. This anomaly was found in the 30 mg/L concentrated caffeine - dichloromethane solution's recording. The outlier was present because the equipment available for use in the lab - the UV/Vis Spectrophotometer - used to measure the absorption was unable to measure absorption higher than 3 a.u. Therefore, a quadratic fit was applied to the already measured data points. Yet, as in the already measured data points, there were no points which had absorption higher than 3 and as the slope rose to the 3 a.u. was very high, the quadratic fit made the absorption peak very high, resulting in an outlier.

Additionally, a hypothesis is not stated in the report because there is no correlation that is sought. Instead, the sought aim is to find the caffeine concentration in different coffee beans which are not related to any correlation. Also, it is very hard to have a definite hypothesis because the coffees in different origins have their caffeine concentrations changing as the environment they live in change, too. Therefore, it was very hard to find any definite prior knowledge and literature information as the concentration of the caffeine in the coffee beans change as their environment change, too.

Strengths and Their Significance
Precise and Elaborate Material Usage: The usage of UV/Vis Spectrophotometer allowed for a more precise and possible analysis of caffeine as the UV/Vis Spectrophotometer can give continual reading of every wavelength starting from 200 nm to 800 nm. Due to this precision and elaboration present in the spectrophotometer, as my data fits into the literature value ranges, the data collected becomes reliable.
Quality of The Data Collected: The data collected as caffeine concentrations were quite qualified and reliable as a peak-simulation procedure was used. With the simulation procedure, it was ensured that the area under the peak (a.u.) was only belonging to the caffeine chemical, and not to any other. Therefore, by the simulator, it was ensured that the only measured concentration would belong to caffeine.

Weaknesses / Limitations	**Improvements**
Dichloromethane (DCM) has a very high viscosity - it is nearly half as small as water's viscosity. Thereby, it is very hard to transfer DCM within between the flasks as it was not able to stay in any of the pipettes used. In the experiment, transferring the DCM was problematic and reflected in the evaluation as a random error. Its error could have affected the result in a way like it could have created faulty solution concentrations both in the solutions created for the calibration curve and coffee's sample solutions leading to altered results and an inappropriate calibration curve.	In order to prevent the hardship of DCM transfer due to its low viscosity, better equipment with better vacuuming could be imported to the school's lab and used. As a substitute, with using the cohesion, the transferring obstacles could potentially be eliminated. The pipette could first be rinsed with DCM, and then emptied for better usage as there will be DCM particles remaining in the pipette which would create the cohesive forces. When more DCM is taken into the pipette, the DCM droplets remaining in the pipette from the rinsing could hold the newly drawn DCM by cohesive forces.
Dichloromethane is a highly volatile liquid. Thereby, it easily turns into a gaseous state. It is even more easy for DCM to turn into a gaseous state when it is heated as the kinetic energy of its particles would easily increase. Also, because DCM is an organic molecule, it can pass through many different substances, too, when in gaseous state. In the experiment, when the DCM solution of coffee beans was created, for caffeine to dissolve, the DCM - coffee bean solutions were stirred and heated. In this process, they were covered with parafilm and a watch glass on top. Yet, still some of the DCM may have leaked out from the covered beakers throughout the stirring - heating process. By the leakage, the amount of DCM used for dissolving each coffee bean would change, changing the concentration of caffeine dissolved as the volume of the solution would decrease. This would in return affect the final results, making the caffeine measured more concentrated as more coffee would dissolve in the solutions. In the	To prevent the DCM gas leakage, better equipment such as beakers with gas-leakage-inhibiting lids can be imported to the school's lab and used. But, it should be of the utmost importance to use a lid that is not organic for the DCM to not dissolve through it. As a substitute, an Erlenmayer flask could have been used with a silicone stopper in order to avoid DCM leakage. The silicone stopper would inhibit the gas from leaving the flask. Thereby, the amount of DCM in which coffee beans dissolve - a controlled variable - wouldn't be affected. Thus, the final results would be accurate, especially valid in the case where a comparison can be done.

experiment, high volatility of the DCM making it leak out was problematic and reflected in evaluation as a random error.	
UV/Vis Spectrophotometer is the device used to measure the absorbance of the caffeine. Even though many of the UV/Vis Spectrophotometers have a maximum range of absorbance measurement higher than 3 arbitrary units, the UV/Vis Spectrophotometer in the school does not have a maximum range higher than 3 arbitrary units. Therefore, readings higher than 3 arbitrary units weren't able to be taken which restrained the measured absorbance values - the ones higher than 3 arbitrary units. In the experiment, the UV/Vis Spectrophotometer not being able to measure higher than 3 arbitrary units was problematic and reflected in evaluation as a systematic error.	As an improvement to the problem regarding UV/Vis Spectrophotometer two different ways can be implemented. The first way is to import a UV/Vis Spectrophotometer with maximum range higher than 3 arbitrary units to the school's lab. The other way can be to dilute every solution to a lower concentration before measuring them. After diluting the sample dichloromethane - caffeine solutions, every coffee solution's absorbance could have been measured while being in the range of maximum 3 arbitrary units, and still, a comparable concentration of caffeine would be obtained.
UV/Vis Spectrophotometer device is used with an apparatus named cuvette. The sample from which the absorbance data is desired to be measured is poured into the cuvette and the cuvette is inserted into the machine. For the poured solution's mere absorbance to be found, there should be no impurities or other chemicals in it. Yet, within the sample's measurement taking processes, the cuvette is rinsed with distilled water. Due to this rinsing, water may be present in the cuvette as an impurity with the sample solution possibly altering the obtained data, making it either less or more concentrated. In the experiment, using cuvette possibly having impurities in it was problematic and reflected in evaluation as a random error.	To prevent any impurity such as water to be a part of the absorbance measurement of the desired solution, the cuvette rinsed with distilled water can be left out for a long time to get all of the water to dry out. As a substitute, for cleaning of the cuvette, an alcohol with high volatility point such as ethanol can be used for fast evaporation of the cleaning material from the cuvette. Thus, the cuvette would both be clean and become ready rapidly.

Extensions

As extensions, several actions can be taken to improve and positively alter this experiment:

- A new independent variable which also has caffeine in its composition can be added such as different teas so that a comparison within between coffee and tea can be carried out, too. This extension would be effective because other than coffee, tea is one of the most bought and drunk beverages both for the awakeness and for entertainment purposes. With the comparison of tea and coffee, a different type of beverage could have been presented to the readers for them to choose within between.
- A new dependent variable could be added: measuring the acidity of the coffee samples. As the caffeine content measurement aimed to find the most caffeine concentrated coffee bean origin for people to choose, the acidity could have had the same aim. As different people could have different taste preferences, acidity, if it had been measured, could have added a new parameter for deciding which coffee to drink.

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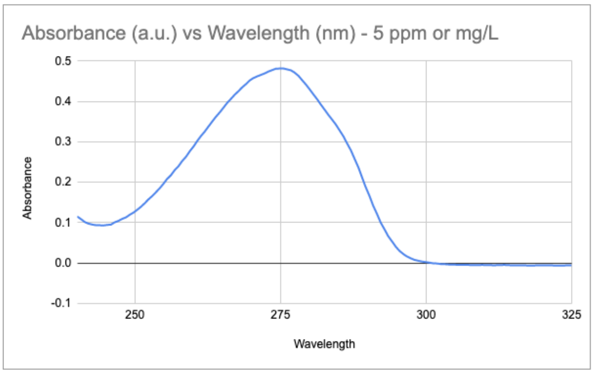
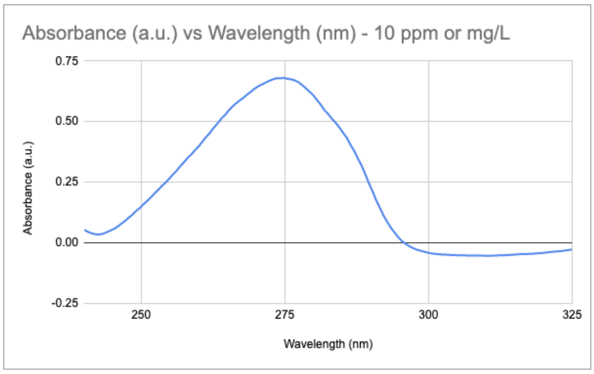
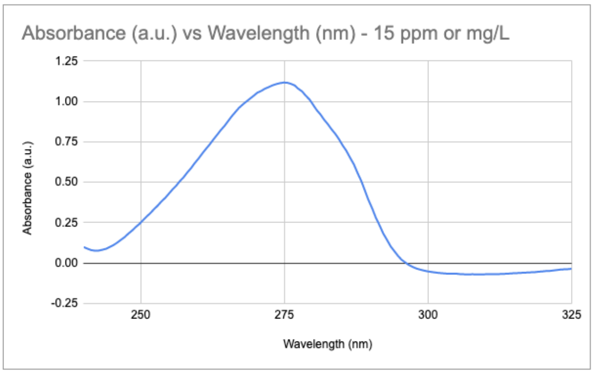
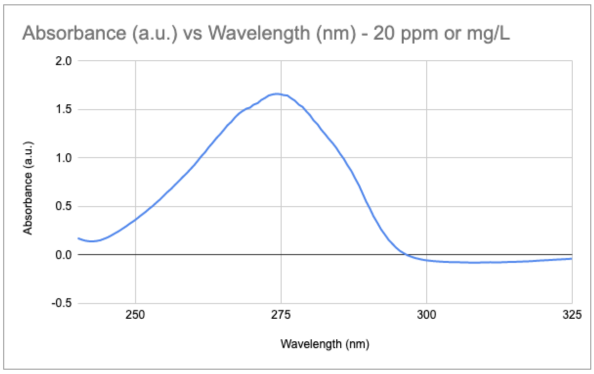
Appendix

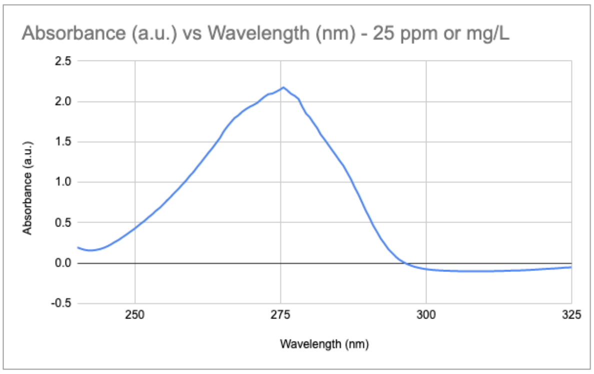
Appendix A: Calibration Curve Raw Data

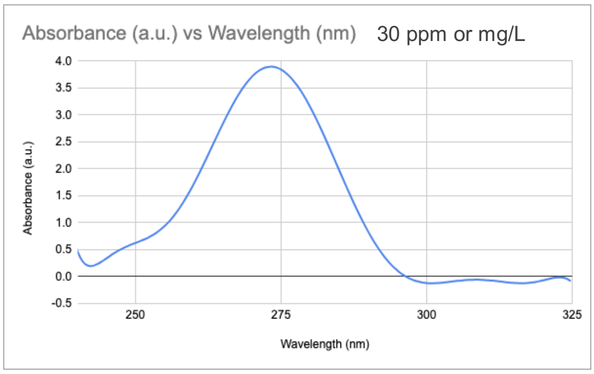




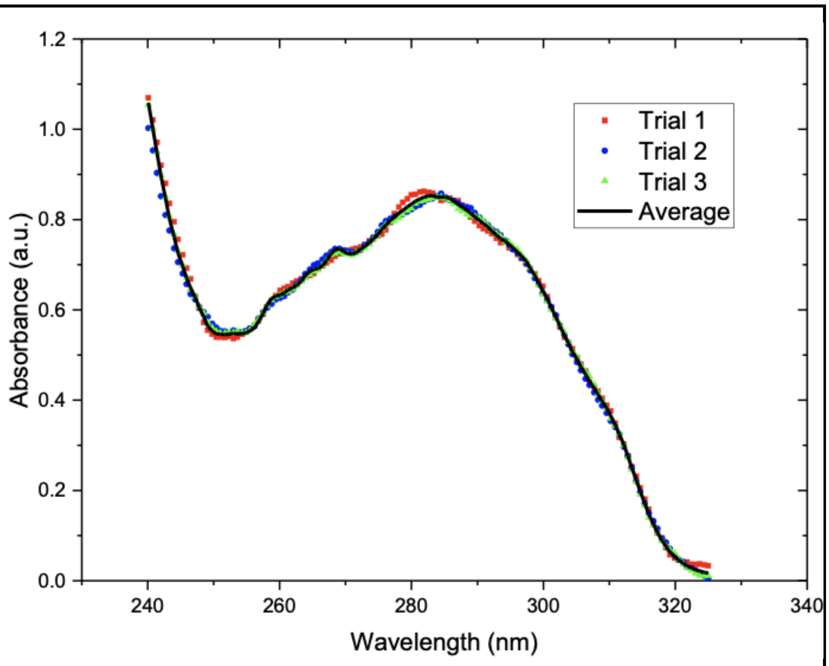
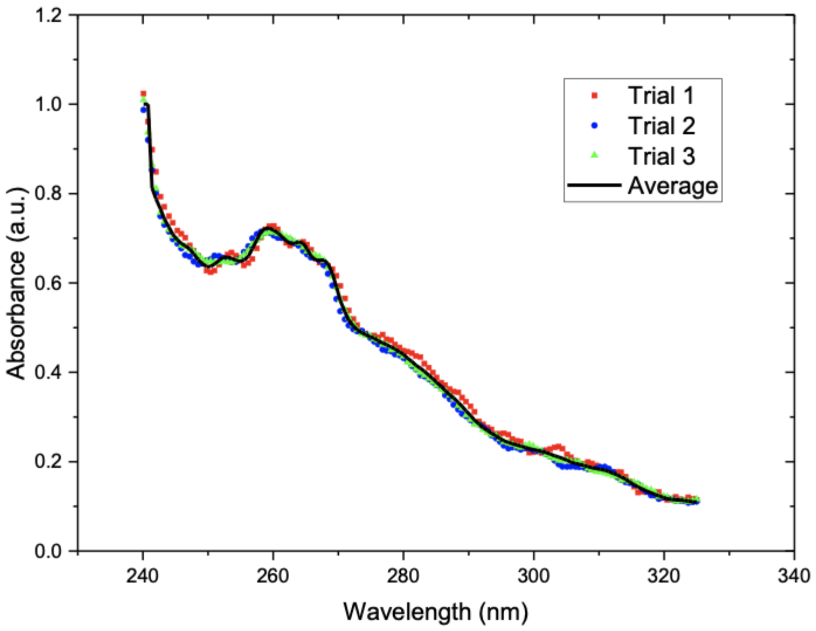


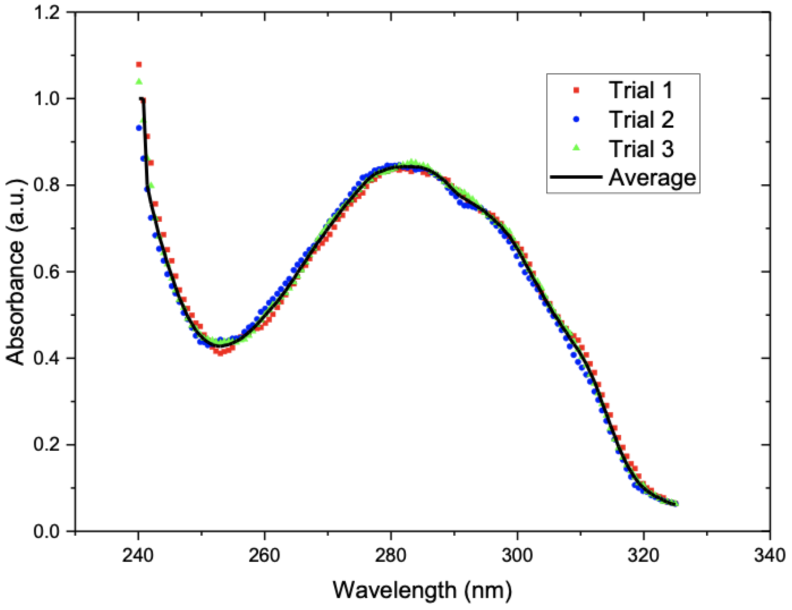
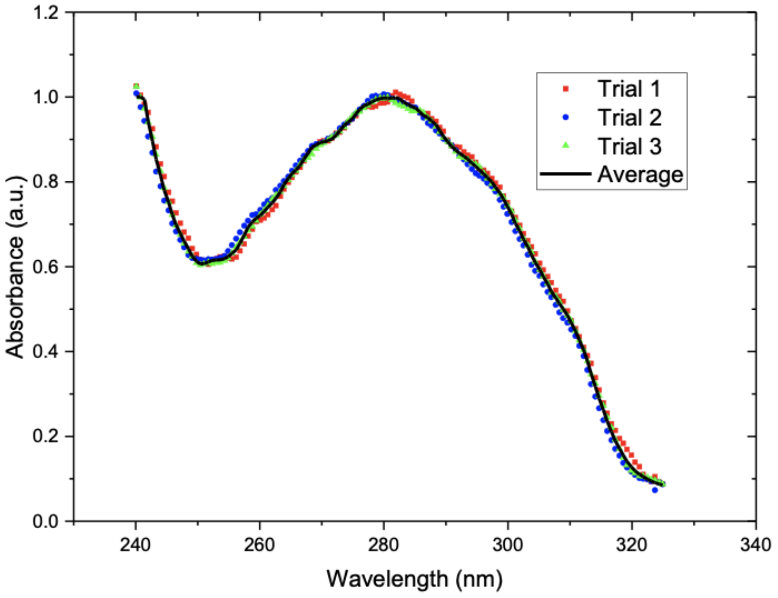






Appendix B: Coffee Sample Raw Data:

Indian Coffee Beans	
Indonesian Coffee Beans	

Honduras Coffee Beans	
El Salvador Coffee Beans	
Costa Rica Coffee Beans