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What is the Variation in Raw Caffeine Concentration Between Different Brands of Yerba Mate and Coffee Extracts?

Introduction:

Growing up in an Argentine family, I have regularly consumed *yerba mate*, a caffeinated drink, as it is used in the tea beverage *mate* which forms a part of the argentine culture. Additionally, my parents' frequent consumption of coffee has turned into somewhat of a refined passion for them. Personally, curious about the differences in concentration of caffeine in coffee versus *yerba mate*, I set out to find the concentration of caffeine within each drink.

Figure 1. An image of a *mate* beverage.[1]

Figure 2. An image of a coffee beverage.[2]

Caffeine is a central nervous system stimulant which reduces drowsiness and increases mental and physical processes.[3] It is classified as an alkaloid (nitrogenous base) and exists as a white solid at room temperature.[4] Caffeine appears in many beverages such as coffee, *mate*, and pop drinks, and is "the most widely used psychoactive drug in the world." [5]
The tea beverage *mate* is made using *yerba mate*, a plant which contains three xanthines, purine bases found in most body tissues and fluids, including caffeine.[6] Coffee is a widely popular drink that contains caffeine and is formed from roasted and brewed coffee beans. A commonly used technique to extract caffeine from caffeinated beverages relies on the addition of a solvent immiscible with water such as dichloromethane (CH_2Cl_2).[7] As the caffeine has a greater solubility within the dichloromethane (140mg/mL) than within a water-based solution (22mg/mL), it would flow out of the yerba mate or coffee solution and into the solvent.[8]
This addition causes the caffeine to move into the solvent which can then be separated from the coffee.[9] By heating the solvent at its boiling point, the solvent is evaporated, and the pure caffeine can be extracted. My experiment was based upon this principle in order to extract the pure caffeine from varying solutions of mate and coffee.

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[1] Cetron, Joshua. 2014. "I.MU.SE.O » El «tabaco» Del Sur: La Historia De Obsesión Con La Yerba Mate En América Colonial".
[2] "Join Us for Coffee Hour On The Lanai! | University of Hawai'i At Mānoa Department Of Biology".
[3] "Caffeine: The Chemistry Behind the world's Most Popular Drug". The University of Kansas.
[4] "Experiment 9: Extraction of Caffeine". Indiana State University.
[5] Harvard
[6] Pubchem
[7] "Experiment 9: Extraction of Caffeine". Indiana State University.
[8] "Experiment 9: Extraction of Caffeine". Indiana State University.
"Extraction Of Caffeine From Tea (Theory): Biochemistry Virtual Lab II : Biotechnology And Biomedical Engineering : Amrita Vishwa Vidyapeetham Virtual Lab".
[9] "Experiment 9: Extraction of Caffeine". Indiana State University.

The results of this experiment may potentially have real world ramifications for both the beverage industry and the average consumer, as data would be obtained to quantify the amount of pure caffeine within different brands of coffee and yerba mate.

Figure 3. The molecular structure of caffeine.[10]

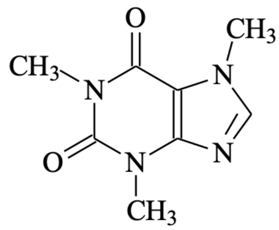
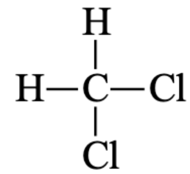


Figure 4. The molecular structure of dichloromethane.[11]



Research Question:

What is the variation in raw caffeine concentration between different brands of yerba mate and coffee powder?

Variables:

Independent variable:

Brand of yerba mate and coffee powder.

As the amount of raw caffeine is directly dependent upon the caffeinated solution, the brand of the substance was varied in order to observe the changes in caffeine concentration. By using 5 different brands (2 different brands of coffee and 3 different brands of yerba mate), the reliability of the results was increased as a larger data set would reduce random error and allow for more accurate observations.

Dependent variable:

Mass of raw caffeine obtained.

The mass of the raw caffeine was chosen as the dependent variable as its procurement is necessary to calculate the concentration of caffeine within the original solutions.

Controlled variable:

1. *Mass of initial extract (yerba mate/coffee powder).*
   Since the mass of caffeine is proportional to the mass of the total extract, changing the mass of the extract will affect the amount of caffeine obtained, and thus its concentration.
   Method: 8.0 x 10 g measured for each extract.
2. *Volume of water added to solution.*
   As the concentration of the caffeine is reliant on the original volume of the solution, a change in this original volume will affect the concentration of caffeine. By diluting the caffeinated solution, the concentration of raw caffeine will decrease.
   Method: A total of 6.0×10^2 mL of hot water was added to the original solution. This volume would allow for 5 trials to be conducted using the same liquid solution.

[10] "Experiment 9: Extraction of Caffeine". Indiana State University.
[11] Ibid.

3. *Volume of dichloromethane added to solution.*
   Increasing the volume of dichloromethane may affect the mass and subsequently concentration of caffeine obtained from the caffeinated solution as a greater volume of dichloromethane may separate more raw caffeine from the liquid solution.
   Method: A total of 25mL of dichloromethane is used to wash the solution and then is filtered out and recollected using a separatory funnel. This volume was chosen as it allows for the solution to remain below the separatory funnel's total 150mL capacity. Furthermore, the approximately 20% of each sample of 125mL of caffeinated liquid and dichloromethane is a significant percentage which increases the reliability of the data.
4. *Number of distillations.*
   Similar to the volume of dichloromethane added, by increasing the number of distillations, increasing the number of distillations may separate more raw caffeine from the liquid solution.
   Method: Two distillations using 15mL and 10mL dichloromethane respectively are performed. The number of distillations was not increased beyond two as two distillations are sufficient to provide reliable data. Furthermore, more distillations would require the use of more dichloromethane, which would decrease the total amount of dichloromethane available for future trials and impact the total number of trials able to be performed.

Method:

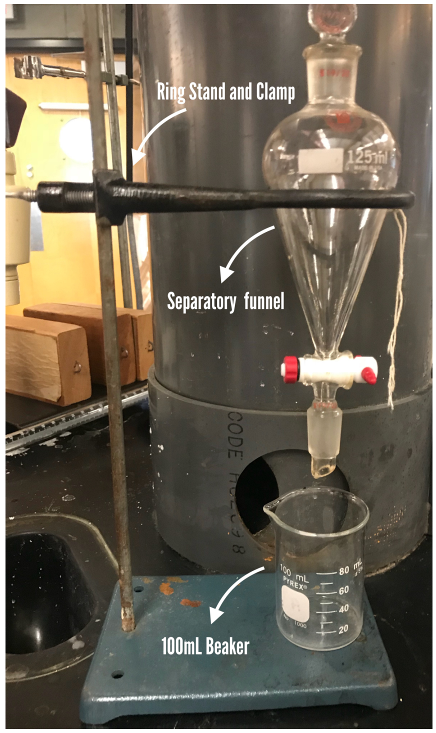
Materials and Reagents:

Table 1. Summarizes the use of Materials and Reagents.

Materials	Reagents
Hot Plate	Yerba Mate extract (8.0 x 10g)
6.0×10^2 mL water	Coffee powder extract (8.0 x 10g)
250mL Beaker (x2)	Dichloromethane CH_2Cl_2 (140mL)
100mL Beaker (x5)	
100mL graduated cylinder	
10mL graduated cylinder	
Separatory funnel	
Ring Stand and Clamp	
Digital Scale	
French Press	
Gloves	

Photograph of Set-Up:

Figure 5. A labeled photograph taken by the student shows the initial setup for the experiment.



Experimental Procedure:

1. Measure 8.0 x 10g of substance and place inside the French Press.
2. Bring 6.0×10^2 mL of water to a boil and pour into the French Press.
3. Filter out the solid substance using the French Press to obtain a liquid caffeinated solution.
4. Pour 140mL of dichloromethane into a 250mL beaker.
5. Measure and record the mass of a 100mL beaker.
6. Pour 1.0×10^2 mL of the liquid solution into a 100mL graduated cylinder.
7. Measure and record volume of solution and transfer it into a separatory funnel.
8. Using a 10mL graduated cylinder, measure 15mL (1.0 x 10mL and then 5mL) of dichloromethane, pouring into a separate 250mL beaker after measuring.
9. Add 15mL dichloromethane to separatory funnel, cap separatory funnel, and shake lightly to prevent an emulsion from forming.
10. Collect the clear dichloromethane layer into a 100mL beaker, without adding any of the unclear layer directly above it.
11. Repeat steps 8-10 with 1.0 x 10 mL of dichloromethane, collecting the dichloromethane into the same beaker.
12. Boil the remaining dichloromethane solution at 135°C on a hot plate under a fume hood to evaporate the dichloromethane.
13. Bring the beaker off of the heat, let it cool for 5 minutes, and measure and record the new mass of the beaker.
14. Subtract the beaker's original mass from the new mass in order to obtain the mass of the raw caffeine obtained.
15. Use the recorded value of the liquid solution in order to find the concentration of the raw caffeine.
16. Repeat steps 5-15 four more times to obtain 5 runs per liquid solution.

Photographs of Procedure:

Figure 6. Shows steps 4 & 8 of the procedure in which the dichloromethane is being measured.

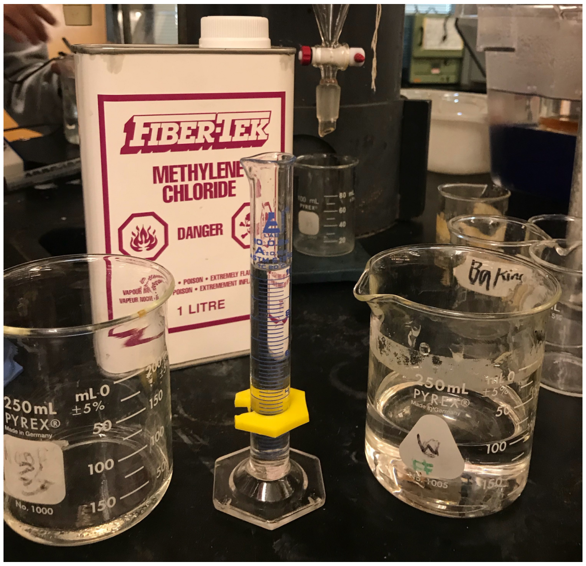


Figure 7. (Left) Shows the liquid solution (Step 6.)
Figure 8. (Middle) Separatory funnel with liquid solution
Figure 9. (Right) Depicts the filtration process in which dichloromethane is slowly seeped out of the separatory funnel (Steps 8-11)

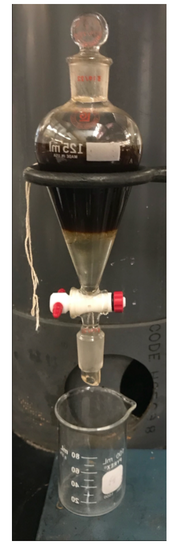


Figure 10. The boiling process to remove excess dichloromethane (Step 12.)

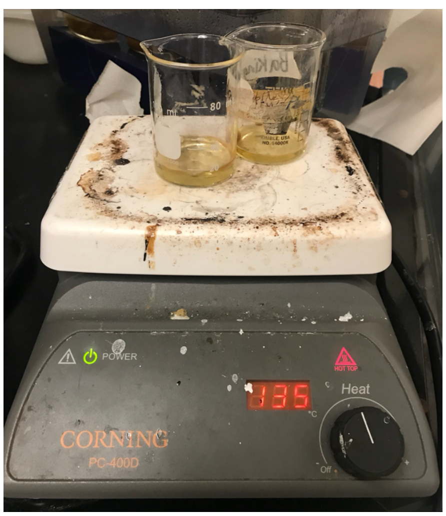


Figure 11. Measuring the mass of the jar with the resulting raw caffeine (Step 13.)

Risk Assessment:

Safety Considerations: Dichloromethane is hazardous if it comes in contact with eyes or skin, or if ingested or inhaled, as it may cause serious eye and skin irritation, and damage to organs[12]. Thus, gloves and goggles were worn at all times to prevent damage to skin and eyes, and the experiment was conducted under a fume hood to minimize the inhalation of harmful fumes.

Ethical Considerations: No ethical considerations were necessary for this experiment.

Reagent Disposal: As dichloromethane qualifies as a toxic chemical, in order to reduce potentially negative environmental impacts, the disposal procedure was comprised of pouring the excess dichloromethane into a toxic waste container for proper disposal. The remaining coffee or yerba mate liquid solutions were rinsed down the sink with water.

[12] “WHMIS Classification for Methylene Chloride - CNESST”.

Raw Data:

Table 2. Information about Coffee Solutions

Solution Number	Brand Name	Mass of initial coffee powder (g) (±0.01g)	Volume of Water (mL) (±10mL)
1	JJBean Espresso	80.11	600
2	Blenz Arabica	79.78	600

Table 3. Volume and Mass of Jar for Coffee Solution #1.

Trial	Volume of Solution (mL) (±0.5mL)	Mass of Empty Jar (g) (±0.01g)	Mass of Jar with Raw Caffeine Obtained (g) (±0.01g)
1	100.1	56.96	56.98
2	99.7	57.16	57.19
3	100.2	50.79	50.83
4	101.3	50.00	50.01
5	91.8	57.17	57.24

Table 4. Volume and Mass of Jar for Coffee Solution #2

Trial	Volume of Solution (mL) (±0.5mL)	Mass of Empty Jar (g) (±0.01g)	Mass of Jar with Raw Caffeine Obtained (g) (±0.01g)
1	99.7	49.92	49.92
2	99.6	52.67	52.68
3	100.0	56.84	56.85
4	100.4	49.13	49.14
5	100.8	52.67	52.67

Table 5. Information about Yerba Mate Solutions

Solution Number	Brand Name	Mass of initial yerba mate extract (g) (±0.01g)	Volume of Water (mL) (±10mL)
1	La Tranquera	80.24	600
2	Amanda	79.41	600
3	CBSé	79.62	600

Table 6. Volume and Mass of Jar for Yerba Mate Solution #1.

Trial	Volume of Solution (mL) (±0.5mL)	Mass of Empty Jar (g) (±0.01g)	Mass of Jar with Raw Caffeine Obtained (g) (±0.01g)
1	101.1	49.37	49.37
2	100.4	57.16	57.16
3	99.8	58.20	58.20
4	99.8	49.82	49.83
5	100.2	56.98	56.98

Table 7. Volume and Mass of Jar for Yerba Mate Solution #2.

Trial	Volume of Solution (mL) (±0.5mL)	Mass of Empty Jar (g) (±0.01g)	Mass of Jar with Raw Caffeine Obtained (g) (±0.01g)
1	100.1	49.33	49.34
2	99.3	48.88	48.90
3	102.1	50.00	50.00
4	101.1	57.17	57.17
5	100.2	48.89	48.90

Table 8. Volume and Mass of Jar for Yerba Mate Solution #3.

Trial	Volume of Solution (mL) (±0.5mL)	Mass of Empty Jar (g) (±0.01g)	Mass of Jar with Raw Caffeine Obtained (g) (±0.01g)
1	99.7	48.88	48.89
2	100.3	57.15	57.17
3	100.0	49.99	50.00
4	99.8	49.33	49.45
5	101.2	48.88	48.89

Qualitative Observations:

Table 9. Qualitative Observations of Reagents.

Reagent	Qualitative Observations
Coffee powder extract	Fine, brown particles. Earthy smell.
Yerba Mate extract	Leaf-like, green solid solute varying in area between ~0-4 mm^2. Earthy smell.
Dichloromethane	Clear, colourless liquid. Strong smelling, acute odour.

Processed Data: [13]

Table 10. Mass and Concentration of Raw Caffeine Obtained from Coffee Solution #1.

Trial	Mass of Raw Caffeine (g) (±0.02g)	Concentration of Raw Caffeine (mg/L)
1	0.02	200 ± 200
2	0.03	300 ± 200
3	0.04	400 ± 200
4	0.01	100 ± 200
5	0.07	800 ± 300

Sample Calculations: Mass = (56.98 ± 0.1g) - (56.96 ± 0.1g) = 0.02 ± 0.02g

[13] Although uncertainties throughout this section may be larger than a data's value, it is evident that the data values must remain positive as a negative concentration is not possible.

Concentration = $\frac{0.02 \pm 0.02g}{100.1mL}$ x $\frac{1mL}{10^{-3}L}$ x $\frac{1mg}{10^{-3}g}$ = $\frac{20000 \pm 20000\ mg}{100.1mL}$ = 199.800 ± 199.800 x ($\frac{20000}{20000}$) = 199.800 ± 199.800 = 200 ± 200 mgL^{-1}

Table 11. Average Mass and Concentration of Raw Caffeine Obtained from Coffee Solution #1.

Coffee Solution	Average Mass of Raw Caffeine (±0.02g)	Average Concentration of Raw Caffeine (mg/L)
1	0.03	400 ± 300

Sample Calculations: Average Mass = $\frac{0.02+0.03+0.04+0.01+0.07}{5} \pm \frac{0.10}{5} = 0.034 \pm 0.02g = 0.03 \pm 0.02g$

Average Concentration = $\frac{199.80+300.90+399.20+98.72+762.53}{5} \pm \frac{199.80+200.60+199.60+197.43+217.86}{5} = 352.23 \pm 203.058g = 400 \pm 300\ mgL^{-1}$

Table 12. Mass and Concentration of Raw Caffeine Obtained from Coffee Solution #2.

Trial	Mass of Raw Caffeine (g) (±0.02g)	Concentration of Raw Caffeine (mg/L)
1	0.00	0 ± 0
2	0.01	100 ± 200
3	0.01	100 ± 200
4	0.01	100 ± 200
5	0.00	0 ± 0

Table 13. Average Mass and Concentration of Raw Caffeine Obtained from Coffee Solution #2.

Coffee Solution	Average Mass of Raw Caffeine (±0.02g)	Average Concentration of Raw Caffeine (mg/L)
2	0.01	100 ± 200

Table 14. Mass and Concentration of Caffeine Obtained from Yerba Mate Solution #1.

Trial	Mass of Raw Caffeine (g) (±0.02g)	Concentration of Raw Caffeine (mg/L)
1	0.00	0 ± 0
2	0.00	0 ± 0
3	0.00	0 ± 0
4	0.01	100 ± 200
5	0.00	0 ± 0

Table 15. Average Mass and Concentration of Raw Caffeine Obtained from Yerba Mate Solution #1.

Yerba Mate Solution	Average Mass of Raw Caffeine (±0.02g)	Average Concentration of Raw Caffeine (mg/L)
1	0.00	0 ± 100

Table 16. Mass and Concentration of Caffeine Obtained from Yerba Mate Solution #2.

Trial	Mass of Raw Caffeine (g) (±0.02g)	Concentration of Raw Caffeine (mg/L)
1	0.01	100 ± 200
2	0.02	200 ± 200
3	0.00	0 ± 0
4	0.00	0 ± 0
5	0.01	100 ± 200

Table 17. Average Mass and Concentration of Raw Caffeine Obtained from Yerba Mate Solution #2.

Yerba Mate Solution	Average Mass of Raw Caffeine (±0.02g)	Average Concentration of Raw Caffeine (mg/L)
2	0.01	100 ± 200

Table 18. Mass and Concentration of Raw Caffeine Obtained from Yerba Mate Solution #3.

Trial	Mass of Raw Caffeine (g) (±0.02g)	Concentration of Raw Caffeine (mg/L)
1	0.01	100 ± 200
2	0.02	200 ± 200
3	0.01	100 ± 200
4	0.12	1200 ± 200
5	0.01	100 ± 200

Table 19. Average Mass and Concentration of Raw Caffeine Obtained from Yerba Mate Solution #3.

Yerba Mate Solution	Average Mass of Raw Caffeine (±0.02g)	Average Concentration of Raw Caffeine (mg/L)
3	0.03	400 ± 200

Figure 12. A graph displaying the processed data.

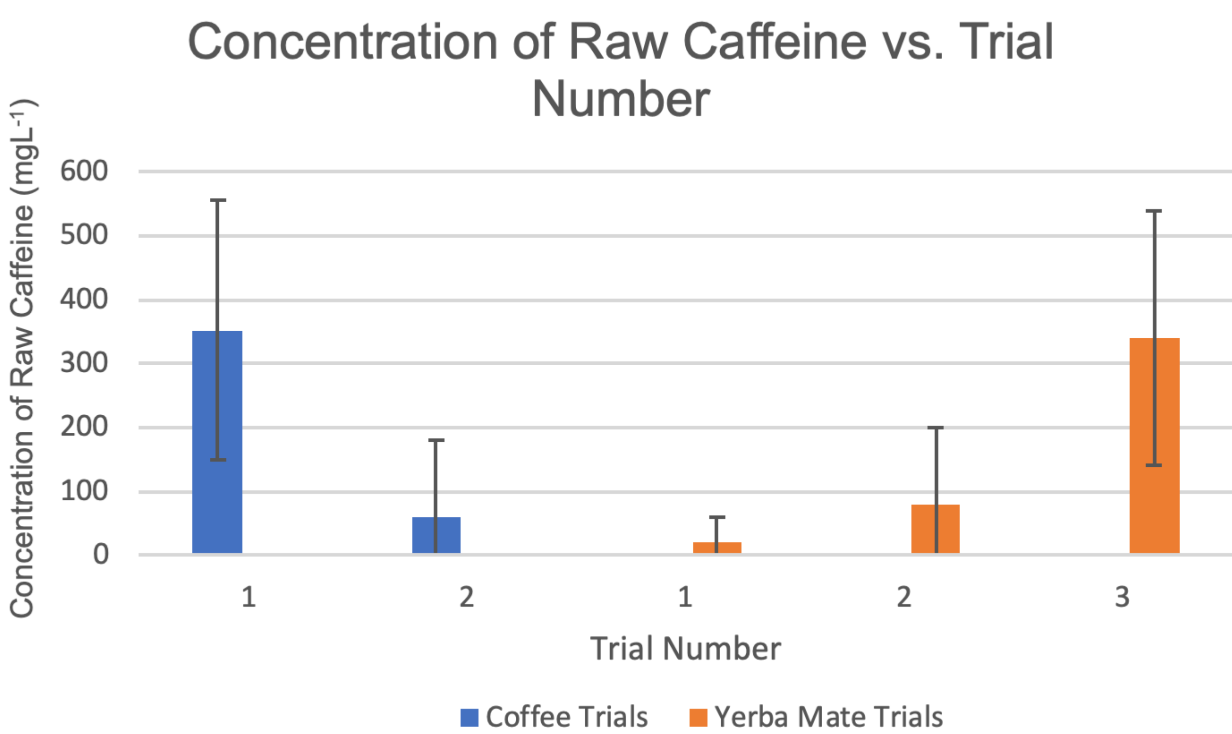


Table 20. Average Concentration of Raw Caffeine for Coffee and Yerba Mate solutions.

Solution	Average Concentration of Raw Caffeine (mg/L)
Coffee	200 ± 200

Yerba Mate	100 ± 200

Sample Calculation: Average Concentration = $\frac{20.04 + 80.22 + 340.18}{3} \pm \frac{40.08+120.16+199.60}{3} = 146.81 \pm 119.95\ mgL^{-1} = 100 \pm 200g$

Taking the accepted values of the concentration of coffee and yerba mate to be 1.00 x 10^2 mg per cup and 8.0 x 10 mg per cup respectively[14], the total error of our results may be calculated as follows:

Total error = $\frac{|accepted\ value - experimental\ value|}{experimental\ value}$ x 100%

Coffee accepted value: $\frac{1.00 \times 10^2\ mg}{1\ cup}$ x $\frac{4.22675\ cups}{1\ L}$ = 422.675 mgL^{-1}

Yerba mate accepted value: $\frac{8.0 \times 10^1\ mg}{1\ cup}$ x $\frac{4.22675\ cups}{1\ L}$ = 338.14 mgL^{-1}

Coffee Total error = $\frac{|206.12-422.68|}{206.12}$ x 100% = 105.07% = 105%

Yerba Mate Total error = $\frac{|146.82-338.14|}{338.14}$ x 100% = 130.32 = 130%

Table 21. Total Error for Coffee and Yerba Mate Solutions

Solution	Total Error (%)
Coffee	105
Yerba Mate	130

Evaluation:

Conclusion:

As shown in Figure 8., the concentrations of the raw caffeine in the yerba mate and coffee solutions were obtained successfully and are summarized below. Total % Error has been added to each of the data values in order to compare it to its theoretical concentration.

Table 22. Summary of Solution Type, Brand Name, and Corresponding Concentration.

Solution Type	Brand Name	Concentration of Raw Caffeine (mg/L)	Total Error (%)
Coffee	JJBean Espresso	400 ± 300	20
Coffee	Blenz Arabica	100 ± 200	600
Yerba Mate	La Tranquera	0 ± 100	1600
Yerba Mate	Amanda	100 ± 200	300
Yerba Mate	CBSé	400 ± 200	0.6

The large total error is due to the correspondingly large uncertainty within the concentration of raw caffeine.

Strengths:

The values of raw caffeine obtained throughout both the coffee and yerba mate solutions were shown to be consistent over different samples, as they showed low variation. This consistency adds to the reliability of the data.

The number of samples per trial (five) was an effective choice as it helped reduce random error while allowing the experiment to be performed within a reasonable span of time.

The high concentration of the caffeinated solution: 80g/600mL=130gL^{-1} allows for a more effective calculation of the amount of raw caffeine within the caffeinated beverage. Since the precision of the digital scale lowers the accuracy of our measurements, a more concentrated initial solution would logically yield a greater amount of raw caffeine and increase the measurements' accuracy. Furthermore, as a standard serving of the caffeinated solution used is less concentrated then the

[14] Berkeley. 2013. "Caffeinated Tea: Mate". Sather Health. July 23, 2013.

concentration used, the experiment provides a good upper bound for the amount of raw caffeine within the liquid solutions.

Weaknesses:

The concentration of caffeine is not consistent over a given solution type; thus, this experiment does not provide reliable results to compare both solutions.
The large uncertainty presented for each data sample detracts from the validity of the data as the large range of possible values do not give an accurate measurement for the mass of raw caffeine.
Although attempts were made to reduce emulsions by lightly shaking the capped separatory funnel, emulsions still formed when the dichloromethane mixed with the liquid solution within the separatory funnel. These emulsions likely induced the introduction of unwanted molecules within the filtered dichloromethane solution. These unwanted molecules, molecules other than raw caffeine, may have added mass to the raw caffeine concentration data, creating a systematic error and reducing the reliability of the results. The formation of an emulsion is likely the cause of the outliers found within trial 5 of the coffee solution #1 (Table 10.) and trial 4 of the yerba mate solution #3.

Extensions: [15]

Adding sodium carbonate to the solid extract (yerba mate or coffee) before using the French Press to isolate the liquid solution, would make the molecules in the caffeinated solution more water-soluble. This increased solubility would allow for fewer impurities when the dichloromethane is filtered through the separatory funnel, as fewer unwanted molecules would separate into the dichloromethane solution. Thus, a more accurate value of raw caffeine may be obtained.
A saturated salt solution could have been added to the filtered dichloromethane solution in order to separate the water layer from the organic solvent instead of simply boiling the water off.
Additionally, molecular sieves can be added to the filtered dichloromethane solution. These sieves would allow for a further removal of water particles before the boiling and evaporation of the dichloromethane takes place. This purification process may decrease systematic error and yield more accurate results.
Furthermore, to more accurately measure the amount of raw caffeine within coffee and yerba mate solutions, a larger data set must be used.

Limitations:

Table 23. A summary of the experiment's limitations and corresponding potential improvements.

Limitations	Improvement
Precision of digital scale	Obtain a digital scale accurate to one thousand of a gram and use this scale instead. This would decrease the uncertainty by 10 times. By decreasing the random error, more accurate data would be obtained.
Product loss to surroundings during the evaporation of the filtered dichloromethane solution	The use of a distillation may reduce the amount of product loss to surroundings as the distillation process would occur in a more closed environment.
Impurities within the dichloromethane solution	As detailed in the **Extensions** section, the addition of sodium carbonate, a salt solution, or molecular sieves could decrease the impurities within the dichloromethane solution, reducing

[15] "How to Extract Caffeine from Coffee". 2015. YouTube. NileRed.

	systematic error and increasing the accuracy of the data.

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