

# Quantitative analysis of caffeine content



Caffeine was extracted from decaffeinated coffee and normal tea using DCM as the solvent and compared with prepared solutions of caffeine using reverse phase HPLC. The experimentally determined result was a caffeine concentration of 7.598? g/ml for decaffeinated coffee compared with 5.9? g/ml reference and 0.4049mg/ml for tea against 0.26mg/ml[1]\*.

### **Introduction**

Caffeine (1) is the most commonly consumed non-prescription stimulant. It is a member of the xanthine (2) family[1] and is structurally related to other plant alkaloids such as theobromine (3), commonly found in cocoa beans and chocolate.

The average person in the UK drinks over three cups of tea a day,[2] and so quantitatively knowing the amount of caffeine that is contained in a cup of tea is particularly important. Caffeine acts on certain adenosine receptors in the brain[3], although there is evidence that action on these specific receptors may stimulate others such as dopamine receptors, commonly associated with reward[4]. More chronic effects of caffeine consumption include irritability, impaired concentration, muscle-aches, nausea, vomiting and blurred vision[5].

HPLC denotes high pressure liquid chromatography. In this experiment, reverse phase HPLC was used, meaning that partially polar organic solvents were used as a mobile phase against a non-polar stationary phase, in this case, octadecyl silane chains[6]. HPLC is a useful tool in analysing quickly and accurately relative abundances of various chemicals contained within a sample.

The experiment was undertaken to evaluate the caffeine content in an average cup of tea and an average cup of decaffeinated coffee.

### **Experimental**

The experiment was carried out according to the University of Bristol Second Year Laboratory Manual. Results were analysed by reverse HPLC using an isocratic solvent system consisting of 10 mM ammonium acetate, methanol and tetrahydrofuran at a ratio of 90: 8. 5: 1. 5. The non-polar phase featured octadecyl silane chains at a particle size of 5  $\mu$  m. The injection volume was 10  $\mu$  l, flow rate was 1. 5ml min<sup>-1</sup> with UV monitoring at 273nm.

### **Preparation of Tea**

One tea bag was added to 200ml of boiling deionised water and boiled (ca. 10 minutes) with occasional agitation. Tea bag was removed and solution allowed to cool to room temperature. Sodium chloride (5g, 0. 856moles) added with calcium hydroxide (1g, 0. 0135moles) twice filtered by Büchner filtration. 20ml transferred to extraction funnel, extracted with DCM (20ml) three times. Magnesium sulphate added to dry DCM thoroughly of all water. Evaporated to dryness via rotary evaporation. Remaining solid dissolved in DCM and made up to 20ml.

### **Preparation of Decaffeinated Coffee**

1. 50g of Decaffeinated Coffee granules was added to 200ml of boiling water and allowed to cool to room temperature. Calcium hydroxide added (1g, 0. 0135moles) to decaffeinated coffee solution. Solution twice filtered via Büchner filtration. 20ml of solution transferred to extraction funnel and extracted with three equivalents of DCM (20ml). Magnesium sulphate added

to dry the DCM, Büchner filtered and evaporated to dryness via rotary evaporation. Remaining solid dissolved in DCM and made up to 20ml.

### **Results and Discussion**

The tea solution was originally brown and transparent, and the coffee was black. Addition of calcium hydroxide changed the colour of both solutions to an opaque dark brown. Tannins are precipitated by lime water, and so this is likely to be what precipitated out in the tea and coffee solution.[7] This precipitate was then removed by Buchner filtration. It was also found that both solutions, coffee especially, readily formed emulsions and bubbles in the extraction funnel, and so a large quantity of magnesium sulphate was needed to remove all water from the extractions. This may have interfered with the full extraction of the caffeine.

The absorption intensity for Tea was 7108493 units. Substituting this into equation 1 allows us to calculate the caffeine concentration in Tea. The solved concentration is 0.4049mg/ml.

The absorption intensity for Decaffeinated Coffee was 79233 units. Substituting into equation 1 allows us to calculate the caffeine concentration in decaffeinated coffee. The solved concentration is 0.007598mg/ml.

### **Errors in solution concentration**

The error for the balance used to measure out the caffeine powder for the standard solutions was  $\pm 0.00005\text{g}$ . The error for the 20ml volumetric flasks used was  $\pm 0.1\text{ml}$ . Substituting these to find maximum and minimum values into the moles equation allows for accurate error analysis.

Substituting these values into the data and plotting onto a graph grants the ability to draw the same graph above with error boxes. It also allows for maximum and minimum gradients to be drawn with the maximum and minimum values. From this information precise errors in the concentration of tea and decaffeinated coffee can be calculated.

Substituting maximum values into a graph enables the gradient and intercept to be calculated, and hence the positive error in caffeine concentration of the tea and decaffeinated coffee solutions. The same can be done with minimum values.

Substituting in values of absorbance for tea gives a maximum value of 0.4069mg/ml and minimum value of 0.4029mg/ml. For decaffeinated coffee the maximum value of 7.639? g/ml and minimum value of 7.558? g/ml.

Literature values for the caffeine content of decaffeinated coffee are in the region of 0.5-1.5mg per 177.44ml<sup>1</sup>, which is between 0.0003-0.

009mg/ml. This is close to the recorded value of 7.598? g/ml  $\pm$  .

The literature values for the caffeine content in tea are 15-75mg/ml per 177.

44ml, which is between 0.088-0.440mg/ml. This is close to the recorded

value of 0.4049mg/ml  $\pm$  . Both of the obtained values are within the range given in reference; however both results are in the upper end of values that should be obtained. This could be due to poor measurement of either the reference caffeine peaks or instant coffee measurement.

## **Conclusion**

The isolation of caffeine from tea and instant coffee was successful and the values obtained from the quantative analysis of a sample by reverse HPLC

compared favourably to reference values. More accurate values for caffeine content could be obtained by larger quantities of instant coffee samples and by repetition of assays involving caffeine extraction from tea. The importance of defining the quantity of caffeine in decaffeinated coffee is important since the concentration must remain low.

### **Acknowledgements**

I would like to thank my demonstrators Jack Davidson and Chun Zhu for helping me with the experiment and my lab partner Ben Gough.

### **References**

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