

Lab sheet #6**-Determination of caffeine content in tea and soft drink-****Method:****First: Sample preparation:**

1. 10 ml of (soft drink samples or hot water extract of tea samples) is taken in separating funnels, and 10 ml of chloroform was added to each sample.
2. The separating funnel should be shaken vigorously for 5 min while shaking, open the cover from time to time to release any pressure within the funnel.
Be sure funnel is pointing away from you before opening.
3. The solutions then allowed to separate for 10 min at room temperature.
4. Only the lower chloroform layer will be collected for further analysis in a test tube or flask.
5. This chloroform layer will be diluted with pure chloroform (as shown in the table) appropriately to read absorbance.
6. Absorbance at 270 nm against pure chloroform as blank.

Second: Preparation of caffeine standard:

Tubes	Caffeine standard (100 µg/ml)	Sample	Chloroform
S1	0.1	--	2.9
S2	0.2	---	2.8
S3	0.3	----	2.7
S4	0.4	----	2.6
S5	0.5	----	2.5
S6	0.6	----	2.4
S7	0.7	----	2.3
S8	0.8	----	2.2
Tea 1	----	0.1	2.9
Tea 2	----	0.3	2.7

Results:

Tubes	Absorbance at 270 nm	Caffeine Concentration (µg/ml)
S1		
S2		
S3		
S4		
S5		
S6		
S7		
Tea 1		
Tea 2		

Calculations:

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