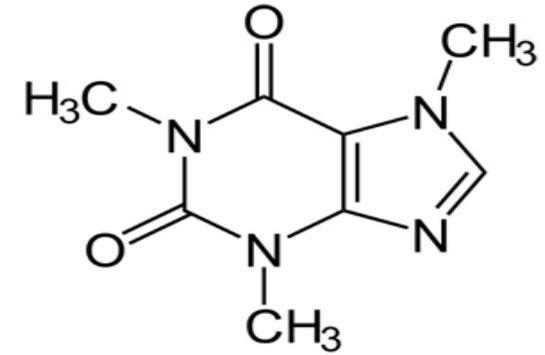


Determination of caffeine content in tea and soft drink



Caffeine

- Caffeine, the common name for **1,3,7-trimethylxanthine**,
- It belongs to a group of methylxanthene.

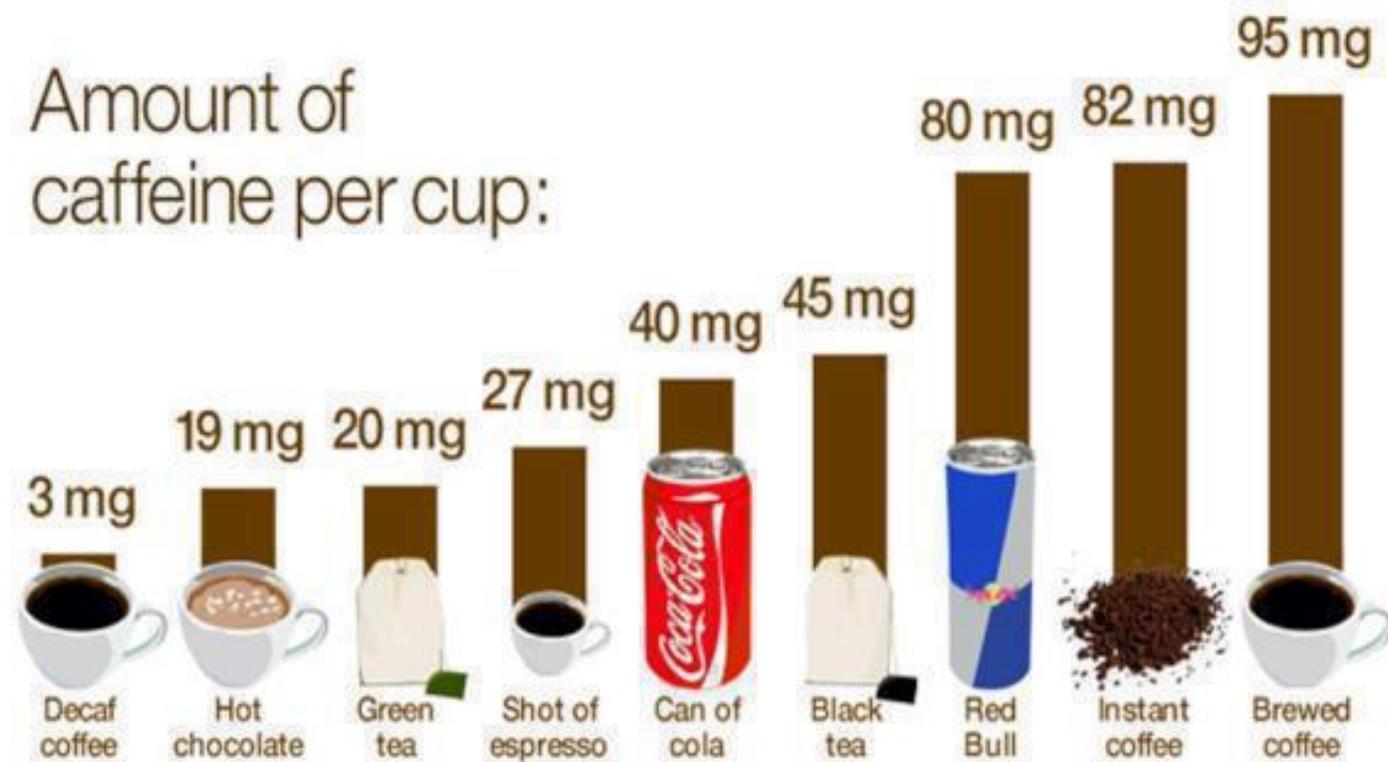


Sources of caffeine

- Caffeine is a chemical that is found **naturally** in the **leaves and seeds** of various plants.
- **Natural sources of caffeine** include coffee beans, cocoa beans, kola nuts, tea leaves and fruits of more than 60 plants.
- **Tea leaves** contains 1.5% to 3.5% caffeine
- **Roasted coffee beans** contain 0.75% to 1.5% caffeine
- **Cocoa bean** contains 0.03% to 1.7% caffeine
- Caffeine can be added to **energy drinks** and some **carbonated drinks** and **drug** products.
- Various **carbonated beverages** contain caffeine in the amount 30 to 60 mg per 355 ml.



Amount of
caffeine per cup:



The effect of caffeine

- Caffeine's main effect on your body is to **make you feel more awake and alert for a while**, but it can also cause **problems**.
- Many studies confirm caffeine's (if it **consumed properly**) ability to **enhance mood** and, exercise performance, the speed at which information is processed, awareness, **attention**, and reaction time.
- **Non proper consuming** of caffeine can make you shaky, make it **hard to fall asleep**, your heart beat faster , raise your **blood pressure** , cause **headaches**, nervousness, In massive doses caffeine is **lethal**.

A **fatal dose** of caffeine is more than 10 grams (about 170 mg/kg body weight).

Caffeine is classified as a central nervous system stimulant :

- 1- An increase in heart rate.
- 2- Constriction of blood vessels.
- 3- Relaxed air passages to improve breathing.
- 4- Ease of muscle contraction.



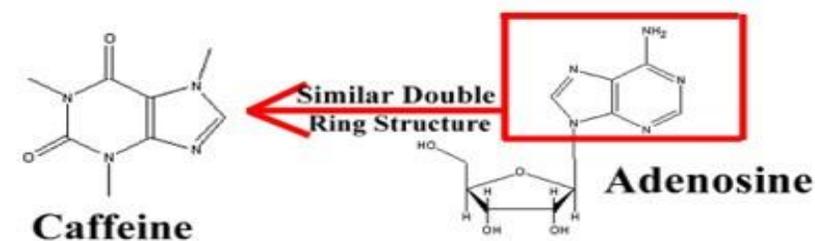
Mechanism of action

Adenosine is a central nervous system neuromodulator that has specific receptors.

When adenosine binds to its receptors, **neural activity slows down**, and you feel sleepy. Adenosine thus facilitates sleep and dilates the blood vessels.

1- **Caffeine** acts as an **adenosine-receptor antagonist**. This means that it binds to these same receptors, but without reducing neural activity. Fewer receptors are thus available to the natural “**braking**” action of adenosine, and neural activity therefore speeds up.

2- **Caffeine** also causes the pituitary gland to secrete hormones that in turn cause the adrenal glands to produce more adrenalin so it **increases your attention level** and gives your entire system an extra burst of energy.



Objective

Determination of caffeine content in tea and soft drink using direct absorption of caffeine at 270 nm.



Principle

Even though caffeine is soluble in water, it is more soluble in chloroform. Therefore, caffeine can be extracted by chloroform from the aqueous mixture **using Liquid-liquid extraction** involves the distribution of a substance between two immiscible liquid phases.

Caffeine absorb light at **270 nm directly**

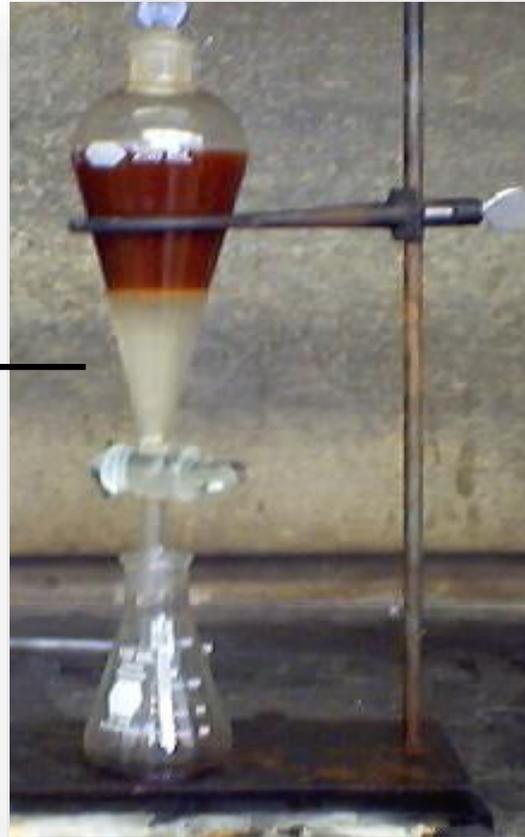
Note that this method will give a **general estimation** of caffeine concentration, it will not give an accurate concentration of caffeine in the sample.

Method:

1-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 (your caffeine sample).
- 6- Absorbance at 270 nm against pure chloroform as blank.

Chloroform layer
(which must be collected) which
contain **caffeine**



* Each group will either has tea or soft drink sample.

Method:

2-Preparation of caffeine standard curve:

Tubes	Caffeine standard (100µg/ml)	Chloroform	Sample
1	0.1	2.9	--
2	0.2	2.8	---
3	0.3	2.7	----
4	0.4	2.6	----
5	0.5	2.5	----
6	0.6	2.4	----
7	0.7	2.3	----
8	0.8	2.2	----
S1 (tea or oft drink)	---	2.9	0.1
S2		2.7	0.3

} Try different concentration of the same sample

Result

Tubes	Absorbance at 270 nm	Caffeine Concentration $\mu\text{g/ml}$
1		
2		
3		
4		
5		
6		
7		
Tea sample1		
Tea sample 2		
Soft drink sample1		
Soft drink sample2		

- Plot absorbance against protein concentration (standard curve).
- determine the caffeine concentration in the sample from the standard curve.
- Calculate the concentration of caffeine in ($\mu\text{g/ml}$) .

Calculations

- **Concentration ($\mu\text{g/ml}$)** = conc. From curve x dilution factor

H.W

What is the benefit of the presence of caffeine in plants ?

Which contain more caffeine Arabic coffee or American coffee and why?

Does **coffee roasting** change caffeine content? what its effect ?

