



**PHARMACOPEIA ANALYSIS  
OF CAFFEINE, ISOLATION OF  
CAFFEINE & COLORIMETRIC  
QUANTITY DETERMINATION**

# Pharmacopoeia Analysis

- It is an analysis to determine whether the active or adjunct substances used in pharmacy complies with the standards reported in the pharmacopoeia.

# **COFFEINUM (TF 1974)**

**1,3,7-trimetilksantin**

# Experiments

- Skills
- Solubility
- Recognition reactions
- Reaction
- Foreign alkaloids

## **SKILLS**

- White
- Crystal structure
- Hot

## **SOLUBILITY**

Its solubility of water, ethanol, ether and chloroform.

# RECOGNITION REACTIONS

## Recognition Reaction A

### Murexide Test

Caffeine+ HCl + KClO<sub>3</sub>  
(Potassium chlorate)



Evaporated to dryness  
(in water bath).



The capsules are exposed to NH<sub>3</sub> vapour.



**Purple** color is formed.



This color disappears  
with the addition of  
alkali.

## Recognition Reaction B

The solution in water forms a white precipitate with a 10% solution of tannic acid in water.

## Recognition Reaction C

The solution in water does not precipitate with iodine TS. Caffeine is converted to enol form by addition of dilute HCl, reacts with iodine and brown precipitate occurs. With the addition of NaOH, caffeine passes into keto form and the precipitate disappears.

### Reaction

When heated with 2 parts of water, it gives a solution which is neutral to the turnus and becomes clear when cooled.

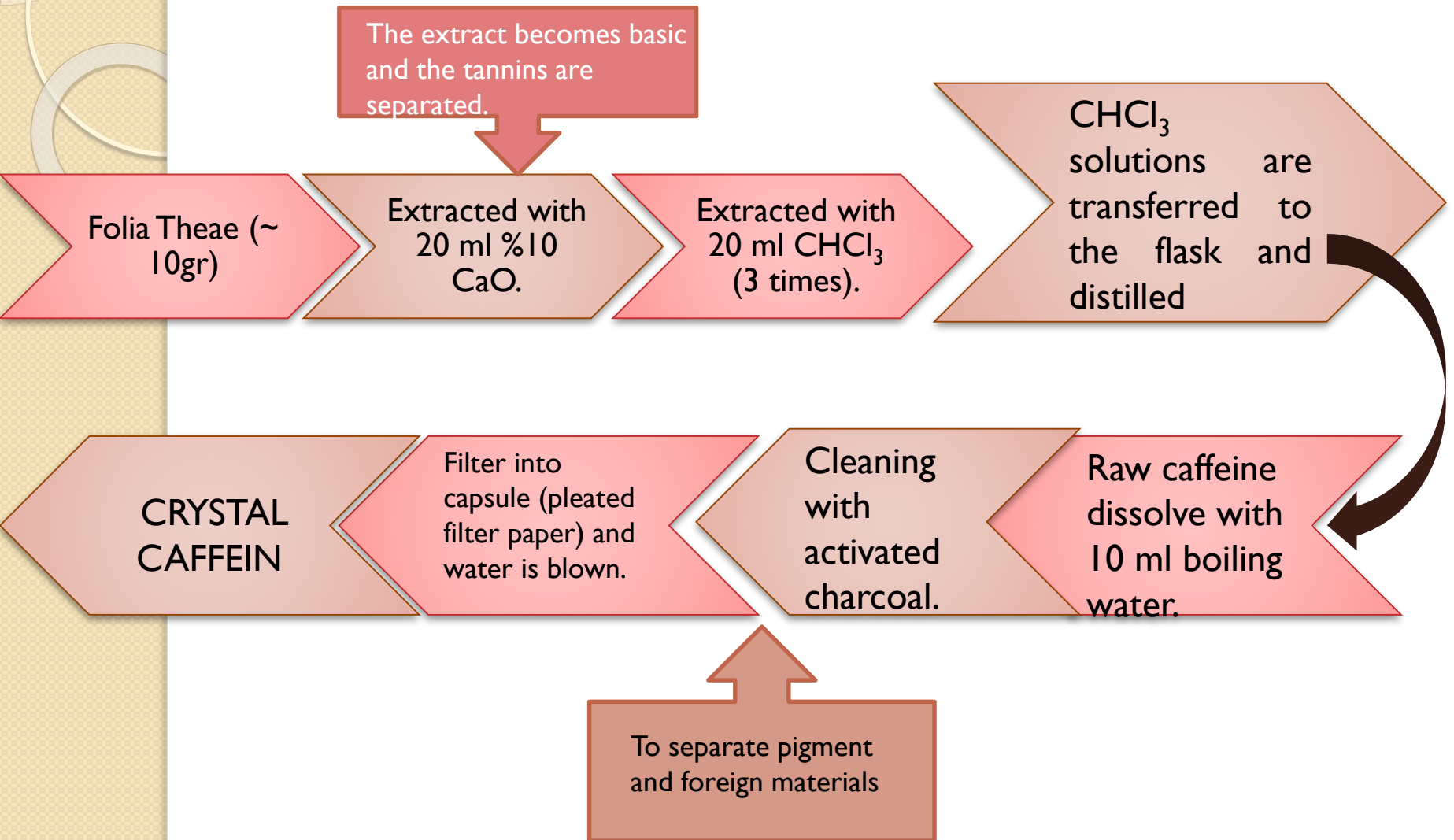


## **Foreign alkaloids**

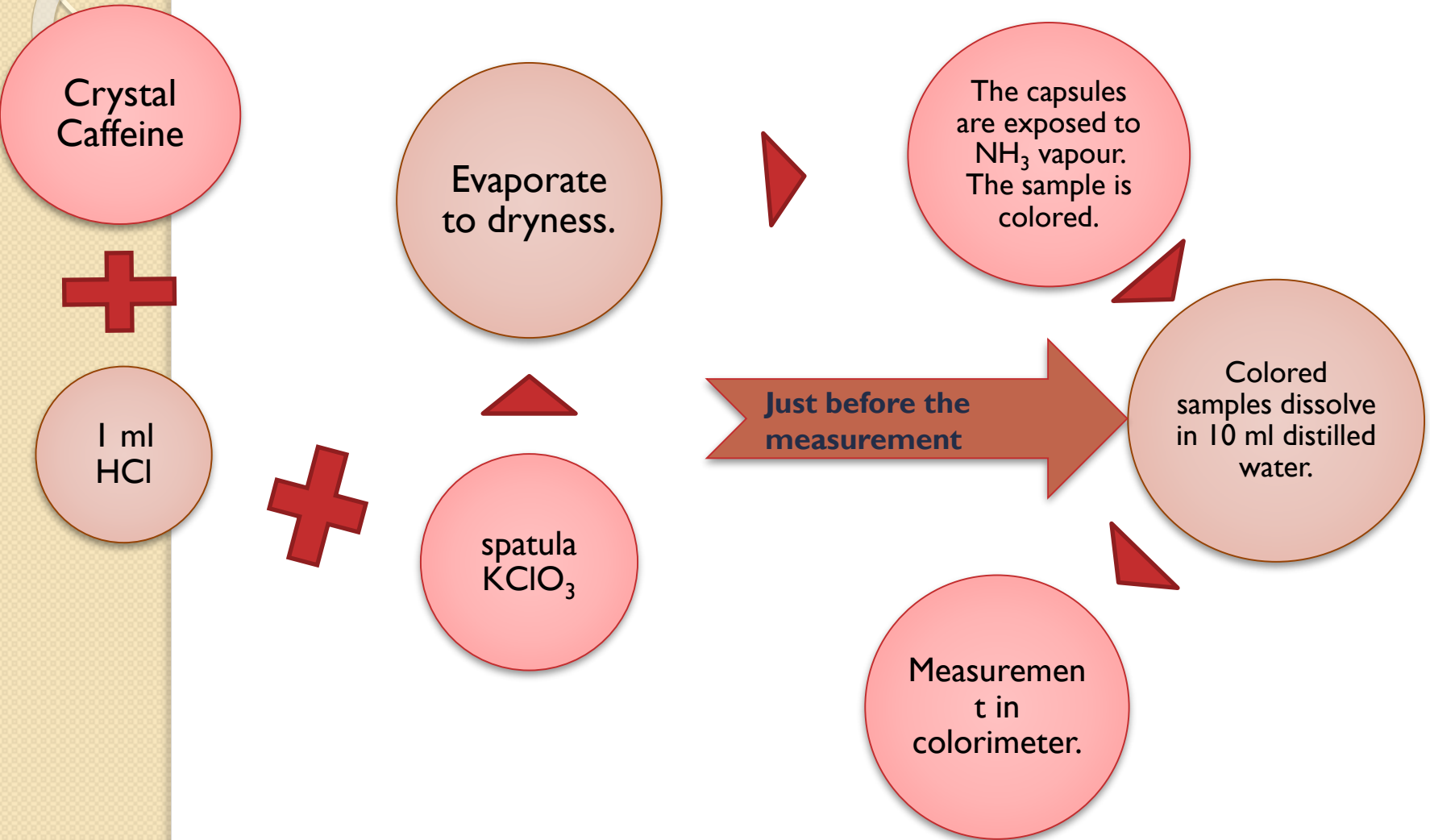
The saturated solution in water does not form precipitate with Mayer's reagent (K-Mercury iodide).



# ISOLATION OF CAFFEINE



# COLORIMETRIC QUANTITY DETERMINATION OF THE CAFFEINE



# CHOLORIMETRIC METHOD

Based on absorbing light. The concentration of an unknown colored substance with unknown concentration is determined by comparison with a standard solution colorimetry, the device used for this purpose is also called a colorimeter.

# MEASUREMENT

- Standard solution known to the concentration in one of the cuvettes in the colorimeter, the concentration of unknown sample is put into another.
- These cuvettes are moved up and down with the help of screws.
- The rays passing through the glass bars of the same type and size come into the lens. The color of the colors is achieved by changing the layer thickness of the eyes and screws and the eyes.
- Read operation at the same time. Colors are seen diagonally, reading is done straight.

Lambert-Beer law is used when calculating.  
Equalization of color intensities means equalization of absorbances.

$$A_1 = A_2$$
$$\epsilon \times I_n \times c_n = \epsilon \times I_s \times c_s$$

$\epsilon$  = Absorption coefficient (cm<sup>-1</sup> . g<sup>-1</sup> . L)

$I$  = Solution layer thickness(cm)

$c$  = Concentration (g/L)

$$I_n \times c_n = I_s \times c_s$$

$$C_n = \frac{I_s \times C_s}{I_n}$$

# CALCULATION

The color intensity of the sample solution is compared to the color intensity of the colored solution in the same color intensity by comparing the color intensity in the colorimeter..

$c_s$ : 0.1 g/L

$l_n$  ve  $l_s$ : read value in colorimeter

**10,.. g drug**  
**100**

**$c_n$  g caffeine**  
**?**

# WHAT SHOULD BE CONSIDERED IN THE COLORIMETRIC METHOD

- Solutions should be clear and freshly prepared.
- The color of the colorimetric method should be colored, if not colored.
- The color tone of the solutions should not be too light or too dark.
- Used bathtubs and glass rods should be very clean.