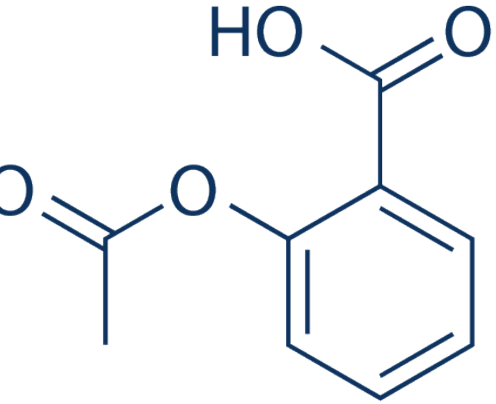
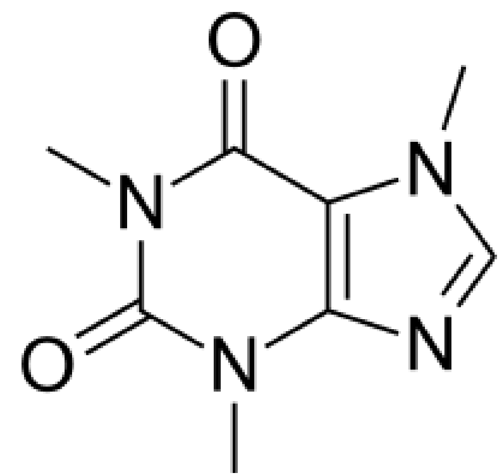



# The determination of Aspirin and caffeine in a mixture by UV spectroscopy



- **<u>Experiment Objective:</u>**
- To appreciate the ability of UV spectrometry in the analysis of a two component mixture.

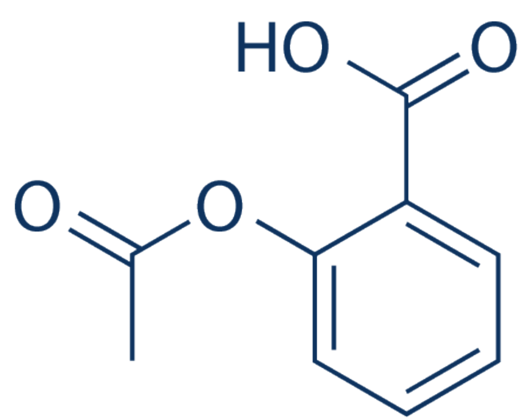
# I. Introduction and theory

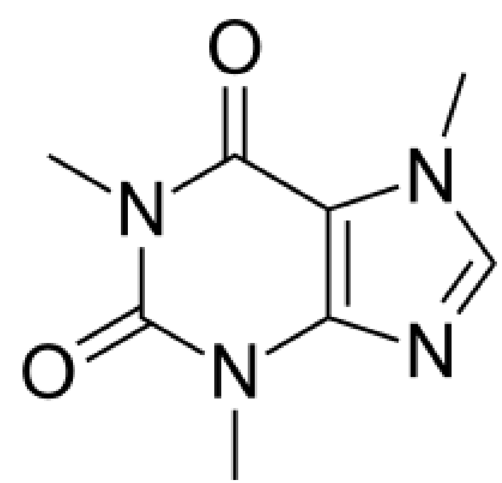
# Introduction and theory

- UV spectroscopy is generally a non selective technique i.e. presence of more than one substance in the sample would make it difficult to determine the amount of each substance accurately (interference).
- However, in few cases the spectra of two co-present substances in a pharmaceutical formulation might be sufficiently different to allow their accurate determination by UV spectroscopy simultaneously.

# I. Introduction and theory

- Aspirin and caffeine represent such an example, where both compounds can be determined simultaneously in their tablet formulation.



# I. Introduction and theory

- Aspirin and caffeine are common components of proprietary analgesics. Such products are subjected to strict quality control (QC) procedures to ensure consistency within specified limits.
- The procedures involve various instrumental techniques of which ultraviolet visible spectrometry is of particular importance

# I. Introduction and theory

- For a dilute mixture, where the components do not chemically interact in solution and where absorbance curves of each display **clearly defined and separate maxima** quantitative analysis based on Beer- Lambert law is feasible.
- A two component mixture such as aspirin and caffeine in a proprietary analgesic may be analyzed by making absorbance measurements **at two characteristic maxima** (one for each component) and solving for the following pair of simultaneous equations:
- $A_1 = A^{\%}{}_{1a} C_a b + A^{\%}{}_{1c} C_c b$ at $\lambda$ $max_1$
- $A_2 = A^{\%}{}_{2a}{}' C_a b + A^{\%}{}_{2c} C_c b$ at $\lambda$ $max_2$

# I. Introduction and theory

**Note that:**

- **$A_{\lambda\ max1}$ is a sum of two absorbance**

  **= Absorbance due to first component (aspirin) + Absorbance due to second component (caffeine)**

- **$A_{\lambda\ max1}$ =**

  **(A1%*B*C) of first component + (A1%*B*c) of second component**

# I. Introduction and theory

- A1: Mixture absorbance at $\lambda\ max_1$
- A%1: Caffeine absorptivity at $\lambda\ max_1$

$$\bullet\ A_1 = A^{\%}_{1a} C_a b + A^{\%}_{1a} C_c b \quad \text{at } \lambda\ max_1$$

- Aspirin absorptivity at $\lambda\ max_1$
- $C_a$: Aspirin concentration
- $C_c$: Caffeine concentration

$$\bullet\ A_2 = A^{\%}_{2a}{}' C_a b + A^{\%}_{2c} C_c b \quad \text{at } \lambda\ max_2$$

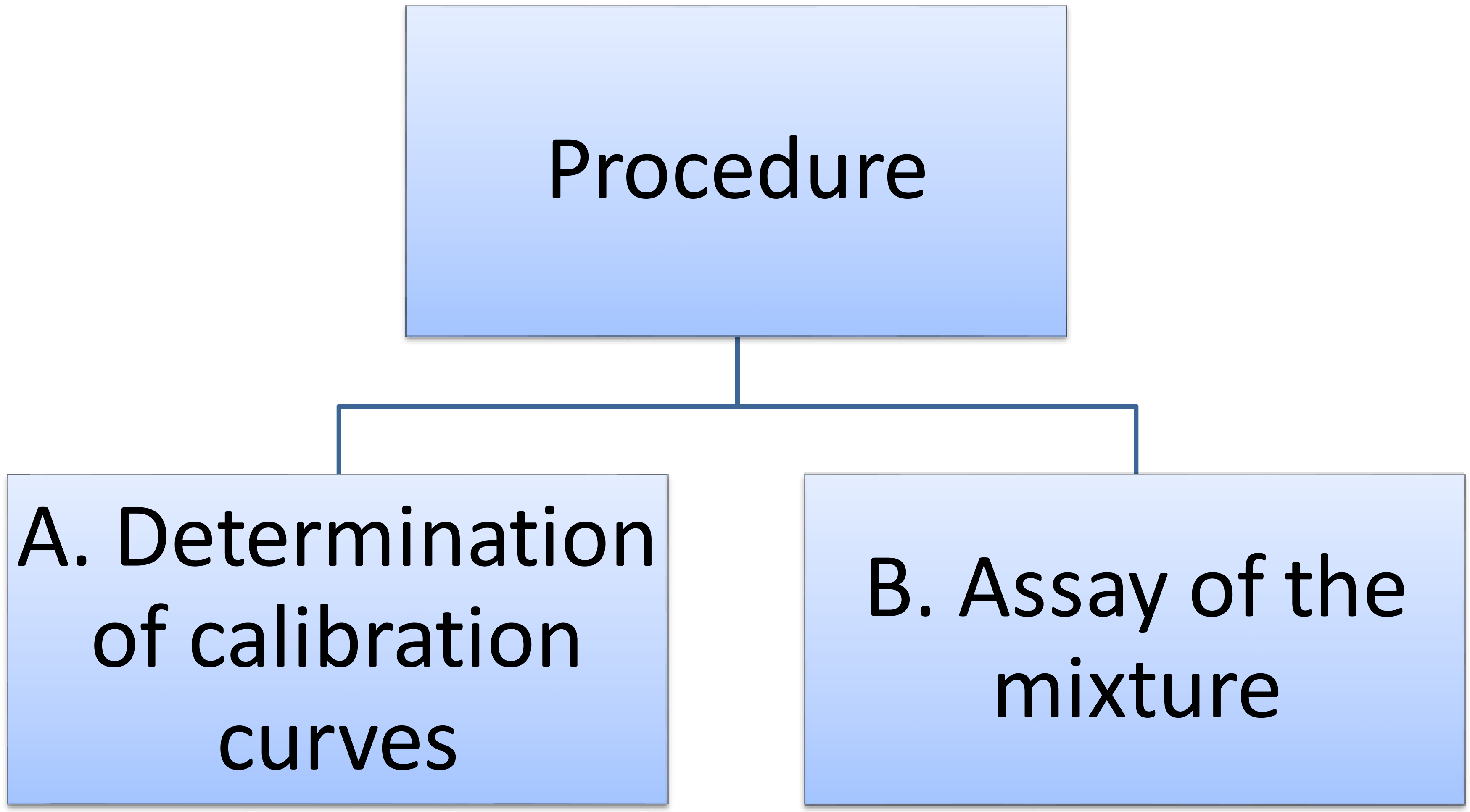
- A2: Mixture absorbance at $\lambda\ max_2$
- A%2: Aspirin absorptivity at $\lambda\ max_2$
- A%2 :Caffeine absorptivity at $\lambda\ max_2$

# II. Practical part

# You will need the following Glassware

- (8 )25ml Volumetric flasks
- (1) 100ml Volumetric flasks
- (1) 50ml Volumetric flasks
- 0.5 ml volumetric pipette
- 1 ml volumetric pipette

- **Chemicals**
  - Solvent:
    - methanol
  - Analytes:
    - Aspirin and caffeine mixed powder
    - Aspirin standard
    - caffeine standard
- **Instrument**
  - UV visible spectrophotometer


Procedure
A. Determination of calibration curves
B. Assay of the mixture

# A. Determination of calibration curves

# A. Determination of calibration curves

- **1. Calibration curve for aspirin**
- <u>1.1 Prepare standard solutions of aspirin</u>

- **Put 375mg Aspirin in 250ml flask**
- **Add 50ml methanol to dissolve the aspirin**
- **Add 20 drops of 4M NaOH**
- **Complete the volume up to 250ml with methanol**


**Aspirin Stock solution**

**From stock pipette the following volumes in 25ml volumetric flasks and complete the volume with methanol**

0.25 ml

0. 5 ml

1 ml

1.5 ml

**Scan this solution to determine lambda max of aspirin**

# A. Determination of calibration curves

- **2. Calibration curve for caffeine**
- <u>1.2 Prepare standard solutions of caffeine</u>

- **Put 95mg caffeine in 250ml flask**
- **Add 50ml methanol to dissolve the caffeine**
- **Add 20 drops of 4M NaOH**
- **Complete the volume up to 250ml with methanol**


caffeine Stock solution

**From stock pipette the following volumes in 25ml volumetric flasks and complete the volume with methanol**

0.25 ml

0. 5 ml

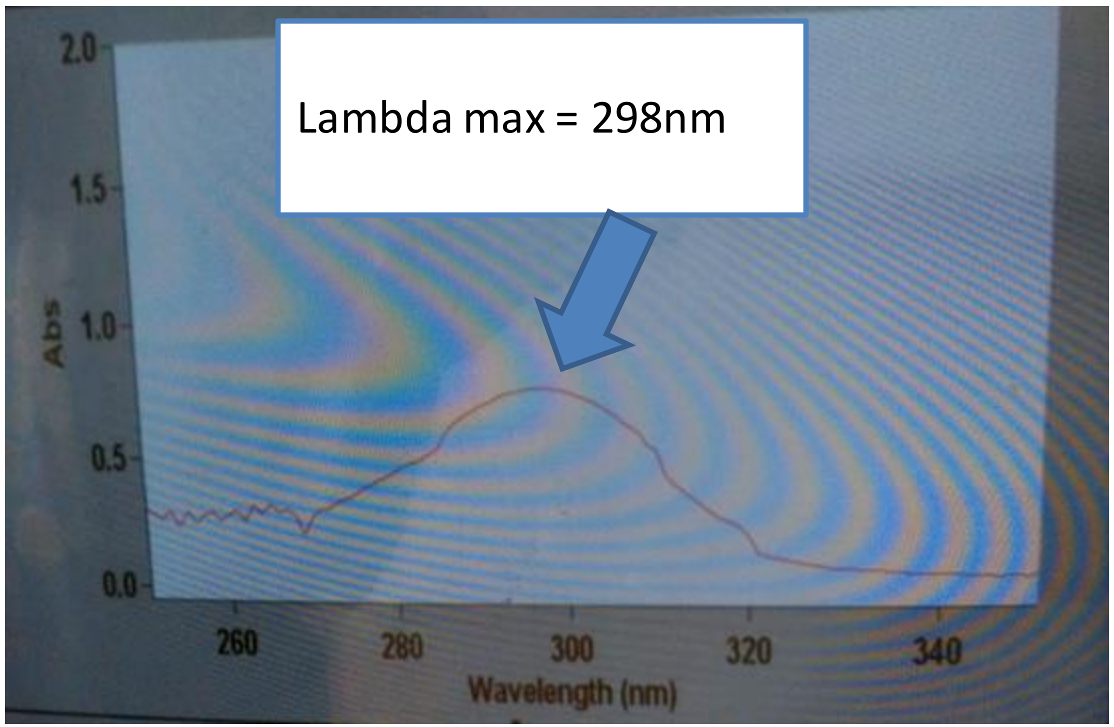
1 ml

1.5 ml

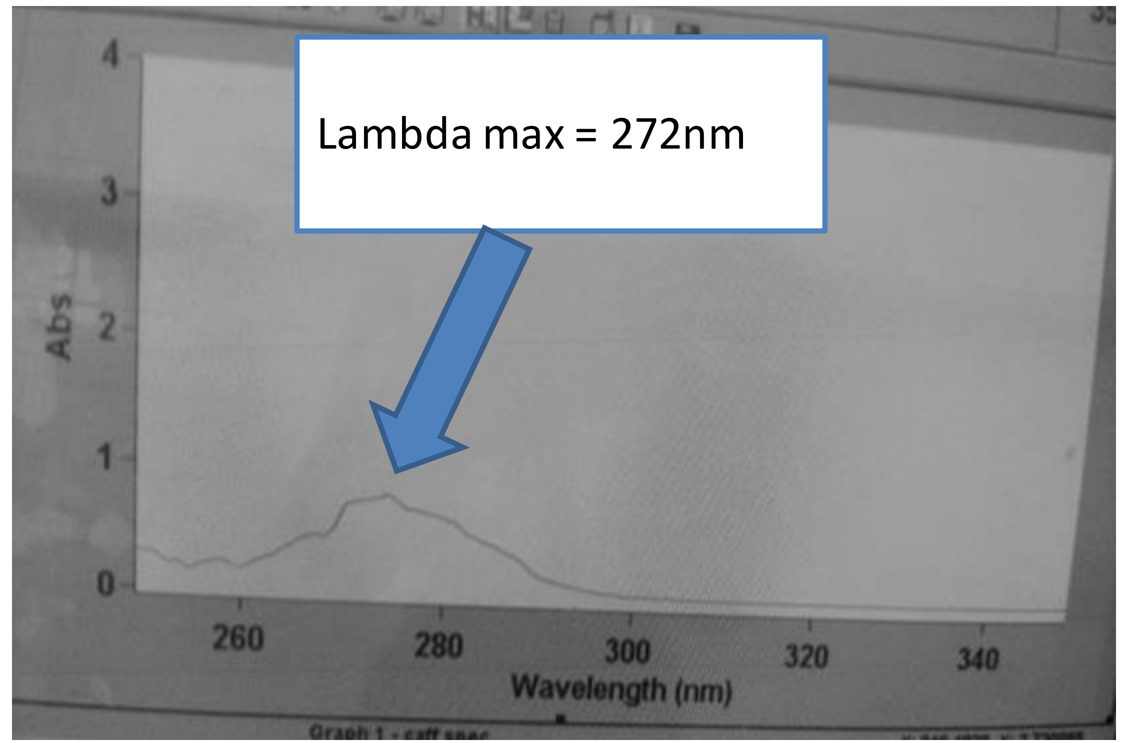
**Scan this solution to determine lambda max of caffeine**

# UV Scan results

**Aspirin spectrum**

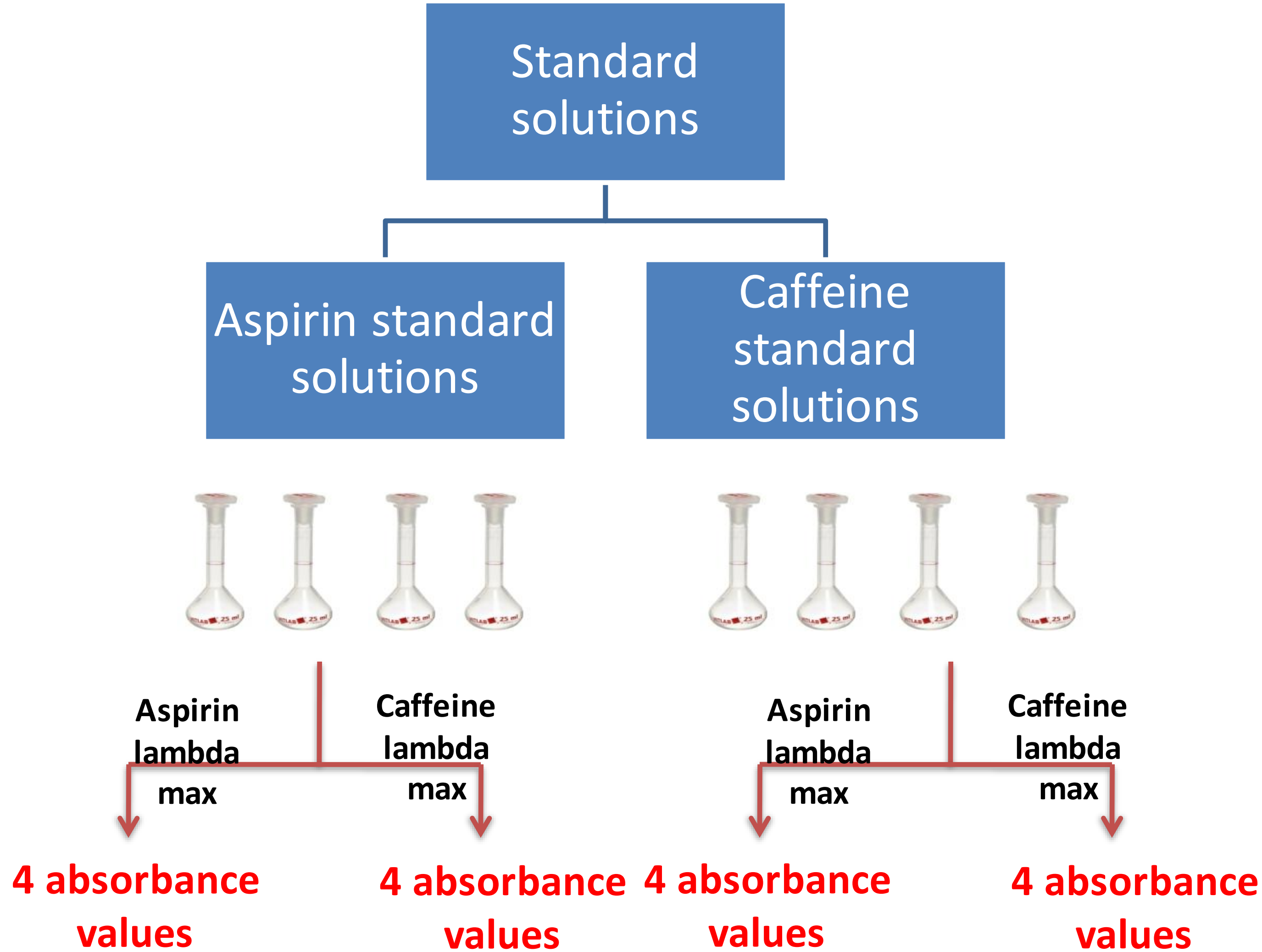



**Caffeine spectrum**



# A. Determination of calibration curves

- By Now you should have two sets of standard solutions one for aspirin and one for caffeine, and two lambda max one for aspirin and one for caffeine.
- For each set of standard solutions **measure the absorbance at the two lambda max.**



# A. Determination of calibration curves

- **Using the absorbance values you have you can draw 4 calibration curves, from each you will get an absorptivity value.**

| Aspirin standard solutions | | | Caffeine standard solutions | | |
|---|---|---|---|---|---|
| **Standard Number** | **Absorbance at aspirin lambda max** | **Absorbance at caffeine lambda max** | **Standard Number** | **Absorbance at aspirin lambda max** | **Absorbance at caffeine lambda max** |
| 1 A | 0.02 | 0.12 | 1 C | 0.0024 | 0.101 |
| 2 A | 0.31 | 0.16 | 2 C | 0.0136 | 0.37 |
| 3 A | 0.9 | 0.27 | 3 C | 0.03 | 0.82 |
| 4 A | 1.37 | 0.38 | 4 C | 0.0505 | 1.25 |

↓ Calibration curve 1 ↓ Absorptivity 1

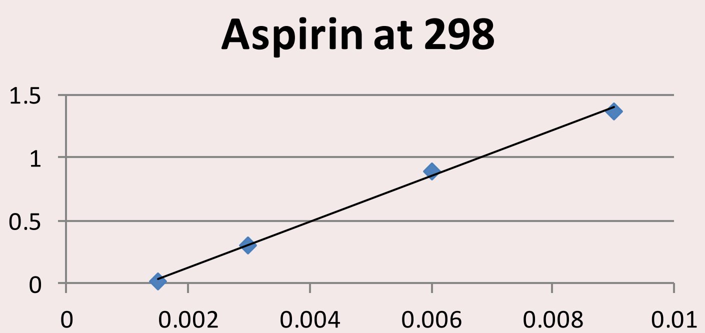
↓ Calibration curve 2 ↓ Absorptivity 2

↓ Calibration curve 3 ↓ Absorptivity 3

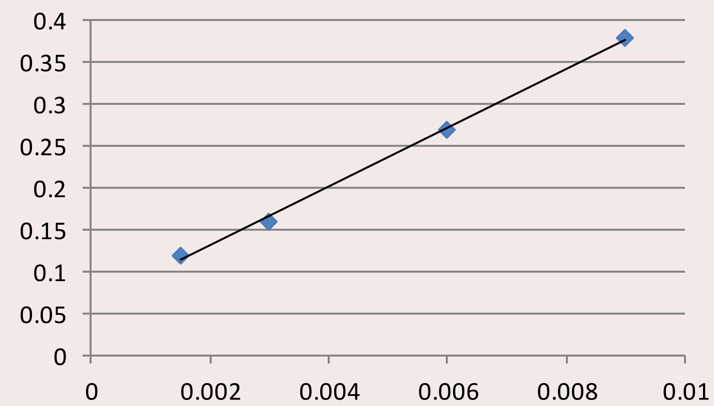
↓ Calibration curve 4 ↓ Absorptivity 4

# A. Determination of calibration curves

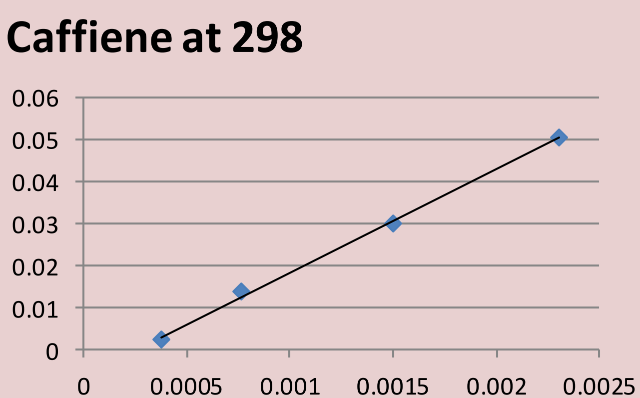
**Calibration curve 1**
**( Aspirin standard at aspirin lambda max)**
**Absorptivity (A1% Asp)**



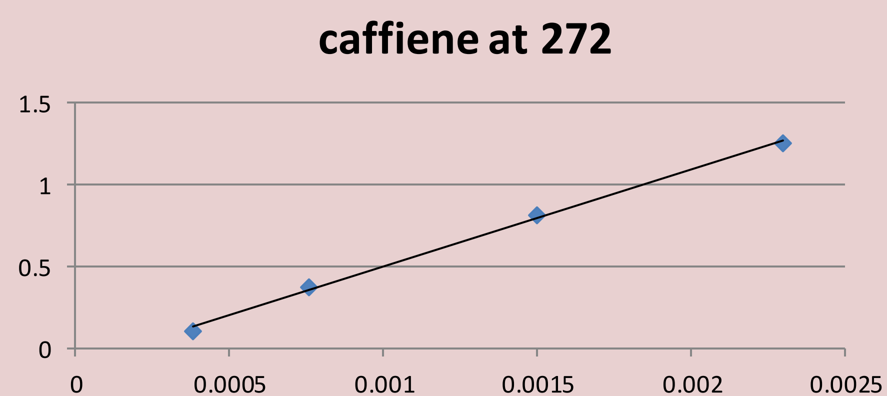

**Calibration curve 2**
**( Aspirin standard at caffeine lambda max)**
**Absorptivity (A2% Asp)**




**Calibration curve 3**
**(caffeine standard at aspirin lambda max)**
**Absorptivity (A1% caff)**




**Calibration curve 4**
**(caffeine standard at caffeine lambda max)**
**Absorptivity = (A2% caff)**

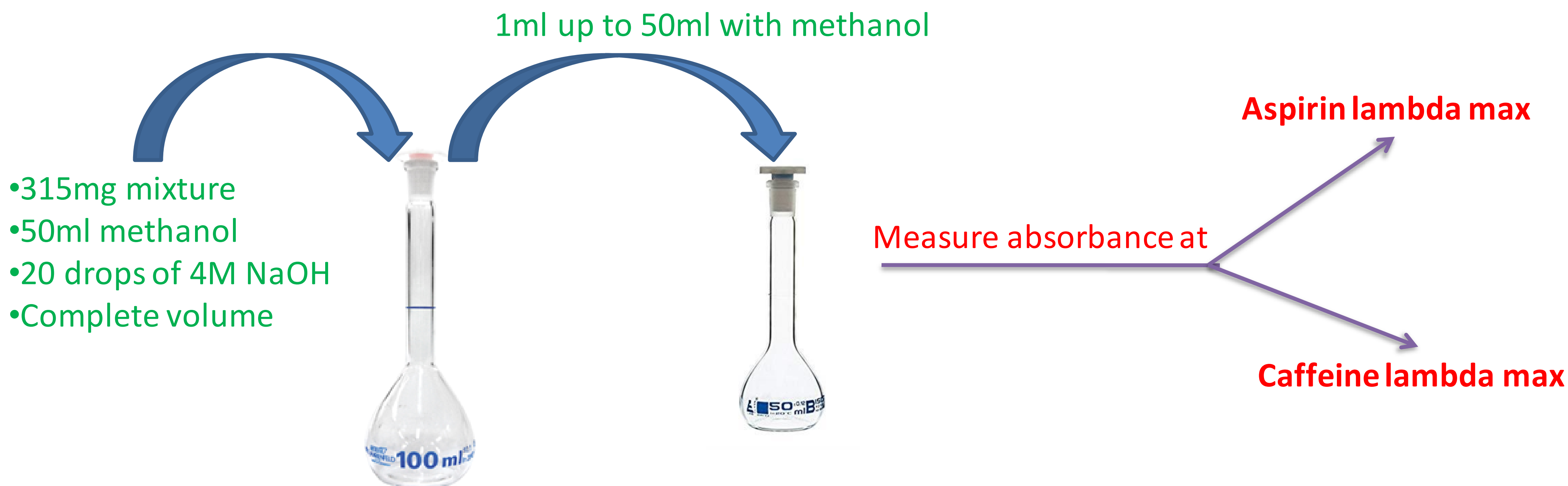



**Attention ! Draw the calibration curves to get the absorptivity using the provided results by yourself. :P**

# B. Assay of the mixture

# B. Assay of the mixture

1. Weigh 315mg of the provided mixture.
2. Dissolve in 50ml methanol and add 20 drops of 4M NaOH.
3. Complete the volume up to 100ml volumetrically.
4. Dilute 1ml up to 50ml with methanol
5. Read the absorbance at the two lambda.



# Calculations

- Summary your results:
- Absorptivity values
  - Aspirin At 298= ----------------
  - Aspirin At 272= -----------------
  - Aspirin At 298= ----------------
  - Aspirin At 272= -----------------
- Mixture absorbance
  - At 298= ----------------
  - At 272= ----------------

# Calculations

- Substitute your results into the simultaneous equations:

A1: Mixture absorbance at $\lambda\ max_1$

A%1: Caffeine absorptivity at $\lambda\ max_1$

- $A_1 = A^{\%}_{1a} C_a b + A^{\%}_{1a} C_c b$ at $\lambda\ max_1$

Aspirin absorptivity at $\lambda\ max_1$

$C_a$: Aspirin concentration

$C_c$: Caffeine concentration

- $A_2 = A^{\%}_{2a}{}' C_a b + A^{\%}_{2c} C_c b$ at $\lambda\ max_2$

A2: Mixture absorbance at $\lambda\ max_2$

A%2: Aspirin absorptivity at $\lambda\ max_2$

A%2 :Caffeine absorptivity at $\lambda\ max_2$

# Calculations

- **For example:**
- **$\lambda$ max$_1$ =272nm** **$\lambda$ max$_2$ = 298**
- **A1= 0.70** **A2=0.995**
- **$A^{\%}_{1a}$ = 35.1** **$A^{\%}_{1c}$ =595.72**
- **$A^{\%}_{2a}$ = 181.24** **$A^{\%}_{2c}$ = 24.62**

$$0.70 = 35.1\ C_a + 595.72\ C_c \quad \text{at } \lambda = 272\text{nm}$$
$$0.995 = 181.24 C_a + 24.62\ C_c \quad \text{at } \lambda = 298\text{nm}$$

You need to solve this pair of equations to get the values of Cc and Ca

# Calculations
# How to solve simultaneous equation?

**To solve by ordinary mathematics follow the link below**

- https://www.youtube.com/watch?v=it3vYdV_oyc


**To solve using excel follow the link below**

- https://www.youtube.com/watch?v=gSNa3fQX0WQ

# Calculations

- Solve the equations to get the values of $C_a$ and $C_C$
- Multiply $C_a$ and $C_C$ by the dilution factor to get the concentration before dilution.
- Convert the concentration values into amount
    - Weight (g) = concentration (g%) * volume(ml)

# Calculations

- The solution for previous example :
- **By solving simultaneous equation using excel:**
  - Ca= 0.0053g%
  - Cc= 0.000859g%

| Ca | Cc | | constant |
|---|---|---|---|
| 35.1 | 595.27 | | 0.7 |
| 181.24 | 24.62 | | 0.995 |
| | | | |
| -0.00023 | 0.005562 | Ca = | 0.005373 |
| 0.001693 | -0.00033 | Cc = | 0.000859 |

- **The concentration before dilution:**
  - Ca=0.00537*50= 0.2685 g%
  - Cc= 0.000859* 50= 0.043 g%
- **Amount in g**
  – Aspirin= 0.2685*100/100= 0.2685g (268.5mg)
  – Caffiene= 0.043g*100/100= 0.448 gm (44.8 mg)