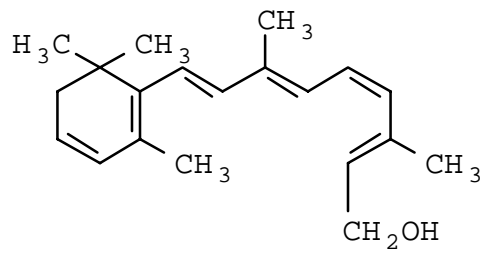
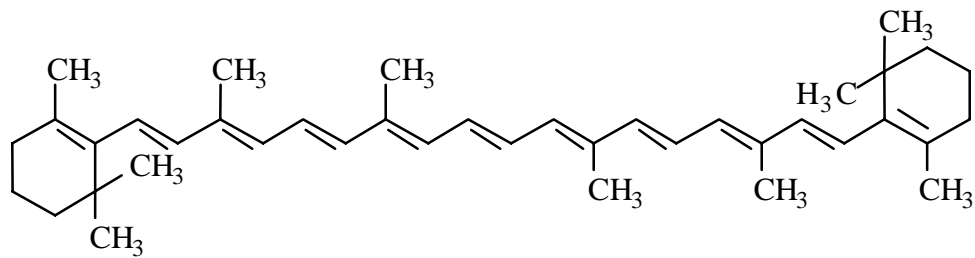


VITAMINS

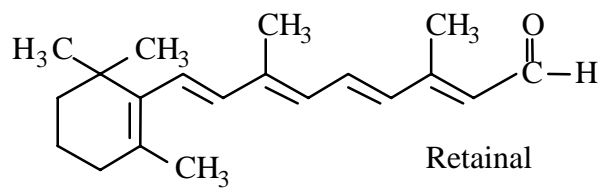
Vitamin A



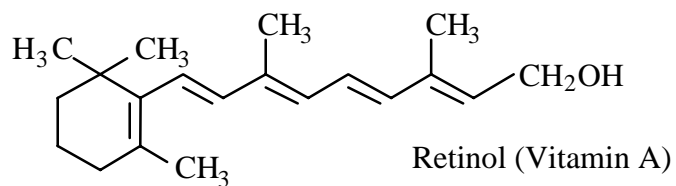
β - Carotene



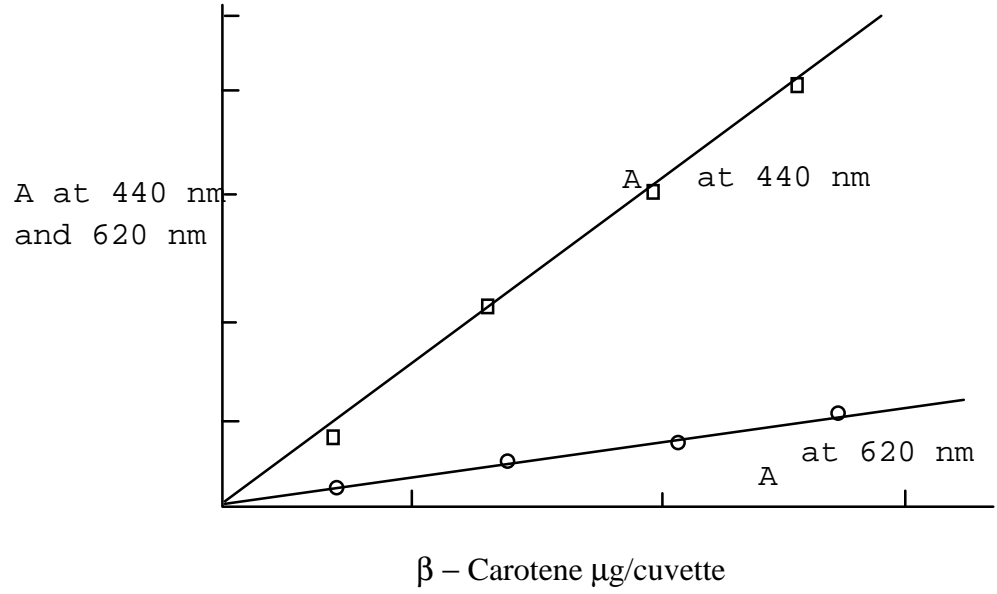
Oxidation
↓



\rightleftharpoons - 2H

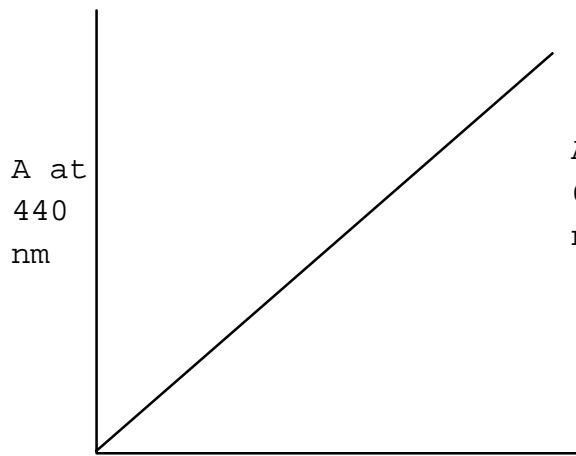


β -CAROTENE STANDARD ABSORBANCES AT 440 AND 620 nm

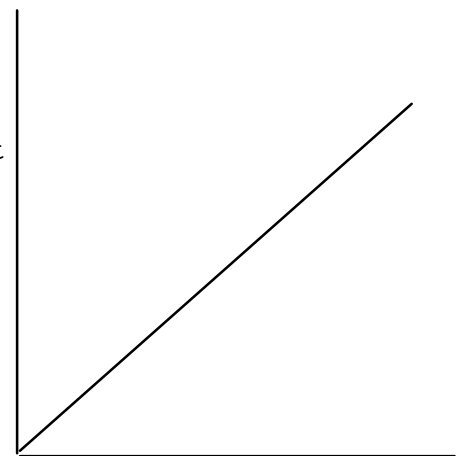


Use absorbances at 440 nm and then convert this to absorbance at 620 nm and subtract from the absorbance at 620 nm to determine the absorbance at 620 due to Vitamin A.

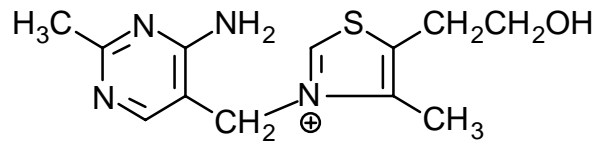
Carotenoid Absorbance at 440nm



Vitamin A Absorbance at 620 nm

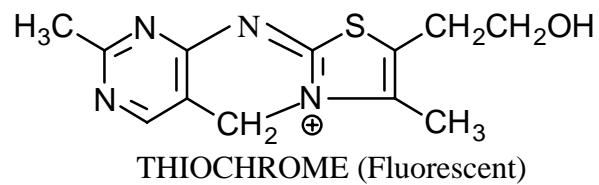
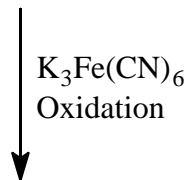
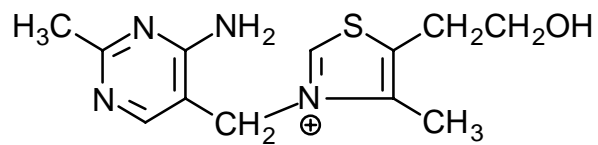


THIAMIN DETERMINATION



PYRIMIDINE

THIAZOLE



Excite thiochrome at 365 nm and measure the absorbance at 435 nm

Thiamin Determination in Foods

5 g enriched flour

75 ml 0.1 N HCl

Make volume to 100 ml

Digest at 100⁰C for 30 min

Centrifuge and filter

Oxidation of Thiamine

Tube 1

5 ml Sample

2.5 g NaCl

3 ml K_3FeCN_6

13 ml Isobutanol

Centrifuge

Tube 2

5 ml Sample

2.5 g NaCl

3 ml NaOH

13 ml Isobutanol

Centrifuge

Tube 3

5 ml Standard (thiamin)

2.5 g NaCl

3 ml K_3FeCN_6

13 ml Isobutanol

Centrifuge

Tube 4

5 ml Standard (thiamin)

2.5 g NaCl

3 ml NaOH

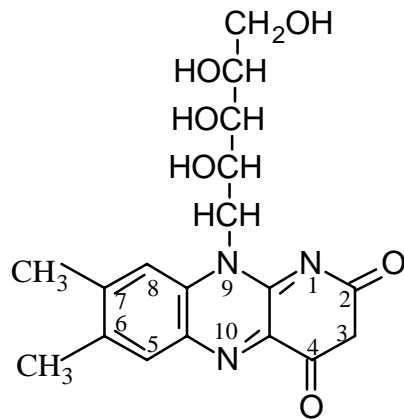
13 ml Isobutanol

Centrifuge

Thiamin Standard Solution = 0.2 $\mu\text{g/ml}$

$$\text{Calculation of } B_1 (\mu\text{g of } B_1/5 \text{ ml assay sample}) = \frac{\text{Tube 1 - Tube 2}}{\text{Tube 3 - Tube 4}}$$

RIBOFLAVIN DETERMINATION



6,7 Dimethyl-9-D-1-Ribitylisoalloxazine

Fluorometric Method

Autoclave in 0.1 N HCl for 30 min

Adjust pH to isoelectric point to precipitate proteins

Filter

Determination: Test Tubes

10 ml sample

+ 1 ml of riboflavin standard or 1 ml H₂O

+ 1 ml acetic acid

+ 0.5 ml KMnO₄ (4%)

+ 0.5 ml 3% H₂O₂ -- KMnO₄ color should disappear in 10 seconds.

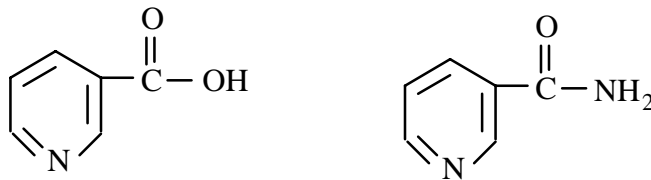
+ 20 mg powdered Na₂S₂O₄

Calculation

mg Riboflavin/ml Final Sample Solution

$$= \frac{[F(\text{sample}) - F(\text{blank})] \times 0.1 \times}{F(\text{sample} + \text{standard}) - F(\text{sample})}$$

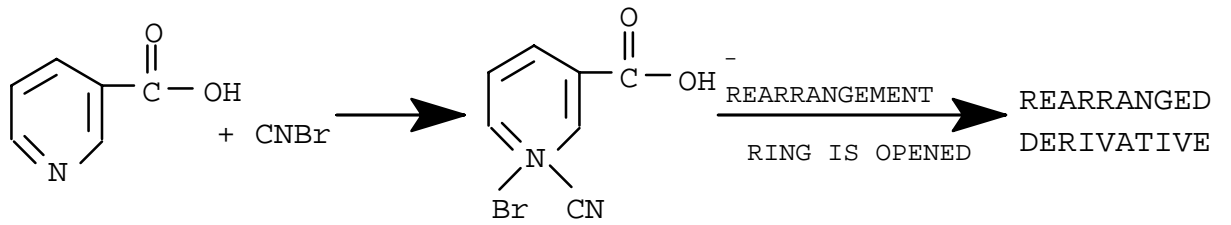
NIACIN



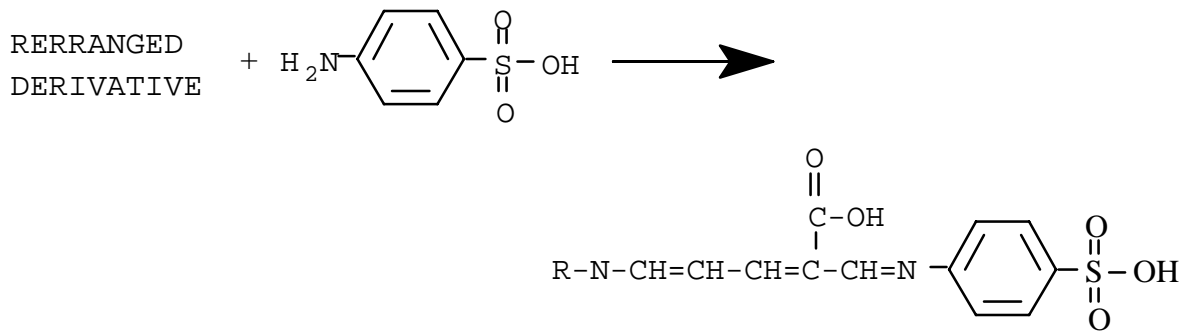
Niacin Determination

1. Digestion and Hydrolysis
Sample + Ca(OH)₂ $\xrightarrow[2 \text{ Hours}]{\text{Autoclave}}$
2. PPT Protein with (NH₄)₂SO₄
Centrifuge and Filter
3. Rupture of Pyridine Ring with CNBR (Cyanogen Bromide)
4. Color Formation with Sulfanilic Acid
5. Determine the Absorption at 470 nm
6. Plot the Standard Curve of Niacin vs. Concentration
(Straight Line of Best Fit)

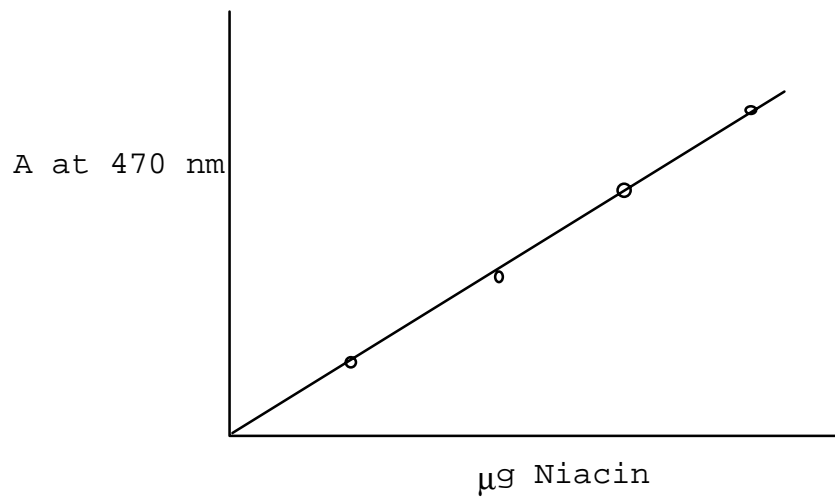
1.



2.

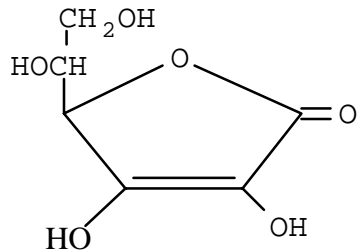


STANDARD CURVE

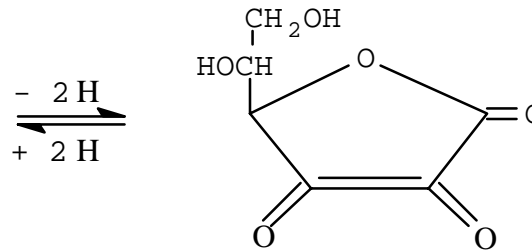


VITAMIN C

Ascorbic Acid



Dehydroascorbic Acid



HSCH₂CH₂(SH)CH₂OH 2,3-DIMETHYLPROPANOL

Reducing agent or converting dehydroascorbic acid to ascorbic acid

Titrimetric Method for Reduced Vitamin C

1. Extract with metaphosphoric acid (HPO₃) in HOAC.
2. Titrate with 2, 6-dichloroindophenol (blue).

At end point, rose pink (2, 6-dichloroindophenol in acidic condition).

$$\text{mg Ascorbic Acid/g} = (A-B) \times \frac{F}{E} \times \frac{V}{X}$$

A = ml of 2,6-dichloroindophenol for sample titration

B = ml of 2,6-dichloroindophenol for blank

F = mg of ascorbic acid equivalent to 1 ml of indophenol standard solution

V = Initial assay solution volume

E = Number of grams

X = Volume sample aliquot titrated