Estimation of Ascorbic Acid by volumetric method

Ascorbic acid otherwise known as Vitamic C is antiscorbutic. It is present in citrus fruits, gooseberry, bittergourd etc. in high amount. Generally it is present in all fresh vegetables and fruits. It is water soluble and heat-labile vitamin. The method described below is easy, rapid and a large number of samples can be analyzed in a short time.

Materials

1. Oxalic Acid (4%)

2. Dye Solution: Weigh 42mg sodium bicarbonate into a small volume of distilled water. Dissolve 52mg 2,6-dichlorophenol indophenol in it and make up to 200ml with distilled water.

3. Stock Standard Solution: Dissolve 100mg ascorbic acid in 100ml of 4% oxalic acid solution in a standard flask (1mg/ml).

4. Working Standard: Dilute 10ml of stock solution to 100ml with 4% oxalic acid. The concentration of working standard is 100ug/ml

Principle

Ascorbic acid reduces the 2, 6-dichlorophenol indophenol dye to a colorlss leuco-base. The ascorbic acid gets oxidized to dehydroascorbic acid. Though the dye is a blue coloured compound, the end point is the appearance of pink colour. The dye is pink colour in acidic medium. Oxalic acid is used as the titrating medium.

Procedure

1. Pipette out 5ml of the working standard solution into a 100ml of conical flask.
2. Add 10ml of 4% oxalic acid and titrate against the dye (V_1 ml). End point is the appearance of pink colour which persists for a few minutes. The amount of dye consumed is equivalent to the amount of ascorbic acid.
3. Extract the sample (0.5-5g depending on the sample) in 4% oxalic acid and make up to a known volume (100ml) and centrifuge.
4. Pipette out 5ml of this supernatant, add 10ml of 4% oxalic acid and titrate against the dye (V_2 ml).

Calculations

Amount of ascorbic acid mg/100ml sample

$$\frac{0.5\text{mg}}{V_1\text{ml}} \times \frac{V_2\text{ml}}{5\text{ml}} \times \frac{100\text{ML} \times 100}{\text{Wt. of the sample}}$$

References

1. Sadasivam, S and Balasubraminan, T (1987) In : Practical manual in Biochemistry. Tamil Nadu Agricultural University Coimbatore p14
2. Harris, L. J and Roy, S.N (1935) Lancet 1, 462