EXPERIMENT 34 DETERMINATION OF VITAMIN A CONTENT IN GHEE BY HPLC

Structure

34.0 OBJECTIVES

After attending to this experiment, we shall be able to:

- learn to perform determination of vitamin A content in ghee by HPLC.

34.1 INTRODUCTION

Structurally vitamin A is all trans-retinol. Vitamin A deficiency leads to change in the tissue of the eye (xerophthalamia) and eventual irreversible blindness. Vitamin A is necessary for vision, growth, immune response, reproduction and embryogenic development. Vitamin A normally occurs in foods originated from animals but not from plants. However, vegetables and fruits are a good source of pro-vitamin A (carotenoids).

34.2 PRINCIPLE

The ghee sample is saponified using ethanolic KOH and the unsaponifiable matter is extracted with diethyl ether. The vitamin A content is then quantified by HPLC.

34.3 REQUIREMENTS

Apparatus

HPLC- Waters/Agilent/HP/Shimadzu or any other model

Hot plate

Micropipette (20-200 µl)

Vortex shaker

Weighing balance

Water bath

Rotary evaporator

Reagents

Ethanol-Distilled

Potassium hydroxide-AR grade

Glycerol-AR grade

Diethyl ether-Extra pure

Standard Vitamin A palmitate (Fluka)-High purity

Sodium sulphate (Anhydrous)

34.4 PROCEDURE

Sample Extraction

Accurately weigh 5 g of sample into a dried iodine flask. Add 50 ml of 14% alcoholic KOH and 10 ml of glycerol into the flask. Place flask on thermostatically controlled water bath at 65-70°C and attach condenser and reflux the sample for 1hr. Rinse condenser with 95% ethanol. Remove the flask from the reflux assembly and close with stopper and cool solution to room temperature. Pour the content into a 250 ml separating funnel. Add 100 ml diethyl ether to it and stopper the funnel and shake vigorously for 45 sec. Let the layer separate out and collect the aqueous layer into a beaker and ether layer into another separating funnel. Pour again the aqueous layer into separating funnel and extract with 50 ml of diethyl ether. Repeat the extraction once again. Wash the extracted ether with water till the aqueous layer becomes colourless with phenolphthalein. Filter ether into 250 ml flask through sodium sulphate. Evaporate ether in flask to dryness on rotary evaporator at 40°C. Dissolve the dried residue in mobile phase. Treat the standard vitamin A palmitate as described for test sample using 2.5 mg of standard.

Instrument Conditions

Column - Bonda Pack C18 (30 cm × 3.9 mm)

Column temperature- Ambient

Mobile phase - Acetonitrile:Methanol:Dichloromethane *(7:0.5:3)*

Detector- UV detector

Wavelength - 310 nm

Injection volume - 20 µL

Retention time - 3.5 to 3.8 min

34.5 CALCULATION

$$\text{Vitamin A, IU/g} = \frac{\text{Area of sample} \times \text{conc. of std} \times \text{dilution} \times \text{potency}}{\text{Area of std} \times \text{sample weight (g)}}$$

34.6 RESULTS AND INFERENCE

The difference between the results of two determinations of sorbic acid carried out simultaneously or in rapid succession by the same analyst shall not exceed 1.0 IU/g. Vitamin A is used to fortify margarine and vanaspati. It is added to margarine and vanaspati at the minimum level of 3000 and 2500 IU/100 g, respectively. The vitamin A level in some foods of animal origin is given below:

Food Products	**Vitamin A (IU/100 g)**
Butter	2363-3452
Cheddar cheese	553-1078
Boiled eggs	165-488
Milk	110-307

34.7 PRECAUTIONS

- For saponification, rinse the flask with ethanol after KOH addition in order to prevent joint of flask with condenser from freezing together.
- Saponify the sample under inert and dark condition or avoid exposure to sunlight.
- Transfer the solvent carefully during extraction so that no loss of vitamin A.
- During extraction, avoid the formation of emulsion, which may cause low recovery of vitamin A.
- Prepare standard solutions accurately.