16.2 Simultaneous estimation of vitamins A & E – HPLC method

Reagents

- Absolute alcohol
- Potassium hydroxide (AR grade)
- Distilled water
- Petroleum ether (40-60ºC)
- Acetonitrile HPLC grade
- Tetrahydrofuran HPLC grade
- Water HPLC grade
- Nitrogen gas
- Vitamin A and E standards

Apparatus

- Amber colored conical flask
- Amber colored vial
- Separating funnel
- Water bath shaker

Fig. 16.1 Separating funnel

Procedure

Extraction

- Saponify about 1 g feed sample with 10 ml of 95% ethanol and 2 ml of 60% KOH in 50 ml conical flask.
- Keep the content on the boiling water both till one or two bubbles appears.
- Thereafter, cool by ice bath for 5 minutes and add 10 ml of petroleum ether.
- Shake the contents for 15 minutes in water bath at 39ºC and collect the extract in the vial.
- Repeat the same for three times.
- Collect pooled ether extract and treat with 10 ml of 0.5 N KOH in a separating funnel (Fig. 16.1).
- Wash the solution with distilled water for three times to remove the excess alkali.
- Finally collect ether and filter through sodium sulfate. Dry the ether extract under nitrogen gas.
- Residue is ready for injection to HPLC.

Operating conditions

Mobile phase	-	Acetonitrile: Tetrahydrofuran: Water (47:42:11)
Flow rate	-	1.5 ml/min
Column	-	C 18 ODS (150 x 40 mm)
Oven temperature	-	17ºC
Detector	-	UV/PDA
Wave length	-	325 nm (vit A)
Wave length	-	290 nm (vit E)
Total run time	-	6 minutes.

Calculations

$$\text{Vitamin A/E } (\mu g/g) = \frac{Ve \times SA \times SdC}{Vi \times SdA} \times \text{purity of vitamin}$$

Where,

Ve - Volume in which the dried was dissolved
SA - Sample area from peak
SdC - Standard concentration (Vit A/E)
Vi - Volume injected
SdA - Standard area from the peak

Reference: Rajiv Chawla and Harjit Kaur 2001. Isocratic HPLC method for simultaneous determination of beta carotene, retinol and alfa tocopherol in feeds and blood plasma. *Ind. J.Dai.Sci.* 54**:** 84-90.