16.3 Estimation of vitamin D_3 – HPLC method

Reagents

- Diethyl ether
- Acetone
- Dichloromethane
- Methanol
- Di-sodium hydrogen orthophosphate
- Acetonitrile HPLC grade
- Nitrogen gas
- Vitamin D_3 standard

Apparatus

- Beaker
- Amber colored vial
- Freezer

Procedure

Extraction

- Add about one gram feed sample with 5 ml diethyl ether and keep it in freezer with acetone containing beaker till the lower portion freezes.
- Collect the supernatant in another vial.
- Repeat the same for three times.
- Add 4 ml mixture of dichloromethane and methanol (3:1) in the collected supernatant of each vial.
- Collect again supernatant from above mixture and treat with 0.1 M of phosphate buffer.
- The supernatant of ether extract is collected and dried under nitrogen gas.
- Residue is ready for HPLC injection.

Operating conditions

Mobile phase	-	Acetonitrile (100%)
Flow rate	-	2 ml/min
Column	-	C 18 ODS (150 x 40mm)
Oven temperature	-	25ºC
Detector	-	UV/PDA
Wave length	-	265 nm
Total run time	-	10 minutes.

Calculation

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

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: Vitamin Analysis for the Health and Food Sciences. By Ronald R Eirenmiller, W.O.Landen 1998.