

ABSTRACT

Vitamins are essential constituents of diet and are necessary for normal metabolic functions of the body. A large number of vitamins have been isolated, characterized and then synthesized. Vitamins are of two types (1) Fat soluble vitamins (A, D, E), (2) water soluble vitamins (B_1 , B_2 , B_6 , B_{12} and C). In USP 24 (United States Pharmacopeia 2000), HPLC method for determination of vitamins is developed by using Amperometric Electrochemical detector. In present work UV-Visible spectrophotometric detector is used which made the method cheaper as compared to the method mentioned in literature. Vitamins RS (Reference standards) were obtained from Schazoo Laboratories, Lahore. The sample Theragra-M tablets were obtained from Bristol-Myers Squibb Pakistan (Pvt.) Ltd.

The parameters studied are (1) Buffers of different pH, (2) Mobile phase composition, (3) wavelength, (4) column temperature, (5) flow rate. The column used is Kromasil 100 C_{18} , (octadecyl silane, ODS, chemically bonded to porous silica). The maximum detector response for fat soluble vitamins was obtained under following chromatographic conditions **mobile phase:** Methanol/Acetonitrile (92:08), **Column Temperature:** 30°C, **Flow rate:** 1.00 cm^3/min , **Pressure:** 6.0-6.4 MPa, **Injection Volume:** 20 μL , **Wavelength:** 280 nm, **Run time:** 20 min. The maximum detector response for water soluble vitamins was obtained under following chromatographic conditions **Mobile phase:** Buffer/Acetonitrile/Methanol (03:90:07), **Column Temperature:** 30°C, **Flow rate:** 1.0 cm^3/min , **Pressure:** 9.0-9.8 MPa, **Injection volume:** 20 μL , **Wavelength:** 254 nm, **Run time:** 20 min.

According to USP, for five replicate injections RSD (relative standard Deviation) should be less than 2.0%. The relative standard deviation for fat soluble vitamins were (1) Vitamin A: 1.43% (2) Vitamin D: 1.05% (3) Vitamin E: 1.01% and for water soluble vitamins (1) Vitamin B_1 : 0.85% (2) Vitamin B_2 : 1.35% (3) Vitamin B_6 : 0.95 % (4) Vitamin B_{12} : 0.75% (5) Vitamin C : 0.99%.

All parameters studied by HPLC are in accordance with the international protocols of United States Pharmacopeia. The method developed for the determination of vitamins appear to be more valuable as it is cheaper than that mentioned in literature. Moreover, this method exhibits good linearity, accuracy and precision.