

DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR DETERMINATION OF RETINYL ESTERS IN HUMAN SERUM USING MONOLITHIC COLUMN

Absorption test of vitamin A is used for monitoring of intestinal permeability and may represent a sensitive indicator of intestinal damage. In this study, a simple and rapid reversed-phase high-performance liquid chromatography (RP-HPLC) procedure for selective and sensitive determination of retinol, α -tocopherol, retinyl-palmitate and retinyl-stearate in blood serum has been developed and used for the monitoring of intestinal mucosal damage in cancer patients treated with cytotoxic drugs after absorption test of vitamin A.

The HPLC instrumentation Series 200 LC from Perkin Elmer (Norwalk, USA) with diode-array detector (DAD) was used for the analysis. Separation of retinol, α -tocopherol, retinyl-palmitate and retinyl-stearate were performed using the monolithic column Chromolith Performance RP-18e, 100 x 4.6 mm (Merck, Darmstadt, Germany). The gradient elution was used at the flow rate 3 ml min⁻¹; mobile phase methanol:water (95:5) in 0-2.1 min and methanol:2-propanol (60:40) in 2.1-4.9 min. The total time of analysis was 6 min. The injection volume of sample was 20 μ l and analysis was done at ambient temperature. The detection of retinol, α -tocopherol and retinyl esters was carried out at 325 nm, 295 nm and 330 nm respectively. Method was completely validated.