

ABSTRACT

The solid phase extraction (SPE) is an effective, reliable and selective modern method for sample preparation of biological material before chromatographic analysis.

In this graduation thesis the new SPE method for isolation of vitamin D2 (ergocalciferol), D3 (cholecalciferol) and its metabolite 25 - (OH) D3 (calcidiol) simultaneously with vitamin A (retinol) and E (α -tocopherol) in human serum was developed. The vitamins were subsequently determined by validated high performance liquid chromatography method (HPLC) using tocol as internal standard.

The developed SPE procedure:

Firstly 250 μ l of human serum was pipetted to glass tube and 1000 μ l of cool ethanol (4°C) was added. Sample was shaken and left 10 minutes in a refrigerator at 4°C for deproteinisation. After centrifugation (4 000 x g, 15 minutes, 4°C) all supernatant was carefully removed and applied into pre-treatment SPE column (firstly washed with 1 ml of methanol, then with 1 ml of distilled water). During these two steps the sorbent must not be dried up. The vitamins were eluted by 1.5 ml of methanol and then 2.0 ml of n-hexane. The organic solutions were evaporated in the vacuum concentrator at a temperature of 45 ° C. The residue was dissolved in 250 μ l of methanol and analyzed by using HPLC method.

HPLC method:

Mobile phase - A: methanol : water : 2-propanol (75:15:10) flow rate 3 ml/min, 0 - 3 minutes, B: methanol : water (95:5) flow rate 3.5 ml/min, 3 - 3.5 minutes; column – monolithic column Chromolith Performance RP-18e, 100 x 4,6 mm + Speed ROD RP-18e, 50 x 4,6 mm MERCK (Darmstadt, Germany); injection volume 20 μ l; diode array detection - 25-(OH)D₃, D₂, D₃: 264 nm, retinol: 325 nm, α -tocopherol: 295 nm, internal standard tocol: 295 nm.

The new developed SPE method for simultaneous analysis of cholecalciferol, ergocalciferol, calcidiol, retinol and alpha tocopherol in human serum can improve the diagnostic possibilities of patients with disorders of renal function, osteoporosis and also for oncological patients in Faculty Hospital in Hradec Králové.