

Abstract

In this diploma work the new HPLC method for simultaneous determination of vitamins D₂, D₃ and their metabolites (1,25(OH)₂D₃, 25(OH)D₃) using the internal standard was developed.

During the suggested assessment the monolith column Chromolith Performance RP-18e, 100 x 4,6 mm was used. The detection was carried out with the help of a diode array detector at wavelength 265 nm for vitamins D and its metabolites, 295 nm for the internal standard tocol. The mixture of methanol : acetonitrile : water in percentual representation 12,5 : 85 : 2,5 was used as the mobile phase. The flow rate of the mobile phase was 1,5 ml.min⁻¹ and the injection volume of the sample was 20 µl. The total time of the analysis was 3,5 minutes including the equilibration of the column.

This method was developed and partially optimised with the standards of vitamins D and will be validated for biological material.