

## **Abstract**

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**Title of the diploma thesis:** Development of UHPLC method for separation of eight structurally related derivatives of vitamin E

The aim of this diploma thesis was to develop UHPLC method for the separation of eight derivatives of vitamin E. They include a group of tocopherols and tocotrienols. To achieve the aim ultra-high performance liquid chromatography was used. In process of optimization the most important thing was the choice of stationary phase, consistency of mobile phase, flow speed, temperature of column and the time of separation. The Ascentis Express F5 Column (100 x 3.0 mm, 2.7  $\mu\text{m}$ ), Sigma-Aldrich was the best stationary phase because the separation of all eight derivatives on this column including two problematic pairs:  $\beta$ -T3 with  $\gamma$ -T3 and the pair  $\beta$ -T with  $\gamma$ -T was performed. The mobile phase was a mixture of a methanol with acetonitrile (1:1) and water in the ratio 75:25 with a flow rate 1 ml/min, isocratic elution and PDA and FLR detection. In PDA detection the wavelength was set 290 nm. In more sensitive FLR detection the emission wavelength was 325 nm and the exciting wavelength of exciting was set on 280 nm. The time of separation was less than 8 minutes. The development of the method included validation, where the accuracy and precision of the method was verified by measuring the concentration and determining the RSD for the  $\alpha$ -tocopherol drug drops in the nutritional supplement.

**Key words:** vitamin E; tocopherols; tocotrienols; UHPLC; development of method; separation