

## **ABSTRACT**

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**Title of Diploma Thesis:** Influence of sample preparation method on occurrence of matrix effects in UHPSFC-MS analysis of vitamin E

The goal of this thesis was to optimize the chromatographic method for the determination of 8 different isomers of vitamin E that would provide better analysis results than two existing methods; high speed (HS) and high resolution (HR). New Torus stationary phases (SP) were tested (diethylamine, 1-aminoanthracene, 2-picolylamine and diol under conditions: CO<sub>2</sub>:MeOH 98:2, flow rate 2 ml/min, injection volume 2 µl, column temperature 40°C, BPR 12.9 MPa, make-up solvent 0.1% NH<sub>4</sub>OH in MeOH. The modifiers (ethanol, isopropanol and a 1:1 mixture of acetonitrile/methanol) were tested on the 2-PIC column. The effect of additives in the mobile phase was also compared (0.1% NH<sub>4</sub>OH, 10mM ammonium formate, 10mM ammonium acetate and 2% H<sub>2</sub>O). The next part of the thesis describes the development of the sample preparation method to reduce or eliminate the matrix effects. Dilution method was tested using solvents - H<sub>2</sub>O, 50% acetonitrile (ACN) and 75% ACN. Urine samples were 10, 20, 50 and 100 times diluted by the most suitable solvent and subsequently analyzed under HS and HR conditions. The second sample preparation method was liquid liquid extraction, where hexane, heptane and dichloromethane were tested in different proportions which were used for extraction of urine containing 0.5 µg/ml vitamin E standards in ratio 1:1 or 2:1. The influence of extraction time (5, 10 and 20 minutes) was tested. After sample preparation samples were analyzed under HS conditions and matrix effects were investigated.

**Key words:** vitamin E; tocopherols; tocotrienols; SFC; method development; optimization; dilution; LLE; matrix effects

