

## **Abstract**

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**Title of thesis:** Determination of tyrosine metabolites

The topic of thesis was determination of tyrosine metabolites (epinephrine, norepinephrine, dopamine, L-DOPA) using derivatization reaction and later analysis using High-performance liquid chromatography with fluorescence detector.

The important step of all work was optimization of conditions of derivatization reaction. As derivatization agent it was used FMOC-Cl (fluorenylmethyloxycarbonyl chloride), which reacted with catecholamines together with borate buffer. Firstly, it was set the ideal concentration of agent (2,7 mM), also the time (15 min) and the temperature (40 °C) of reaction. The concentration (100 mM) and pH (8,0) of borate buffer was optimized in the end.

For effective separation it was necessary to set ideal conditions. As silica gel column was used Kinetex EVO C18 with core-shell particles. The compositions of mobile phase was necessary to optimize and resulting mobile phase was composed from formic acid (20 mM) and acetonitrile in proportion 32:68. Used flow rate was 0,6 ml/min. The column was tempered at 40 °C. The injection volume was 5 µl and time of analysis was 19 minutes. The fluorescence detector was set on wavelengths 263 nm ( $\lambda_{ex}$ ) and 313 nm ( $\lambda_{em}$ ).

**Keywords:** Tyrosine, Epinephrine, Norepinephrine, Dopamine, L-DOPA, FMOCl, Derivatization, HPLC