

Abstract

Charles University

Faculty of Pharmacy in Hradec Králové

Department of Pharmaceutical Chemistry and Pharmaceutical Analysis

Candidate: Michaela Gieslerová

Supervisor: PharmDr. Petr Kastner, Ph.D.

Title of thesis: Study of retention properties of selected tyrosine metabolites under HPLC conditions

The aim of this diploma thesis was to study the retention properties of selected tyrosine metabolites under the high performance liquid chromatography using spectrophotometric (285 nm) and fluorimetric detection (emission 279 nm, excitation 320 nm). The total of eight analytes was examined – tyrosine, L-DOPA, dopamine, epinephrine, norepinephrine, homovanillic and vanillylmandelic acid.

Firstly, we studied the effect of mobile phase composition, buffer concentration and pH for the separation of analytes. Analysis of selected analytes was performed using an KINETEX 5 μm EVO C18 column (150 mm x 3 mm, 5 μm ; PHENOMENEX). The best separation showed the mobile phase methanol:100 mM acetic acid (pH adjusted with sodium hydroxide to 4,5); 5:95.

Next, the effect of stationary phase for the separation of analytes was studied with the mobile phase described above. A total of 7 different columns were studied, namely stationary phases with modified silica gel C₁₈ group, PFP group, combined stationary phase (C₁₈ group with aromatic functionality, combination C₁₈ group with PFP) and two columns with C₁₈ group with unspecified polar groups. The best separation was achieved with an ARION C18 polar column (column with C₁₈ group with unspecified polar groups), total runtime was 15 min. All experiments were performed by isocratic elution. The flow rate was 0,3 ml/min. The column temperature was set at 25 °C. The injection volume was 5 μl .

Keywords: HPLC, tyrosine, catecholamines