

Abstract

Faculty of Pharmacy in Hradec Králové, Charles University

Department of Analytical Chemistry

Candidate: Ondřej Tužil

Supervisor: PharmDr. Juraj Lenčo Ph.D.

Title: Optimization of mobile phase composition for analysis of peptides in mixed-mode chromatography

In bottom-up proteomic analyzes performed by liquid chromatography with mass detection, the mobile phase is acidified with weak acids, which do not suppress the intensity of the analytes in the detection to the same extent as commonly used acids for separations without mass detection. Such a mobile phase additive is formic acid, which is most commonly used at a concentration of 0.1 % (v / v). This work was focused on finding the lowest possible concentration of formic acid, which in combination with the multimodal stationary phase of the ACQUITY UPLC CSH C₁₈ column from Waters would still ensure efficient separation of peptides, while increasing the response in the mass spectrometer. First, separations of peptide standards were performed by liquid chromatography with UV detection with decreasing amounts of formic acid in the mobile phase. It has been found that significantly lower concentrations of formic acid can be used for satisfactory peptide separation parameters. The three lowest concentration levels were used for mass detection analyzes, where an increase in the intensity of the peptide peaks was observed. Two concentration levels of formic acid in the mobile phase were then also tested in a bottom-up analysis of tryptic peptides from a *F. turalensis* lysate. The highest number of identified peptides was obtained using 0.02% formic acid in the mobile phase. Compared to the standard concentration of 0.10 %, 11.9 % more peptides were identified.

Key words: peptides, mobile phase, formic acid, LC / MS, CSH, intensity