

ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové

Department of Analytical Chemistry

Candidate: Bc. Ondřej Hývňar

Supervisor: PharmDr. Juraj Lenčo, Ph.D.

Title of diploma thesis: Top-down, middle-up and bottom-up RPLC analyses of biopharmaceuticals using a single column

Nowadays, different columns are needed for top-down, middle-up and bottom-up analyses of biopharmaceuticals using liquid chromatography. The aim of this thesis was to determine whether it is possible to use for all these types analyses a new column BioResolve RP mAb Polyphenyl of the company Waters, intended mainly for top-down and middle-up analyses. The biopharmaceuticals trastuzumab, bevacizumab, panitumumab and aflibercept were analyzed as intact for top-down analysis, reduced, cleaved by IdeS protease, cleaved by IdeS protease and reduced for middle-up analysis and cleaved by endopeptidase Lys-C and trypsin for bottom-up analysis. Top-down and middle-up analyses were performed using liquid chromatography with UV detection. We verified the ability of the column to analyze biopharmaceuticals prepared this way in our conditions. We found that although the column can handle intact biopharmaceuticals, it gives better results in middle-up analyses. Bottom-up analyzes were performed using liquid chromatography coupled with mass spectrometry, where the BioResolve column was compared to Acquity UPLC CSH C₁₈ column developed for bottom-up analyses of proteins. Although the Acquity column gave better results, we can conclude from the results that the BioResolve column is also suitable for bottom-up analyzes. In the last part of this thesis, we tried to unify the mobile phases so that it is possible to perform top-down, middle-up and bottom-up analyses with one system of mobile phases. We found that by adding 10% 1-butanol to component B of the mobile phase acidified with 0.1% FA, we obtain a mobile phase system suitable for top-down, middle-up and bottom-up analyzes compatible with MS.

Keywords: Proteomic analysis, biopharmaceuticals, peptides, LC-UV, LC-MS, reverse phase