

## Abstract

This bachelor thesis is focused on separation of four model proteins – conalbumin, bovine serum albumin, beta-lactoglobulin A and beta-lactoglobulin B – by gradient chromatofocusing high-performance liquid chromatography with two commercial anion-exchange columns. The first column contained a strong anion exchanger (Agilent Bio SAX) and the second one contained a weak anion exchanger (Agilent Bio WAX). Three different mobile phase pH gradients were optimized, one-hour gradient and two half-an-hour gradients. The baseline separation of mixture containing conalbumin and beta-lactoglobulines A and B was achieved on both anion-exchange columns using each of the three optimized gradients and with flow rate 1 ml/min. Three bovine serum albumin isoforms were resolved in all tested systems using a strong anion exchanger as the stationary phase. The highest resolution of mixture containing conalbumin and beta-lactoglobulines A and B was obtained with a strong anion exchanger as the stationary phase in thirty minutes. The highest resolution of bovine serum albumin isoforms was achieved with a strong anion exchanger as the stationary phase in sixty minutes.

Key words: gradient chromatofocusing, protein separation, high performance liquid chromatography