Abstract

This work deals with the development and optimization of conditions of a method that can be used to compare the activity of the enzyme β-*N*-acetylhexosaminidase in hydrolysis of a natural substrate and a chromogenic substrate, which is often used in the study of enzyme kinetics. As a substrate, 4-nitrophenyl-*N,N′*-diacetyl-β-D-chitobioside was selected for cleavage. This oligosaccharide contains bond, which the enzyme cleaves in the natural substrate, and the bond that occurs in the chromogenic substrate. To determine the products arising from enzymatic hydrolysis of 4-nitrophenyl-*N,N′*-diacetyl-β-D-chitobioside, capillary zone electrophoresis was used. First, it was necessary to find the optimal composition of the electrolyte, its pH and concentration. The optimal background electrolyte was a solution of sodium tetraborate at a concentration of 25 mmol/l and a pH of 10.25. Subsequently, repeatability, calibration curves and linearity, limit of detection and limit of quantification were investigated. Repeatability of migration times ranged up to 0.6%, the repeatability of peak areas between 2.5 and 6.3%. Limits of detection were ranging from 0.005 to 0.120 mmol/l. Finally, the optimized method was successfully used to monitor the actual enzyme cleavage.