

Abstract

This work deals with optimization of a method for separation and detection of protamine and insulin using capillary zone electrophoresis. The composition of background electrolyte, the solution pH and the injection method were optimized. Citric acid in a concentration range of 80 to 240 mmol L⁻¹ and chloroacetic acid ranging from 50 to 150 mmol L⁻¹ were tested sequentially. The optimized method uses a fused silica capillary with inner diameter of 50 μm. The total length of capillary is 50.0 cm, effective length is 8.5 cm. The injection of the sample is performed on the short end of the capillary. The method uses chloroacetic acid of 100 mmol L⁻¹ concentration as the background electrolyte. Driving voltage is 20 kV. Sample is injected hydrodynamically with a pressure of 5 kPa for 3 s. The analytes are detected spectrophotometrically at wavelength of 200 nm. The method allows for determination in case of protamines in concentration range between 4 μg mL⁻¹ and 300 μg mL⁻¹ and insulin from 5 μg mL⁻¹ to 300 μg mL⁻¹. The limits of detection are 1 μg mL⁻¹ for protamine and 2 μg mL⁻¹ for insulin. Repeatability of migration times and peak areas tested at 30 μg mL⁻¹ and 200 μg mL⁻¹ concentration levels using hydrodynamic injection showed values of relative standard deviation lower than 6 % suggesting satisfactory repeatability. Electrokinetic injection was also tested by applying voltage of 5 kV during 5 s. Electrokinetic injection is less accurate, but it can be used at lower concentration range. The applicability of the optimized method was tested on a real sample of NPH insulin. Before the detection the suspension of the sample had to be dissolved in acidic environment. The sample was dissolved in aqueous solution of phosphoric acid of 4.5 mmol L⁻¹ concentration. The main advantage of the method is the rapid analysis; separation was done in about 2 minutes and the undemanding sample preparation. The disadvantage is that this method is not able to separate individual protamine peptides.

Keywords

capillary zone electrophoresis, protamine, insulin