Abstract

Unfractionated heparin, which is a widely used anticoagulant, is frequently replaced with low-molecular-mass species. They are used due to their more predictable anticoagulant effect with less bleeding complications and also they have prolonged anticoagulant effect. For monitoring of low-molecular-mass heparin levels, anti–factor Xa assay is used, which has some significant drawbacks.

This work is dedicated to determination of low-molecular-mass heparin, namely Fraxiparine, using affinity capillary electrophoresis. Heparin is a polysaccharide which does not exhibit a significant UV absorption; therefore, its indirect detection method was used. Fraxiparine forms a stable complex with protamine. Protamine is an arginine–rich, positively charged peptide which is used to suppress heparin anticoagulant effect. Because protamine has a complex, not precisely defined structure, it was replaced by well-defined tetraarginine.

The method uses phosphoric acid of 9 mmol L^{-1} concentration with addition of 0.1% (w/v) hydroxyethylcellulose as the background electrolyte. The samples are injected hydrodynamically into the capillary by a pressure of 5 kPa. First, the zone of Fraxiparine was injected, followed by the zone of tetraarginine (5 s). After that, 30 kV voltage was applied for 30 s. During this time the zones migrate through each other, which enables formation of the complex. Fraxiparine was determined from the amount of remaining free tetraarginine detected at a wavelength of 200 nm. The injection time of Fraxiparine was subject to optimization. The total length of the capillary was optimized, too. The optimal method uses a capillary of 40.0 cm total length (effective length was 31.5 cm). Fraxiparine was injected for 120 s, tetraarginine for 5 s. The voltage was reduced to 20 kV (30 s) to make sure that the solutions will not overheat.

After the optimization, different experiments were performed due to the determination of Fraxiparine in blood plasma. Acetonitrile, acetone and trifluoroacetic acid were tested for deproteination of the samples.

Keywords

capillary electrophoresis, low-molecular-mass heparin, affinity interactions