

## **ABSTRACT**

**Charles University in Prague**

**Faculty of Pharmacy in Hradec Králové**

**Department of Biophysic and Physical Chemistry**

**Candidate: Bc. Martina Dvořáková**

**Supervisor: Doc. Ing. Alice Lázníčková, CSc.**

**Consultant: Mgr. Martina Hermannová, Ph.D.**

**Title of diploma thesis: Optimization of preparative LC-MS method for fractionation of oligosaccharides of hyaluronanu**

This diploma thesis deals with optimization of LC-MS method for analysis of hyaluronan oligosaccharides in preparative mode. The theoretical part summarizes available information about biological and chemical properties of hyaluronic acid. Hyaluronic acid is easily enzymatically degradable by mammalian hyaluronidases that produce hyaluronan oligosaccharides. The biological function of these degradation products depend on their molecular weight. High-performance liquid chromatography is mainly used for separation and purification of hyaluronan oligosaccharides. A new method for the determination of hyaluronan oligosaccharides is based on a combination of separation techniques and mass spectrometry. The experimental part deals with optimization of ionisation conditions for electrospray ionization mass spectrometry in positive and negative ion mode. In the first step, we focused on setting of capillary voltage, cone voltage, desolvation temperature, flow rate of desolvation gas and cone gas. In the MS spectra of 4-, 6-, 8- a 10- oligosaccharides of hyaluronan, we observed the presence of singly and doubly charged ions and compared their intensity. LC-MS analysis of hyaluronan oligosaccharides in negative ion mode was more sensitive than in positive ion mode. In the next step, we focused on the optimization of eluent compositions in preparative mode. Addition of 0,1 % formic acid enhanced the production of negative ions and addition of 0,1 % ammonia enhanced the production of positive ions. The best results were achieved using 100% methanol.