

# Abstract

Charles University in Prague, Faculty of Pharmacy in Hradec Králové

**Department:** Pharmaceutical chemistry and Drug Control

**Candidate:** Lenka Teplá

**Tutor:** PharmDr. Pavla Pilařová, Ph.D.

**Title of Thesis:** HILIC separation of acyclovir and its degradation product II

The aim of this thesis is to test the retention behavior of acyclovir and its degradation product - guanine on zirconia-based column with carbon layer ZirChrom® - CARB under hydrophilic interaction liquid chromatography (HILIC). The thesis also completes the knowledge already obtained from the diploma thesis of Lucie Bouzková (2016), which deals with finding suitable conditions for the separation of analytes. The work examines the influence of the concentration of the phosphoric acid, ammonium fluoride, sodium fluoride, triethylamine and phosphate buffers in the mobile phases on the elution of the analytes. The thesis further looks into the influence of the composition of the mobile phases with different ratios of the organic and aqueous components. The effect of increasing temperature on the retention of acyclovir and guanine was also observed. A higher content of organic component in the mobile phase leads to shortening of retention of acyclovir and guanine. Separation is caused by retention of hydrophilic analytes in the aqueous phase of the mobile phase. As the temperature rises, the retention of the analytes decreases and the symmetry of their peaks is improved. The behavior of fluorides and phosphates during the mobile phase is similar to a Lewis base and they compete with Lewis acid binding sites on the surface of the zirconium column. Interactions are based on ligand exchange. Triethylamine is also behaves like the Lewis base. At a higher concentration of triethylamine the retention of both analytes decreases and the carbon layer on the surface of the stationary phase as well as are involved in the separation mechanism. The different types of interactions seem to depend on the conditions of analysis, i.e. the type of the used buffer and its concentration, the organic component used in the mobile phase and the pH.