Abstract

This diploma thesis deals with optimization of separation conditions for the four groups of analytes related to 7-deazaadenosine (each group was composed of four derivatized nucleosides) using hydrophilic interaction liquid chromatography. All the sixteen analytes were synthesized as potentially cytostatically active compounds. The effect of the type of stationary phase in the chromatographic column, the ratio of organic and aqueous parts of the mobile phase, pH of the buffer used as the mobile phase component and the concentration of ammonium acetate in the buffer in the range of 5-50 mM were tested during the optimization process.

Three stationary phases were tested - bare silica (Spherisorb® Silica column), silica-bonded amide (TSKgel Amide-80 column) and silica-bonded native cyclofructan 6 (Frulic-N column). The dimensions of all columns were 250×4.6 mm i.d.; particle size 5 μm. During the optimization of separation the mechanism of HILIC was studied. It was found that the distribution of analytes between the aqueous layer partially coated on the surface of the stationary phase and the mobile phase and also the adsorption of analytes on the stationary phase participated in the retention and separation mechanism in all tested chromatographic systems. The three groups of analytes (SK1, SK3, SK4) were only partially separated into three peaks. The indication of separation into four peaks was observed only for the mixtures of analytes SK1 and SK2 on the Frulic-N column using mobile phase composed of acetonitrile/5mM ammonium acetate pH 7,5 95/5 *(v/v)*. However, the analyses showed low resolutions. The mixture of analytes SK2 were baseline separated into three peaks on the Frulic-N column using mobile phase composed of acetonitrile/50mM ammonium acetate pH 7,5 95/5 *(v/v)*.

Keywords:

HILIC, 7-deazaadenosine, separation, cytostatics