

OPTIMIZATION OF HPLC CONDITIONS FOR ANALYSIS OF CATIONS AND ANIONS IN MINERAL WATERS FOR MEDICINES INTERACTION STUDIES

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Currently the concept has been adopted that all medicines must be washed down with water. However, there are a number of mineral waters in which a sufficiently large amount contains metals of various natures. At the same time, many medicines contain in their composition active groups such as carboxyl, hydroxyl, amino, which can react with metal cations, participating in complexation reactions.

According to the literature data, study of interaction can be performed by different physico-chemical methods, including HPLC method.

The aim of our work was to optimize the conditions of the chromatographic system for simultaneous separation and quantitation of cations and anions within a single run.

HPLC analysis has been carried out using Waters 2695 chromatography system, for detection we used Waters ELS detector 2424. The separation was done with the usage of ZIC-HILIC column (150 × 2.1 mm, particle size 3.5 μm). The two elution solvents were exchanged: the solvent A (buffer) and solvent B (acetonitrile). Ammonium acetate was used as salt for buffer preparation and pH was adjusted with acetic acid.

At the first step, we prepared a solution of sodium chloride in the concentration of 0.05M to investigate the effect of the organic content of the mobile phase to the separation of analytes. According to obtained data, when the amount of acetonitrile was 20% no separation was observed. In the case of a higher concentration of the organic solvent, the retention times of both ions increased substantially. In addition, the resolution between the ions increased with an increasing of organic amount. When the amount of acetonitrile was 80% the fine separation between ions was achieved, but the single for analysis was too long so we decided to use gradient mode for separation and make the time of analysis shorter.

The pH effects of the mobile were investigated across a range approximately 3.0-7.0. These experiments were conducted with constant buffer concentration of 25mM ammonium acetate. Gradient mode was done during all investigations. Amount of organic solvent was changing as follows from 80% to 20 % in 20 minutes and a flow rate of 0.3mL/min was used. Results showed that with increasing pH the retention times of all cations increased also and the retention times of anions decreased. There was no significant effect on the symmetry of peaks, except for Ca²⁺ and Mg²⁺.

The effect of buffer concentration was investigated using two different salts such as ammonium formate and ammonium acetate. The choice of such salts can be explained by decision to use ELS detector. A volatile buffer of some sort is required for this kind of detector.

According to our further investigation ammonium formate in high concentration (100mM) can form precipitation with acetonitrile at its high concentration (about 80%). For this reason, we decided to avoid that buffer.

Our results showed that the changing concentration of ammonium acetate from 10mM to 100mM, the retention times and peak symmetry of ions are dramatically affected. Increasing of buffer concentration made the retention times of anions longer while retention times of cations decreased.

As conclusions, the results of this work will be used in further researches for quantitative and qualitative determination of cations and anions in waters and interaction study.