

## **Abstract**

The aim of this work is to find the optimal separation conditions in high performance liquid chromatography for the analysis of sulfated polyphenolic substances, especially flavonoids, phenolic acids and catechols. Three chromatographic columns were used in the development of the method. ZIC-HILIC column with polar organic phase and C18 and Kinetex PFP columns with reverse phase. We focused in particular on a short time of separation, sharp tailless peaks and separation of all components of the mixture. Using a Kinetex PFP column, the optimal method for separating the test mixtures was found. The use of a mobile phase of 10 mM ammonium acetate/0,1% formic acid and methanol was shown to be the most appropriate, with a mobile phase flow rate of 0,6 ml/min. By modifying the gradient and temperature, all components of the tested mixtures were separated, the peaks were sharp, and the separation time in most cases ranged do not exceed 10 min. The method is also directly applicable in conjunction with a mass detector.

**Keywords:** High Performance Liquid Chromatography (HPLC), flavonoids, polyphenolic substances, sulfation, separation