ABSTRACT

This thesis was focused on a determination of heparin using sequential injection analysis with spectrofluorimetric and spectrophotometric detection. The principle of determination was based on the interaction of heparin with phenothiazine dye. A decrease of fluorescence intensity of dye in its emission maximum was detected. In the case of spectrophotometric detection a decrease of the absorbance of dye was measured. Azure A, azure B and methylene blue were used as representantive selection of phenothiazine dyes. The determination was performed on a laboratory made SIA apparatus, for which a control software in LabVIEW 7.1 graphical programming was created. Two types of flow configuration for spectrofluorimetric detection were implemented.

Type 1: For deionized water as a carrier stream with a injection of heparin and dye zones there were found the following optimal conditions: $c_{dye} = 1 \times 10^{-5}$ mol dm^{-3}; $v_{flow} = 2500$ µl min^{-1}; reaction coil length of 0 cm; injected volume of dye 150 µl and injected volume of sample 150 µl. Dynamic range of calibration curves with an exponential course for the individual dyes in the range of LOQ – 1200, eventually up 1500 IU dm^{-3} were detected. Limits of detection between 7.6 – 39.1 and the limits of quantification between 58.8 – 124.5 IU dm^{-3} were found.

Type 2: For use a dye as a carrier stream with an injection of heparin there were found the following optimal conditions: $c_{dye} = 1 \times 10^{-5}$ mol dm^{-3}; $v_{flow} = 3000$ µl min^{-1}; reaction coil length of 50 cm; injected volume of sample 150 µl. Dynamic range of calibration curves is similar to the first type of measurement. Limits of detection between 20.4 – 62.8 and the limits of quantification between 80.1 – 135.8 IU dm^{-3} were found.