

# Abstract

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Title of diploma thesis: Development of sensitive determination of diclofenac using fluorescent properties of complexes with cyclodextrins

This experimental thesis deals with the development of a sensitive determination using the formation and detection of a complex consisting of an active substance and cyclodextrin (CD) using sequential injection analysis (SIA). A spectrofluorometer was used for subsequent detection. The tested biologically active substance was diclofenac in the form of sodium salt.

In the beginning the conditions for setting the spectrofluorometer, namely the appropriate excitation and emission wavelengths, which were 364,5 and 290 nm, slit size and detection sensitivity were found. After that, 13 CDs were used in order to find the appropriate type and ratio of CD with diclofenac so that the measured fluorescence intensity differs from the intensity of CD alone. This testing divided the CDs into 4 groups. When testing the stability of selected complexes, the increase or decrease in fluorescence intensity over time was monitored to ensure the formation of complexes with CD.

SIA was used to automate the determination, which was connected to a fluorometric detector. For measurements in the flow system, the spectrofluorometer settings were further adjusted and 5 control programs of the SIA system were programmed, which used different mixing of the two aspirated zones, stopping these zones in the mixing coil and a combination of both procedures. During the optimization, different flow rates were also tested, and 30  $\mu\text{l/s}$  was optimal.

For the calibration, a program combining mixing of the aspirated zones by changing the direction of the carrier flow and stopping in the mixing coil was selected from the control programs, and (2-hydroxypropyl)- was selected from the CD $\gamma$ -CD. First, the measurement was carried out in an aqueous environment and then in a culture medium. To find a linear dependence, different

concentrations of CD  $1 \times 10^{-2}$ ,  $1 \times 10^{-3}$  and  $1 \times 10^{-4}$  mol/l were tested. The highest linearity in the aqueous environment was demonstrated for the range of  $2,5 \times 10^{-7}$ – $7,5 \times 10^{-6}$  mol/l diclofenac with  $1 \times 10^{-4}$  mol/l CD and the coefficient of determination  $R^2 = 0,9954$ . Therefore, the following conditions were proposed for calibration in the culture medium: CD concentration was  $1 \times 10^{-4}$  mol/l, diclofenac range  $2,5 \times 10^{-7}$ – $1 \times 10^{-6}$  mol/l. The aspirated zone ratio was 1:1 with a flow rate of  $30 \mu\text{l}$ . Control program 5 was used with a stop time in the mixing coil of 1 min.