

# Abstract

Charles University in Prague

Faculty of Pharmacy in Hradec Králové

Department of Analytical Chemistry

Candidate: Bc. Anežka Holznerová

Supervisor: Doc. RNDr. Dalibor Šatínský, Ph.D.

Title of Diploma Thesis: On-line SPE HPLC method optimization for determination of patulin mycotoxin in apple drinks

The issue of food contamination with mycotoxins is a serious problem worldwide. These substances are highly toxic to humans and chronic effects on the human organism in very low doses may cause long-term medical complications. In our work we focused on one of the most common dietary mycotoxins - patulin, which is found in apples and related products (especially apple juices). High performance liquid chromatography (HPLC) coupled with on-line solid phase extraction (SPE) using a column switching technique for sample treatment was developed for determination of mycotoxin patulin in apple drinks and juices. A volume of 250  $\mu\text{l}$  of juice sample was injected directly into the on-line SPE-HPLC system. After injection of the sample the extraction of patulin from juice matrix was carried out on SPE precolumn. SPE precolumn 25 x 3 mm was filled with Supel MIP Patulin sorbent, which is a specific "molecularly imprinted polymer" (MIP) designated for the selective extraction of patulin from an apple matrix. As the washing solution for removing ballast matrix was selected 1% solution of  $\text{NaHCO}_3$ , which flowed through MIP precolumn at flow rate 2 ml/min for 2.5 minutes. After this period a valve was switched and the residual ballast matrix, retained on the extraction precolumn, together with patulin were further separated on an analytical column Kinetex Biphenyl 150 x 4.6 mm (particle size 5 $\mu\text{m}$ ). The mobile phase composition of 20 % ethyl acetate in acetonitrile with water in ratio 20:80, flow through the column at 1 ml/min in gradient elution. Detection was performed by UV-VIS detector at a wavelength 276 nm. The total analysis time of one juice sample, including its online pretreatment, was less than 9 minutes. The detection limit of this method was found at level 50  $\mu\text{g/l}$ , which is the value corresponding to the maximum allowed level of patulin in apple juices according to EU standards.