ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové

Department of Analytical Chemistry

Candidate: Kristián Kretek

Supervisor: prof. PharmDr. Lucie Nováková, Ph.D.

Title of the diploma thesis: Development of UHPLC-MS/MS and UHPSFC-MS/MS methods for determination of selected ochratoxins

The purpose of this work was development and comparison of UHPLC and UHPSFC analytical methods coupled to tandem mass spectrometry for four common ochratoxins. The experiments were performed using UHPLC system ACQUITY UPLC and UHPSFC system ACQUITY UPC^2, mass spectrometer XEVO TQ-XS was the same in both cases.

First, optimization of ion source parameters and SRM transitions was done. For UHPLC along with ACN, 3 additives of the water-based component of mobile phase were tested – formic, acetic acid and ammonia, with flow rate 0,4 ml/min, gradient from 2 % to 95 % ACN in 5th minute, temperature 40°C and column Acquity UPLC BEH C18. For UHPSFC 6 organic modifiers along with CO_2 were tested: MeOH and MeOH with addition of water, formic acid, ammonia, and ammonium formate, with flowrate 1,5 ml/min, gradient from 2 % to 45 % MeOH in 5th minute, temperature 40°C and BPR pressure 2000 psi. Screening of 13 columns was also conducted. Optimization of MS detection in SFC was done by testing 8 make-up solvents: MeOH, EtOH, MeOH with addition of water, formic acid, ammonia and ammonium formate and acetate. The final UHPLC conditions were: column Acquity UPLC BEH C18 and mobile phase in both modes: ACN + H_2O + 0,01 % acetic acid. The final UHPSFC conditions were: column Acquity UPLC BEH Amide, mobile phase CO_2 + MeOH + 10 mM ammonium formate + 2 % H_2O and make-up solvent MeOH + 5 % H_2O in positive mode and CO_2 + MeOH + 10 mM ammonium formate and MeOH in negative mode. The final UHPSFC-MS/MS method achieved far narrower calibration range and higher lower limit of quantification compared to UHPLC-MS/MS.

Keywords: ochratoxins, UHPLC-MS/MS, UHPSFC-MS/MS, method development, optimization