Abstract

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Title of the diploma thesis: Development of a sample preparation method for the determination of selected ochratoxins in urine by UHPLC-MS/MS

The aim of the work was to develop a method for the determination of ochratoxins A, B, C, and α in urine with the lowest possible quantification limit, and subsequently to carry out the validation of this method, which would be applicable in clinical practice. The method of ultra-high-performance liquid chromatography in a reversed-phase mode in coupled with tandem mass spectrometry was selected for the analyse. Optimalization of sample preparation involved: micro-solid phase extraction in pipette tips (μ-SPE-PT), dilute and shoot (DAS), supported liquid extraction (SLE) and finally salting-out assisted liquid-liquid extraction (SALLE). The method recovery, the interference of the matrix and the limit of quantification of the given method were evaluated.

Method validation was carried out on an ACQUITY UPLC BEH C18 1.7 μm, 2.1 x 100 mm column from Waters, using μ-SPE-PT as sample preparation technique. Method accuracy ranged from 68 to 122 % for all analytes, and method precision exhibited RSD was less than 20% for all analytes. The LLOQ was determined of 0.01 ng/ml for OTA, 0.02 ng/ml for OTB and OTC, and 0.05 ng/ml for OTα. ULOQ was established for analytes to be 20 ng/ml. Evaluation of matrix effects was performed where the requirements were met only for OTA at both concentration levels of 5 and 20 ng/ml and OTB only at 5 ng/ml. For OTα were the matrix effects only 70% on both levels, and for OTC were in the hundreds of percent.

Key words: ochratoxins, urine, sample preparation, UHPLC, μ-SPE-PT, DAS, SLE, SALLE