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Analysis of Mycotoxins in Urine by Liquid Chromatography Coupled with a Q ExactiveTM Ultra-high Resolution Mass Spectrometer

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Introduction

Every year, ingestion of toxic mushrooms causes many illnesses and can lead to death in absence of medical assistance. These intoxications can result from the consumption of some amatoxins like α-amanitin, β-amanitin or phallotoxin, present in several toxic mushrooms like Amanita phalloides. In order to quantify mycotoxins in complex matrixes and be able to give a treatment as quickly as possible, toxicologists must adapt and improve their analytical techniques to make it faster and more reliable. A validated LC-MS method (liquid chromatography coupled with a Thermo Scientific Q ExactiveTM instrument, an ultra-high resolution and accurate mass instrument) for simultaneous quantification of mycotoxins in small urine samples was set up.

Experimental conditions

The method includes a simple solid phase extraction (SPE). Urine samples were first spiked with an internal standard (IS) (Flurazepam) and extracted by SPE with Bond Elut Agilent Certify cartridges (C18, 200 mg, 3 mL). Analytes were then eluted with 3 mL of methanol with 2% of ammoniac. The eluate was evaporated to dryness at 50°C, reconstituted in 200 µL of LC-MS water and injected on LC-MS system. Quantitation was performed by extracting the exact mass of each of the protonated species using a 5 ppm mass accuracy. LC separation was carried out using a gradient on a C18 Accucore column (100 mm x 2.1, 2.6 µm). Molecules were detected in positive mode using the Q Exactive^{TM} mass spectrometer equipped with an Electro Spray Ionization (ESI) source. Analysis was performed in Targeted SIM by injecting only the masses of interest into the mass spectrometer. All acquired data were processed using Xcalibur software.

Results and Conclusion

Separation of analytes was obtained in a 6 min LC gradient with a flow rate of 400 µL.min^{-1}. Retention times for α-amanitin, β-amanitin, phalloidin and IS were respectively 2.2, 2.0, 3.5 and 3.8 min (Fig. 2). Accurate mass specific to each mycotoxin was determined by direct infusion of the molecule into the orbitrap. Measured accurate m/z values were 919.3614, 920.3455, 789.3257 and 388.1586 for α-amanitin, β-amanitin, phalloidin and IS, respectively. Mass spectrum of toxins and Flurazepam showed one abundant peak, corresponding to the $[M+H]^{1+}$ species. Calibration curves were obtained by spiking drug-free urine from 1 to 100 ng/mL. Mean correlation coefficients, r^2, were above 0.99 for each mycotoxin (Fig. 3). The validation procedure included tests for the following parameters: linearity, accuracy, precision (with a variation coefficient less than 20%), recovery (around 90%) and matrix effects according to the currently accepted method validation procedures.

FIGURE1 : Separation of three mycotoxins (100 ng/mL) and flurazepam (50 ng/mL)
Column: C18 Accucore, 2.6 µm, 100x2.1 mm - Mobile phase A: 10 mM ammonium acetate buffer containing 0.1% (v/v) formic acid; mobile phase B: acetonitrile with 0.1% formic acid - 6 min LC Gradient - Flow rate : 400 µL/min.

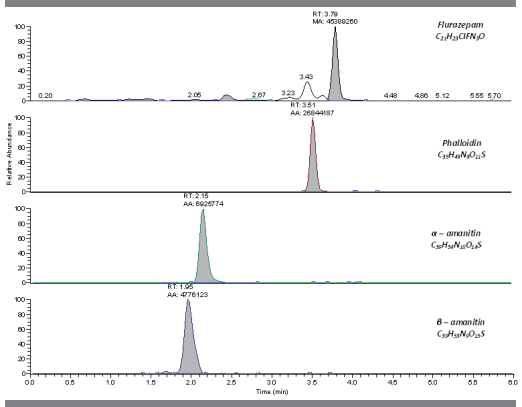
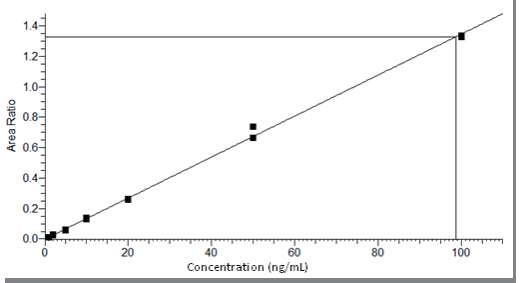


FIGURE 2 : Calibration curve of α-Amanitin in urine (y= -0.00057+0.01346x ; R^2=0.998)



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