

ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové

Department of Analytical Chemistry

Candidate: Jakub Eduard Syřínek

Supervisor: Assoc. Prof. PharmDr. Lucie Nováková, Ph.D.

Title of Diploma Thesis: Optimization of metabolomics workflow: critical evaluation of sample clean-up step

Metabolomics workflow involves following steps: experiment planning, sampling, sample preparation, sample analysis and both data processing and data interpretation. Metabolomics is focused on small molecules with mass lower than 1500 Da. This is reason, why sample preparation of biological samples is critical. This work deals with the optimization of sample preparation method for polyethylene glycol (PEG) elimination from sample. PEG can interfere with metabolites during the ionization in the ion source of mass spectrometer. Solid phase microextraction in pipette tips (μ SPE-PT) was used in this work for PEG elimination. Porous titanium dioxide was employed as a sorbent.

Vaginal swabs were collected from patients suffering from vulvovaginal discomfort caused by *Candida albicans*. Samples were first dissolved in phosphate buffer saline solution. In μ SPE-PT method 5 different composition of binding solution, number of aspiration/expelling repeats in sample loading step, 5 different composition of washing buffer and elution agent and also number of aspiration/expelling repeats in each of these steps were optimized. As well 4 different composition of solution for reconstitution of sample were optimized. Number of aspirate/expel in each step of μ SPE-PT was 1–50. The liquid chromatography separation was carried out on Acquity BEH C₁₈ column using gradient elution with 0.075% formic acid (FA) and 0.075% FA in acetonitrile (ACN) at flow rate of 0.5 mL/min in 13 minutes. Quadrupole time-of-flight high resolution tandem mass spectrometer Synapt G2-Si was employed for the data acquisition. MarkerLynx software was utilized for finding of potential markers. Microsoft Excel software was used for PEG measure evaluation.

Using 0.1% FA as binding solution, water as wash buffer and 50% methanol for elution was found as the most effective workflow for PEG elimination. The best results were obtained with 5 times aspirate/expel for sample loading, 50 times aspirate/expel for washing and 5

times aspirate/expel for elution. 5mM FA in 2% ACN was used for reconstitution after evaporation of sample. After μ SPE-PT 21.62 % of total number of PEG in sample was detected in the eluate. Moreover, 5544 potential markers were found in the eluate. These μ SPE-PT conditions appear as effective tool for PEG elimination in vaginal swabs in untargeted metabolomics experiment.

Keywords: untargeted metabolomics analysis; polyethylene glycol; titanium dioxide; solid phase microextraction pipette tips (μ SPE-PT); matrix effects