[Abstract:0109] OPY-002

Optimization of an *in vitro* gut microbiome biotransformation platform with chlorogenic acid as model compound: from fecal sample to biotransformation product identification

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Until recently, it was believed that the metabolism of xenobiotics occurs mainly by the cytochrome P450 enzyme system in the liver. Recent data clearly show that the gut microbiota play a significant role in the biotransformation of many endogenous molecules and xenobiotics, leading to a potential influence of this microbiotic metabolism on (in)activation and possible toxicity of these compounds. To study the colonic biotransformation of xenobiotics by the gut microbiome, *in vitro* models are often used as they allow dynamic and multiple sampling overtime. However, the pre-analytical phase should be carefully optimized to enable metabolite identification representative for the *in vivo* situation.

During this study, chlorogenic acid was used as a model compound to optimize a ready-to-use gut microbiome biotransformation platform using an *in vitro* gastrointestinal dialysis-model with colon stage together with an instrumental platform using liquid chromatography coupled to high resolution mass spectrometry. Identification of the biotransformation products of chlorogenic acid was performed using complementary suspect and non-targeted screening approaches. Concerning the pre-analytical phase, (i) the influence of different incubation media (Wilkins-Chalgren Anaerobic Broth and phosphate buffer) and incubation times (prior to implementation in the colonic stage of the dialysis model) on bacterial composition and concentration were investigated and (ii) four different sample preparation methods (centrifugation, extraction, sonication and freeze-drying) were evaluated targeting colonic biotransformation of chlorogenic acid.

WCB as incubation medium showed to introduce substantial variation in the bacterial composition of the fecal samples, while the sterile phosphate buffer guaranteed a closer resemblance to the *in vivo* composition. Furthermore, incubation during 24 h in sterile phosphate buffer as medium showed no large increase or decrease in anaerobic bacterial concentration, concluding that incubation prior to the colonic stage is not needed. Concerning sample preparation, centrifugation, sonication and extraction gave similar results, while freeze-drying appeared to be inferior. The extraction method was selected as an optimal sample preparation method considering the quick execution together with a good instrumental sensitivity. The optimized protocol was applied to chlorogenic acid leading to the identification of 23 microbiotic biotransformation products.

This study optimized a ready-to-use platform to investigate colonic biotransformation of xenobiotics by using chlorogenic acid as a model compound. This platform can be used in the future to study to differences in colonic biotransformation of xenobiotics using fecal samples of different patient groups (e.g. patients with metabolic disorders as fecal donors or fecal samples collected after antibiotic treatment).

Keywords: Gut microbiome, in vitro gastrointestinal dialysis model, liquid chromatography-high resolution mass spectrometry

[Abstract:0110] OPY-003

Headspace solid-phase microextraction using magnetic nanoparticles

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Modern strategies of green analytical chemistry tend towards minimization of reagent consumption and searching for safe solvents and materials for the implementation of sample preparation, which is necessary for the analysis of complex sample matrices. From this point of view, magnetic nanoparticles (MNPs) can be considered as perspective and safe material for sorption. Recently, MNPs have been widely used for separation and preconcentration of diverse compounds from various matrixes (biological samples, foods, environmental samples, etc.). As known, magnetic materials as sorbents have several advantages over conventional sorbents for sorption in aqueous phase. The separation and preconcentration processes can be performed directly in aqueous solution containing MNPs, and after sorption MNPs can easily be collected and separated from the liquid phase using an external magnetic field. Due to the large surface-area-to-volume ratios of nanometer (nm)-sized sorbents, MNPs are particularly useful for extracting