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Dansylation isotope labeling liquid chromatography mass spectrometry for parallel profiling of human urinary and fecal submetabolomes

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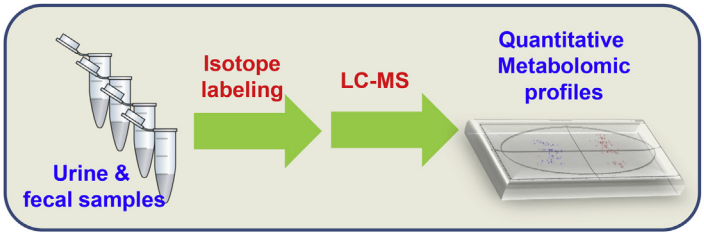
HIGHLIGHTS

- A method of parallel analysis of urine and fecal metabolomes is developed.
- Dansylation isotope labeling LC-MS is used for amine/phenol sub-metabolome quantification.
- Detection of 3089 metabolites in urine and 3012 metabolites in feces for a total of 5372 metabolites.
- Parallel profiling of urine and feces increases the metabolomic coverage.

GRAPHICAL ABSTRACT



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ABSTRACT

Human urine and feces can be non-invasively collected for metabolomics-based disease biomarker discovery research. Because urinary and fecal metabolomes are thought to be different, analysis of both biospecimens may generate a more comprehensive metabolomic profile that can be better related to the health state of an individual. Herein we describe a method of using differential chemical isotope labeling (CIL) liquid chromatography mass spectrometry (LC-MS) for parallel metabolomic profiling of urine and feces. Dansylation labeling was used to quantify the amine/phenol submetabolome changes among different samples based on ^{12}C-labeling of individual samples and ^{13}C-labeling of a pooled urine or pooled feces and subsequent analysis of the ^{13}C-/^{12}C-labeled mixture by LC-MS. The pooled urine and pooled feces are further differentially labeled, mixed and then analyzed by LC-MS in order to relate the metabolite concentrations of the common metabolites found in both biospecimens. This method offers a means of direct comparison of urinary and fecal submetabolomes. We evaluated the analytical performance and demonstrated the utility of this method in the analysis of urine and feces collected daily from three healthy individuals for 7 days. On average, 2534 ± 113 (n = 126) peak pairs or metabolites could be detected from a urine sample, while 2507 ± 77 (n = 63) peak pairs were detected from a fecal sample. In total, 5372 unique peak pairs were detected from all the samples combined; 3089 and 3012 pairs were found in urine and feces, respectively. These results reveal that the urine and fecal metabolomes are very different, thereby justifying the consideration of using both biospecimens to increase the probability of

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finding specific biomarkers of diseases. Furthermore, the CIL LC-MS method described can be used to perform parallel quantitative analysis of urine and feces, resulting in more complete coverage of the human metabolome.

1. Introduction

Urine is widely used for biomarker discovery research, as it contains metabolic signatures of many biochemical pathways associated with a health state. Profiling urine metabolome can generate a rich source of information about the global physiological status of an organism [1–3] and various pathological conditions such as kidney-related diseases [4–6], diabetes [7,8] and cancer [9–11]. Feces are also gaining more attention as a biospecimen for metabolomics since fecal samples contain not only endogenous human metabolites, but also gut microbiota metabolites and residues or metabolites of digested materials [12–15]. Thus, human feces are an excellent source to interrelate the microbiome and human metabolomes [16]. Recent studies have suggested that the gut microbiome performs numerous important biochemical functions for the host, and disorders of the microbiome are associated with many diverse human disease processes [17–20].

Because both urine and feces can be readily collected non-invasively and their metabolomes are likely different, it would make sense to profile both biospecimen simultaneously in order to provide more comprehensive information on the human metabolome. NMR has been used for parallel profiling of urine and feces in animal models such as mouse and rat [21–26] and in human studies [27]. However, NMR detects less than 100 metabolites in total. GC-MS and LC-MS are more sensitive, but only a few studies of metabolomic profiling of urine and feces in parallel have been reported. Cauchi et al. reported the use of GC-MS to profile blood, breath, feces and urine samples of individuals with gastrointestinal diseases and healthy controls, and found only the metabolic data from the fecal samples could discriminate between Crohn's disease and healthy controls [28]. LC-MS was applied to analyze mouse urine and fecal metabolomes in a study of how the intake of prebiotic fibres affects intestinal microbiota in a diet-induced obesity animal model [29] and in a study involving a humanized mouse model [13]. To our knowledge, there is no report of quantitative comparison of the human urinary and fecal metabolomes with a relatively large metabolome coverage (i.e., thousands of metabolites).

In this work, we report a method based on the high-performance chemical isotope labeling (CIL) LC-MS platform for parallel analysis of urine and fecal samples. We have recently shown that in a dansyl labeled human urine sample, more than 1600 unique peak pairs or putative metabolites can be detected in a 25-min LC-MS run [30], while in a dansyl labeled human fecal sample, more than 1700 peak pairs can be found [12]. In this work, we demonstrate that differential isotope labeling of pooled urine and pooled feces followed by LC-MS analysis can be used to relate the concentrations of common metabolites found in the two biospecimen, allowing both qualitative and quantitative comparisons of the two metabolomes. We illustrate the metabolomes of urine and feces are quite different, justifying the parallel profiling to increase the overall human metabolome coverage. Finally, we apply this method to compare the human urine and fecal metabolomes of healthy individuals in a pilot study which could be expanded in the future for discovering disease biomarkers based on parallel profiling of urine and feces.

2. Experimental

2.1. Sample collection and processing

All samples were collected in compliance with prevailing human research ethics guidelines. The study protocol was approved by the Medical Ethics Committee of the 1st Affiliated Hospital of Zhejiang University, Hangzhou, China and the Ethics Approval Board of the University of Alberta, Edmonton, Canada. In this study, urine and fecal samples were collected daily from 3 female healthy volunteers (marked as W, J and S) in 7 separate days. These individuals were chosen randomly. Urine samples were collected twice in a day. One was the 2nd-void morning urine after overnight fasting (12 h), denoted as 2nd-void urine. The other one was urine collected at the same time when the fecal sample was collected which is denoted as urine@feces. The fecal sample was stored immediately after collection in a −20 °C freezer while urine sample was processed as follows. Within 1 h of urine collection, the urine sample was centrifuged at 4000 rpm for 10 min, after which the supernatant was filtered twice through 0.22 μM filter (Agela Technologies, China). The filtered urine was aliquoted into 1.5 mL vials and stored at −20 °C.

For fecal metabolite extraction, fecal samples were subjected to sequential solvent extraction by water and acetonitrile (ACN) as descried before [12]. Briefly, 600 μL water was added into each sample for the first extraction, followed by using 600 μL ACN for the 2nd extraction. The supernatants from the two extractions were combined and dried with a SpeedVac and then stored at −20 °C for further use.

2.2. Derivatization, normalization and mixing

The synthesis of $^{13}C_2$-dansyl chloride has been reported [31] and the dansyl labeling reagents are available from the University of Alberta (MCID.chem.ualberta.ca). Dansylation labeling of fecal and urine samples was done as reported [12,32]. The individual sample was labeled separately using $^{12}C_2$-dansyl chloride and quantified by LC-UV based on absorption at 338 nm [33]. A pooled fecal sample (i.e., fecal-pool) was prepared by mixing the same amount of aliquot from each of the 63 extracts (i.e., triplicate extractions of 21 samples) and a pooled urine sample (i.e., urine-pool) was prepared from 126 extracts. A portion of the fecal-pool or urine-pool was taken and labeled by ^{13}C-dansylation. An aliquot of the ^{13}C-labeled pool was mixed with a ^{12}C-labeled individual sample in 1:1 molar ratio to produce a mixture for LC-MS analysis.

For the comparison of fecal and urine metabolomes, a small portion of the urine-pool or fecal pool was separately labeled using ^{12}C-dansylation and the total concentration of the labeled metabolites was determined by LC-UV. Another pooled sample (i.e., urine-fecal-pool) was prepared by mixing the same amount of the urine-pool and fecal-pool, and labeled using ^{13}C-dansylation. According to the UV quantification results, an appropriate volume of ^{13}C-labeled urine-fecal-pool was combined with ^{12}C-labeled urine-pool or fecal-pool in 1:1 molar ratio to generate a pool-to-pool mixture for LC-MS for analysis.