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An integrated platform for directly widely-targeted quantitative analysis of feces part II: An application for steroids, eicosanoids, and porphyrins profiling

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ABSTRACT

Steroids, especially bile acids, along with eicosanoids and porphyrins in feces play pivotal roles for the clinical diagnosis of various diseases. However, their reliable measurement is extensively obstructed by poor stability, structural diversity, broad content ranges, and tedious sample preparation protocols that account for a majority of the measurement errors. In current study, in-depth component screening was initially carried out by flexibly integrating diverse modes, such as predefined multiple reaction monitoring, stepped multiple ion monitoring, neutral loss scan, and precursor ion scan on a hybrid triple quadrupole-linear ion trap mass spectrometer, which also provided MS^2 spectra *via* enhanced product ion experiments. Meanwhile, a hybrid ion trap-time of flight mass spectrometer served as a complementary tool by providing accurate mass spectral information. Afterwards, because authentic compounds were unavailable for most analytes, an online optimization strategy was then proposed to optimize parameters, including precursor-to-product ion transitions and spectrometric parameters, notably collision energy. Finally, direct analysis of all detected components in feces was carried out by employing a platform integrating online pressurized liquid extraction, turbulent flow chromatography, and LC–MS/MS, and applying those optimized parameters. Seventy-one compounds, including 52 steroids and 13 eicosanoids, together with 6 porphyrins, were found and annotated in a fecal pool, and then relatively quantified in various fecal matrices. The quantitative dataset was subjected for multivariate statistical analysis and significant differences were observed among the quantitative chemome profiles of the fecal matrices from different groups. The findings obtained in the two parts demonstrated that the analytical platform in combination with the work-flow is qualified for not only directly simultaneous measurement of diverse endogenous substances, but widely targeted metabolomics of fecal matrices.

1. Introduction

It has been revealed that either non-targeted metabolomics or targeted metabolomics strategy owns its respective shortcomings [1,2], whereas homolog-focused profiling has been regarded as a feasible choice to bridge specific targeting toward global screening *via* in-depth profiling of selected categories of metabolites [3,4]. The class-specific profiling strategy is expected to be able to circumvent the bottlenecks including information omission resulted from targeted technique as well as the involvement of much redundant information caused by non-targeted measurement.

Fecal samples are favored biological sources for metabolome characterization because they are non-invasive and more accessible [5–7]. A vast number of endogenous substances are distributed in the solid matrices, and the chemical compositions of fecal samples could intrinsically reflect the physiological status as well as the gut functional ecology [8]. Steroids and eicosanoids, along with porphyrins serve as the primary chemical categories in feces. They intervene in a wide range of metabolic pathways and are responsible for various physiological processes, for instance immunity and lipid absorption & metabolism [9] . In addition, those chemical clusters play key roles for various pharmacological activities

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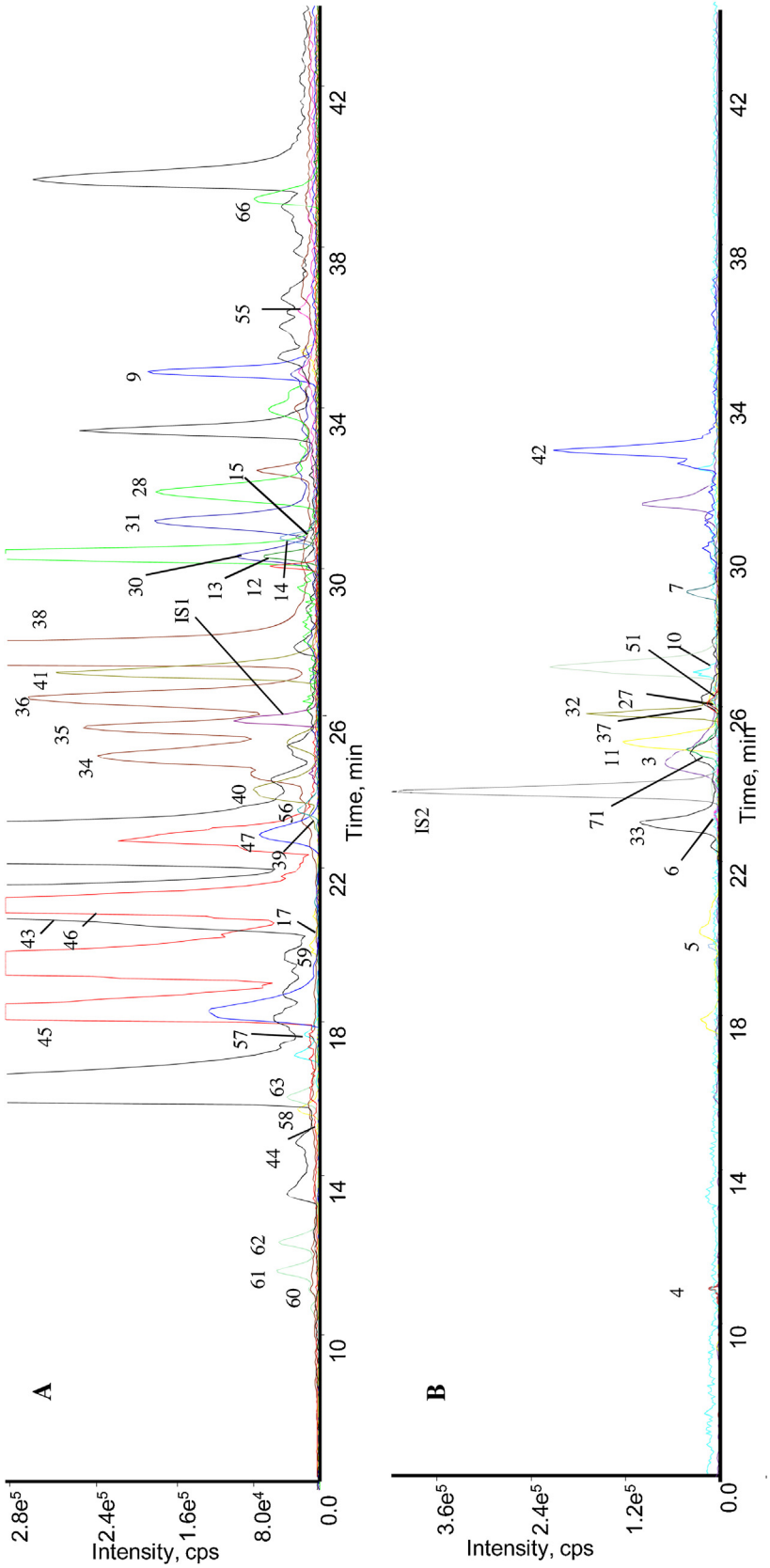

Fig 1. Representative overlaid selected ion current (XIC) chromatograms were obtained using MRM modes on online PLE- TFC-LC-Qtrap-MS. A, a selected fecal sample (G-c-5) with negative mode, and B, a selected fecal sample (G-c-5) with positive mode. The numbers are coded as Table 1.