

**Human urinary metabolic signatures by parallel dual secondary column-dual detection two-dimensional comprehensive gas chromatography: reliable targeted and untargeted comparative analysis.**

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This study investigates the potential of a parallel dual secondary column-dual detection two-dimensional comprehensive GC platform (GC×2GC–MS/FID) in delineating informative metabolic signatures in human urine samples. The platform was firstly tested on a dietary manipulation study on mice and demonstrated to be effective thanks to the synergic combination of full scan mass spectrometry for analytes identification with flame ionization detection for (semi)quantitative pattern comparison [1]. The present study was indeed focused on human urine samples collected from adults (males and females) with type-2 diabetes [2]. Samples submitted to a standard derivatization protocol [3] were analyzed by GC×2GC-MS/FID for profiling and fingerprinting of low-molecular weight metabolites (acids, aminoacids, monosaccharides ect..). Thanks to an effective template matching algorithm that adopts a newly implemented global mapping function [1], the parallel separation patterns from the two detectors (FID and MS) were cross-aligned and untargeted region-features adopted for comparative analysis.

Contemporarily a targeted analysis was run by focusing on known informative metabolites aimed at corroborate and validate the untargeted approach. By comparing targeted profiling with of untargeted fingerprinting results it was possible to select relevant (peak-region) features showing statistically consistent variations between subjects belonging to different Gaussian quartiles. Analytes that maximize the discrimination were, above all, glycine, threonine, phenylalanine, valine, succinic acid, malonic acid, xilitol and ribitol. Interesting results from untargeted analysis suggested the presence of new potential bio-markers able to effectively discriminate subjects.

### **References**

- [1] Bressanello D. et al. *Journal of Chromatography A* 1361 (2014) 265
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- [3] Q. Zhang, G. Wang, Y. Du, L. Zhu, A. Jiye, GC/MS analysis of the rat urine formetabonomic research, *J. Chromatogr. B* 854 (2007) 20–25.