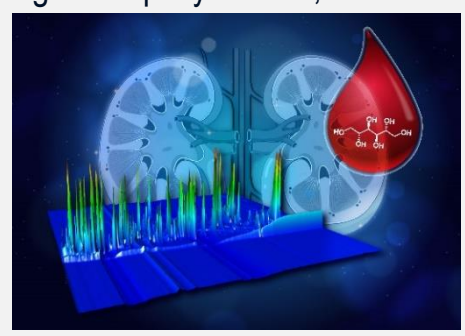
A Validated GC×GC-TOFMS identified Alteration of Plasma Sugar and Sugar Alcohol in Diabetic Mellitus Patients with Kidney Failure

Kassaporn Duangkumpha[1,2], Narumol Jariyasopit[1,2], Kwanjeera Wanichthanarak[1,2], Esha Dhakal[1,2], Pattipong Wisanpitayakorn[1,2], Yougyut Sirivatanauksorn[2], Chagriya Kitiyakara[3,5], Nuankanya Sathirapongsasuti[4,5], Sakda Khoomrung[1,2,6,*]

[1]*Metabolomics and Systems Biology, Department of Biochemistry, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*
[2]*Siriraj Metabolomics and Phenomics Center, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*
[3]*Department of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand*
[4]*Section of Translational Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University Bangkok, Thailand*
[5]*Research Network of NANOTEC – MU Ramathibodi on Nanomedicine, Bangkok, Thailand*
[6]*Center of Excellence for Innovation in Chemistry (PERCH-CIC), Faculty of Science, Mahidol University Bangkok, Thailand*

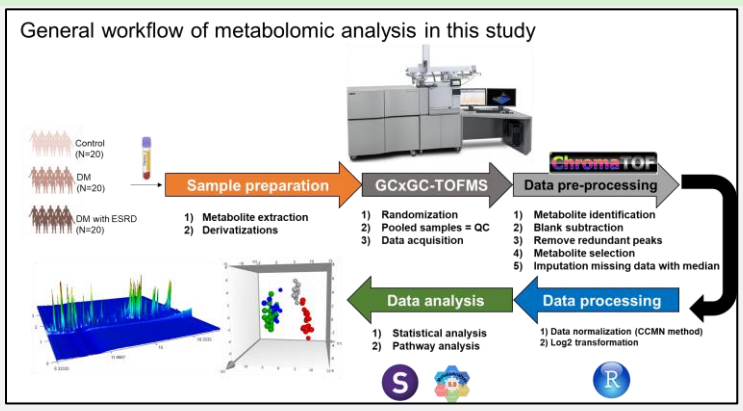
Correspondence: Sakda Khoomrung, Email: sakda.kho@mahidol.edu



Objectives:

1) To implement and validate a GC×GC-TOFMS method to investigate metabolites in plasma from diabetes mellitus (DM) patients with kidney failure (KF).
2) To identify significant metabolites in DM with KF.

Methods: The accuracy and precision of the method were validated with 47 authentic standards consisting of four chemical classes, e.g., sugar, sugar alcohols, amino acids, and free fatty acids. Next, the optimized method) was applied to target a group of metabolites in DM with KF (N= 20), healthy (CON; N=20), and DM (normal eGFR and normoalbuminuria) using GC×GC-TOFMS (LECO Pegasus 4D-HRT).



Results

Part 1: Our validated GC×GC-TOFMS method provided at least twice the numbers of identified metabolites when compared to the traditional GC-TOFMS method (*Figure 1 and Table 1*). With the optimized method, over 98% of reference standards were clearly separated and correctly identified.

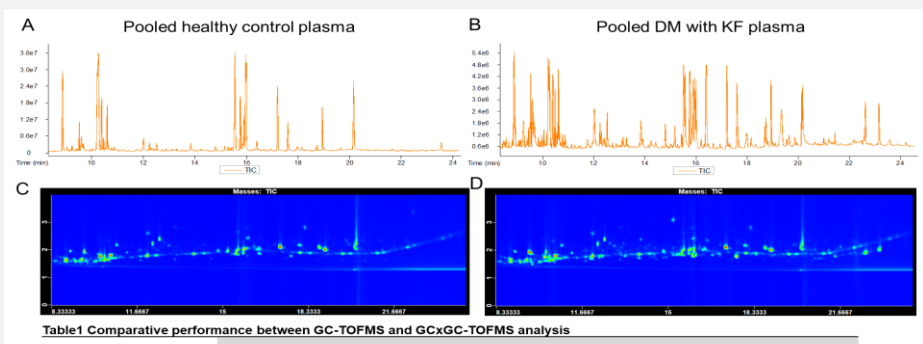


Table1 Comparative performance between GC-TOFMS and GCxGC-TOFMS analysis

	Healthy Control			DM with KF		
	GC-TOFMS	GCxGC-TOFMS	p-value	GC-TOFMS	GCxGC-TOFMS	p-value
No. of detected peak (Mean ± SD)	576.33 ± 8.50	1,028.00 ± 15.62	***0.000002***	723.33 ± 17.47	1,256 ± 35.02	***0.000019***
No. of unknown peak (Mean ± SD)	396.67 ± 12.66	703.67 ± 21.22	***0.000028***	532.67 ± 23.97	874.00 ± 22.07	***0.000054***
No. of annotated peak (Mean ± SD)	178.67 ± 5.03	324.33 ± 6.66	***0.000007***	190.67 ± 9.07	382.67 ± 13.32	***0.000033***

Figure 1 Chromatogram (A,B) and contour plots (C,D) representing plasma metabolomic analysis in both CON and DM with KF groups using GC-TOFMS and GC×GC-TOFMS, respectively.

Results

Part 2: Plasma metabolomic analysis of DM with KF showed distinctly different features compared to the other two groups (*Figure 2*). A group of significant metabolites (sugars and sugar alcohols) including D-xylose, D-arabinose, D-maltose, D-ribose, D-fructose, D-galactose, meso-erythritol, D-arabitol and myo-inositol showed significantly higher concentration ($p<0.0001$) in DM with KF compared to other groups (*Figure 3*). Enrichment analysis demonstrated that these sugar and sugar alcohols are significantly ($p<0.05$) correlated to the altered galactose metabolism and polyol pathway.

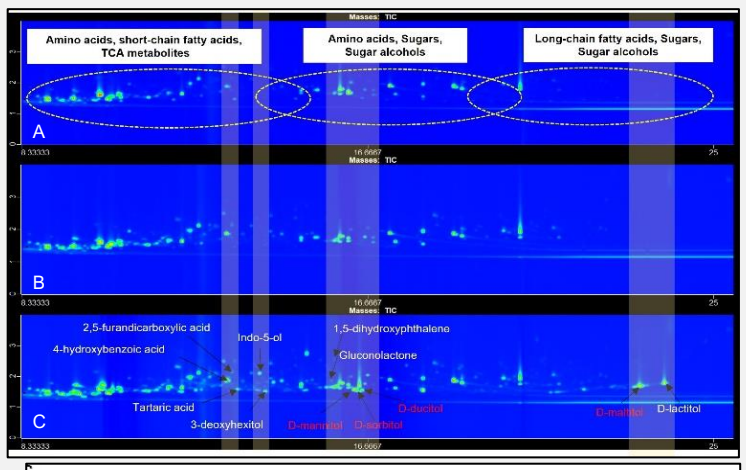


Figure 2 Contour plots of three samples representing the (A) CON, (B) DM, and (C) DM with KF groups; mannitol, sorbitol, dulcitol, and maltitol are the unique metabolites found in the DM with KF group (red letters).

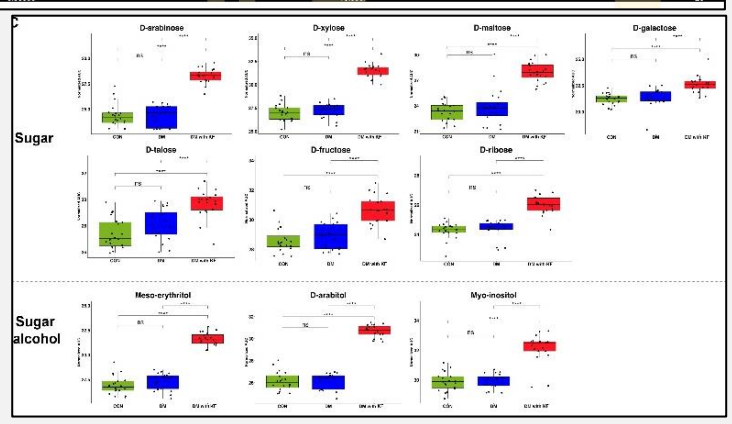
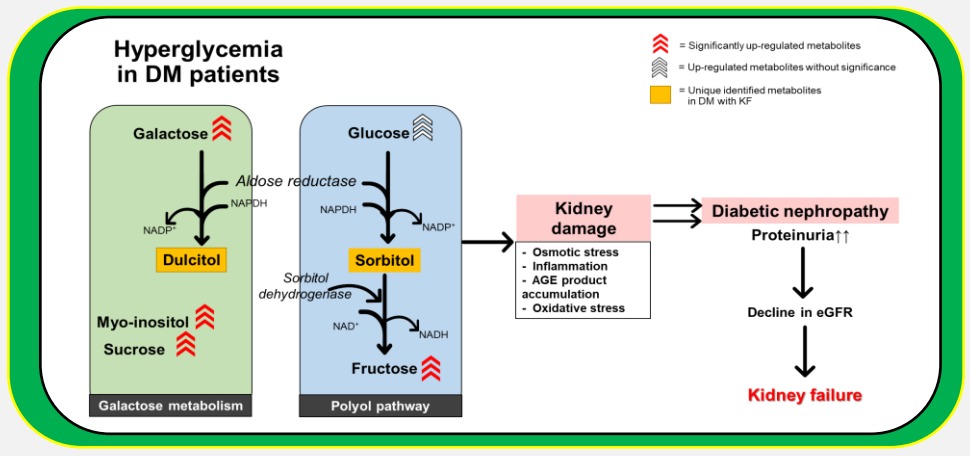


Figure 3 Box plots represent the two main classes of significantly elevated metabolites in the DM with KF group, including seven sugar metabolites and three sugar alcohols. Green, blue and red color boxes represent CON, DM and DM with KF, respectively.



Conclusion: The GC×GC-TOFMS is an excellent technique for metabolite profiling, especially for the analysis of extremely complicated sample matrices. Sugars and sugar alcohol metabolites were identified as the significant metabolites showing elevated concentrations in DM with KF. These significant metabolites were associated with galactose and polyol pathway. The applications of our GCxGC-TOF method could be further extended to study metabolomics in other diseases.

Acknowledgements

SK was supported by Chalermphrakiat Grant, Faculty of Medicine Siriraj Hospital. This research project was supported by postdoctoral fellowship award from Mahidd University (R016320002). This research project was supported by Mahidol University, Grant no. (IO) R016420001 (to SK). The Center of Excellence for Innovation in Chemistry (PERCH-CIC), Ministry of Higher Education, Science, Research and Innovation Thailand, is gratefully acknowledged. This research has received funding support from the NSRF via the Program Management Unit for Human Resources & Institutional Development, Research and Innovation [grant number B16F640099]. N.S. and C.K. were supported by the Research Network NANOTEC program (RNN-Ramathibodi hospital, Bangkok, Thailand), and the Faculty of Medicine Ramathibodi Hospital Mahidol University, Bangkok, Thailand (grant number CF_62006). We thank LECO Thailand team for the technical support.