

## **Analysis of urinary metabolic alteration in type 2 diabetic rats treated with metformin using the metabolomics of quantitative spectral deconvolution <sup>1</sup>H NMR spectroscopy**

### **ABSTRACT**

Metformin has been used clinically as the first-choice drug to treat type 2 diabetes patients. It is not metabolized in liver and the residue which is excreted unchanged in the urine. Identification and quantification of metformin in the urine can be used as a unique marker for the metformin treated diabetic patients. The aim of our study was to identify residual metformin in the urine of type 2 diabetic rats using quantitative spectral deconvolution of <sup>1</sup>H NMR method. Additionally, quantitative assessment of residual metformin and other identified metabolites in the urine, as well as the evaluation of metabolic alteration due to metformin treatment of diabetic condition were also carried out. Partial least square discriminant analysis (PLS-DA) and statistical analysis were used to discriminate the metformin treated type 2 diabetic, the lean, the obese, and the type 2 diabetic rat groups. Furthermore, in order to evaluate the statistical difference of metabolite levels between the groups, ANOVA test was performed. The presence of metformin in the urine of the metformin treated diabetic rats from the <sup>1</sup>H NMR spectral analysis and its identity was confirmed by 2D NMR spectroscopy experiments including the 2D <sup>1</sup>H J-resolved (JRES), and <sup>1</sup>H heteronuclear multiple correlation (HMBC). Based on the PLS-DA model and statistical analysis, it was established that metformin treatment significantly influenced the improvement of glucose and 1-methylnicotinamide metabolism; increase the growth and activity of gut microbiota as indicated by the elevation of trimethylamine (TMA), phenylacetylglycine (PAG) and indoxylsulfate levels; as well as the suppression of tricarboxylic acid (TCA) cycle metabolism as shown by the decreased levels of citrate, 2-oxoglutarate, succinate, and fumarate. This study provided new approach to the quantitative analysis of metformin in urine and further exploration on the effect of metformin treatment on the type 2 diabetic rats revealed useful findings on its metabolic states.

**Keyword:** Metformin; Type 2 diabetic rat model; Urine metabolites; Deconvolution method; Quantitative <sup>1</sup>H NMR-based metabolomics