



Development of an in-House Software Platform and its Application in Untargeted Ultraperformance Liquid Chromatography Coupled to Time-of-Flight Mass Spectrometry Analysis in Metabolomics and Traditional Chinese Medicine Studies

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Editorial

The recent development in high-resolution mass spectrometry (MS) has afforded powerful analytical equipments, e.g., ultra performance liquid chromatography coupled to time-of-flight mass spectrometry (UPLC TOFMS), for rapid qualitative analysis of samples as complex mixtures in biomedical research. This is especially beneficial for the research fields as untargeted metabolomics and pharmaceutical analysis of natural product, e.g., the traditional Chinese medicine. However, a significant bottleneck in deriving biological knowledge from the studies is the process of identification of the samples' components, which is a labor-intensive step that follows data acquisition and analysis and must occur before biological interpretation is possible [1]. To address this bottleneck, several computer software platforms have been developed in recent years for automated data processing [1,2]. In our laboratory, we developed our own software platform in 2012, the operation of which is very simple [3]. Unlike the reported software platforms that address raw LC-MS data and require the Taverna or R environment, our software platform deals only with the dataset of LC-MS features produced after the pre-processing step and requires nothing more than a Microsoft Windows operating system and Microsoft Excel software. One component of our platform is a software named "Searcher", which works as an Excel "add-in" component and can make automatic putative batch identifications by matching the measured m/z data list with a reference m/z list. Another component is a series of in-house, localized, Excel-format databases that contains accurate theoretical m/z values for multiple types of ions commonly observed in the electrospray ionization (ESI). Currently the series includes a HMDB (Human Metabolome Database, <http://www.hmdb.ca/> [4-6]) -derived database (HMDBDDB), a database of observed metabolite signals in UPLC TOFMS analysis of serum of Sprague-Dawley (SDRSDB), a database of observed metabolite signals in UPLC TOFMS analysis of urine of Sprague-Dawley (SDRUDB) and a literature-derived chemical database of the plants of Rehmannia, Angelica, Paeonia and Ligusticum (RAPLDB). The SDRSDB and SDRUDB can be used for distinguishing the signals of endogenous metabolites from exogenous metabolites in serum or urine samples of SD rats. The RAPLDB can be used to detect the signals of reported phytochemicals of the four medicinal plant families. With these two components (i.e., the Searcher software and localized database series), rapid automatic putative batch identification of high-resolution LC-MS data can be achieved with very simple operations, and the obstacle with data analysis in our laboratory was successfully overcome. Below are examples of some research works in our lab using this platform.

Research 1, "Rapid comparison of metabolites in humans and rats of different sexes using untargeted ultra performance liquid chromatography coupled to time-of-flight mass spectrometry and an in-house software platform" [7]. In this study, untargeted UPLC TOFMS and our platform (Searcher software plus HMDBDDB) were used for a rapid comparison of sex differences in urinary metabolites in humans and in urinary and serum metabolites in Sprague Dawley (SD) rats. In addition, the species differences of urinary metabolites between humans and SD rats were also observed. Principle component analysis showed that all the observed metabolite sex differences were more distinct in SD rats than in humans, indicating that the sex differences of human urinary metabolites is small compared with that of SD rats. In SD rats, the observed metabolite sex differences

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were more distinct in urine than in serum, indicating the importance of urine analysis for metabolomics studies. The species differences in the urinary metabolites of humans and SD rats were much more distinct than any of the observed sex differences. Many sex- and species-related markers were discovered and putatively identified. In both humans and SD rats, steroid metabolites appeared to constitute a major sex difference in urinary metabolites. This provides new proof of the special importance of steroid metabolites in sex differences from an untargeted metabolomics investigation, which is rare for sex differences. Contrary patterns involving adrenocortical activity appeared to exist between rodents and humans, which agrees with previous reports. In the serum metabolites of SD rats, sex differences in ascorbic acid or its isomer and pantothenic acid or its isomer, but not in steroid metabolites, were prominent. Human-specific *a*-N-phenylacetyl-L-glutamine and androsterone glucuronide were among the putative identities of the markers discriminating humans and SD rats. This study demonstrated the feasibility of our in-house software platform and provides metabolite-related information on sex and species differences.

Research 2, "Hepatotoxicity and nephrotoxicity assessment of ethanol extract from *Fructus Psoraleae* in Sprague-Dawley rats using a UPLC-Q-TOF-MS analysis of serum metabolomics" [8]. The purpose of this study was to investigate the toxic effect in rats treated with the ethanol extract from FP (EEFP) and to explore the underlying toxic mechanisms using a metabolomics approach. SD male rats were randomly divided into four groups ($n = 6$). Dosages were administered once daily for two continuous weeks. Serum was analyzed by UPLC TOFMS. PCA and partial least-squares discriminate analysis (PLS-DA) models were built to show the difference. Potential biomarkers were screened from S-plots constructed following analysis with orthogonal partial least squares discriminant analysis (OPLS-DA) and identified based on the accurate mass and MSE information obtained from UPLC TOFMS analysis. Compared with control rats, the hematological (white blood cell (WBC), neutrophilic granulocytes (NEUT), monocytes (MON) and biochemical (alanine transaminase (ALT), total bilirubin (TBIL), creatinine (CRE) parameters were significantly increased ($p < 0.05$, $p < 0.01$), liver and kidney showed mild injury in the EEFP 1.62 g/kg group. PCA and PLS-DA enabled four clusters to be visualized. Using our platform (Searcher software plus HMDBDDDB and SDRSDB), ten potential biomarkers contributing to the clusters were identified, which were lysophosphatidylcholine LysoPC (20:4), *p*-Cresol, ascorbic acid, *p*-Cresol sulfate, inosine diphosphate (IDP), phosphatidylcholine (PC (14:1/20:5), PC (14:1/16:1), PC (22:2/18:1), phosphatidylethanolamine PE (22:1/18:1), and phosphatidylglycerolphosphate PGP (18:1/22:6). Those endogenous metabolites were chiefly involved in phospholipid metabolism, amino acid metabolism, purine metabolism and the antioxidant system. The change of hematological parameters, biochemical parameters and plasma metabolic pattern show that long term EEFP exposure at high dose could induce liver and kidney toxicity in rats. Some potential biomarkers like LysoPC, *p*-Cresol, ascorbic acid, *p*-Cresol sulfate and PC have been found to be reasonable in explaining the toxic effects mechanism of EEFP in rats. The work shows that the metabolomics method is a promising tool in the toxic mechanism research of traditional Chinese medicines.

Research 3, "Cardiotoxicity study of Shenfu compatibility in rats based on metabolomics" [9]. This research was to research the effect of *Ginseng Radix et Rhizoma* and *Aconiti Lateralis Radix*

Praeparata combination on cardiac toxicity in rats by UPLC TOFMS, and explore the endogenous markers and molecular mechanism. Data was analyzed using PCA, partial least-squares analysis and our platform (Searcher software plus HMDBDDDB and SDRUDB). Decoctions of different combinations of Shenfu were given to male Wistar rats at a dosage of 20g·kg⁻¹ for 7 days. Serum samples were collected and analyzed to discover the endogenous metabolites affected by drug administration. Results showed that contents of glutathione, phosphatidylcholine and citric acid decreased in the "mixed-decoction" group, while ascorbic acid, uric acid, D-galactose, tryptophan, L-phenylalanine increased. The Shenfu "co-decoction" group showed a similar or weaker trend as compared with the control group, but mostly not significant statistically.

Research 4, "Chemical comparison between decoctions of *Radix Paeoniae Rubra* and *Radix Paeoniae Alba* by UPLC-QTOF-MS" [10]. This research employed the RAPLDB database. To investigate the chemical difference between decoctions of *Radix Paeoniae Rubra* and *Radix Paeoniae Alba*, samples from legal market were compared using UPLC TOFMS. Data were statistically analyzed by PCA and OPLS-DA. Constituents identification methods include comparing with reference compounds, accurate *m/z* value analysis of TOFMS data based on our platform (Searcher plus RAPLDB) for automatic tentative identification, and QTOF-MS/MS fragment analysis. The results indicate that oxypaeoniflorin and albiflorin are the most important differential constituents in the decoctions of *Radix Paeoniae Rubra* and *Radix Paeoniae Alba*, respectively. Moreover, content of compounds such as 8-debenzoylpaeoniflorin, 1'-O-benzoylsucrose, Mudanpioside F, Mudanpioside C/benzoxypaeoniflorin, Mudanpioside E, (1S,2S,4R)-trans-2-hydroxy-1,8-cineole-B-D-glucopyranoside, saccharose, galloylpaeoniflorin/galloylalbiflorin may also be of significant difference between the two kinds of decoctions.

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