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Metabolite profiling of hemodialysate using gas chromatography time-of-flight mass spectrometry

Xin Qi [a,1], Yinan Zhang [a,1], Jiayuan Gao [b], Tianlu Chen [c], Aihua Zhao [a,*], Yucheng Yan [b], Wei Jia [d]

[a] School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200240, PR China
[b] Renji Hospital, Shanghai Jiao Tong University, School of Medicine, Shanghai 200127, PR China
[c] Ministry of Education, Key Laboratory of Systems Biomedicine, Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, Shanghai 200240, PR China
[d] Department of Nutrition, University of North Carolina at Greensboro, North Carolina Research Campus, Kannapolis, NC 28081, USA

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ABSTRACT

Hemodialysis is an important alternative for renal replacement therapy to remove uremic retention solutes (URS) for the uremic syndrome. The metabolites in the hemodialysate directly reflect the efficiency of URS clearance. Here we report a highly sensitive and reliable metabolomic procedure for the measurement of small molecule metabolites in hemodialysate using gas chromatography coupled with time-of-flight mass spectrometry (GC/TOF/MS). The method was developed and evaluated through orthogonal experimental design using multivariate statistical analysis. The optimized method involves the use of methanol and water in the ratio of 3:1 (v/v) for dissolving the lyophilized solid of the hemodialysate after degradation of urea with urease (no less than 50 U) for 10 min. Validation of the method demonstrated a good linearity with regression coefficients greater than 0.99. Relative standard deviations of precision and stability of proposed method were less than 15%, and recoveries ranged from 71.8 to 115.8%. This method was successfully applied in the metabolite profiling of human hemodialysate samples which was able to differentiate the patients treated with high-flux hemodialysis from those with low-flux hemodialysis. The metabolomic results reveal a higher concentration of URS, and thus, better URS removal, from the patients under high-flux dialysis than those under low-flux dialysis.

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1. Introduction

Hemodialysis is frequently used in patients with uremia or chronic kidney disease to remove uremic retention solutes (URS) from their blood circulation [1,2], as an alternative for renal replacement therapy. Over 100 URS have been identified to be involved in uremic syndrome [3–6]. The blood concentrations of the URS have been extensively investigated [7], partially owing to the poor availability of urine samples in end-stage renal diseases (ESRD) [8]. Additionally, the accumulation of *p*-cresol or indole sulfate in patients is believed to be involved in the development of cardiovascular diseases, which generally occurs in the late stages of chronic kidney diseases due to renal failure [9–11]. Therefore, the URS profile in blood samples directly reflects the treatment effect and adequacy of hemodialysis. Gas chromatography coupled with mass spectrometry (GC/MS), high-pressure liquid chromatography, liquid chromatography coupled with electrospray ionization (LC/ESI/MS), or LC tandem mass techniques have been previously applied for the measurement of URS in the blood [12–15]. Hemodialysate contains considerable metabolites, which are similar to the blood compositions. Previous studies also suggested that the hemodialysate is a biofluid sample more suitable for rapid analytical measurement than other biomatrices such as blood or tissue, due to the absence of large proteins [16]. Previous approaches for hemodialysate analysis focused only on a few URS at one time, which were not able to address global metabolite changes resulting from hemodialysis. It was not until recently that an LC/MS-based metabolomics approach was used for the analysis of uremic toxins for chronic renal failure rats [14]. Metabolite profiling of hemodialysate by a metabolomics approach, such as GC time-of-flight MS (GC/TOF/MS), has not been reported so far. In this study, the protocol for measuring the hemodialysate by GC/TOF/MS metabolomics approach coupled with multivariate statistical analysis has been firstly developed. The protocol was applied in the analysis of hemodialysate samples from human subjects under treatment with either high-flux or low-flux hemodialysis.

* Corresponding author. Tel.: +86 021 34204829.
E-mail address: zhah@sjtu.edu.cn (A. Zhao).
[1] These authors contributed equally to this work.

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2. Materials and methods

2.1. Reagents and materials

Standard compounds, including methoxyamine, L-2-chlorophenylalanine, heptadecanoic acid, and 21 reference standards: 2-aminobutyric acid, isoleucine, leucine, lysine, proline, threonine, tryptophan, valine, 3-hydroxybutyric acid, citric acid, malic acid, pyruvic acid, hippuric acid, indole-3-acetic acid, arabitol, glycerol, threitol, myo-inositol, sorbitol, hexadecanoic acid and oleic acid used for this study were obtained from Sigma–Aldrich (St. Louis, MO, USA). Ultrapure water generated from the Milli-Q system (Millipore, USA) was used in the experiments. Methanol, acetonitrile, acetone and pyridine were of chromatographic grade and purchased from Merck Chemicals (Germany). Anhydrous sodium sulfate (analytical grade) was purchased from China National Pharmaceutical Group Corporation (Shanghai, China). N,O-bis-trimethylsilyl-trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) was purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Hemodialysate samples

The study plan including protocols for specimen collection and analyses was approved by the local Ethics Committee and informed consent was obtained from all participants prior to the study. 60 patients under hemodialysis at a stable physiological condition were recruited in this study. 30 patients received hemodialysis with a high flux dialyzer (Fx60, 1.4 m^2, polysulfone-PVP, Helixone®, Fresenius AG, Bad Homburg, Germany) and 30 patients with low flux dialyzer (REXEED13L, 1.3 m^2, olysulfone-PVP, REXBRANETM, ASAHIKASEI KURARAY medical Co. Ltd., Japan). The two dialyzers were operated with a 4008S machine (Fresenius, Fresenius Medical Care, Germany). The hemodialysis treatment was performed either three times per week for 4 h or 2 times per week for 5 h at the Blood Purification Center of Renji Hospital, affiliated to Shanghai Jiao Tong University. The ratio of the patients who received three times to those who received two times per week was 13–17 under high-flux dialysis, and 17–13 under low-flux dialysis. Heparin sodium was used as anticoagulant during the treatment. An aliquot of 20 ml of waste hemodialysates used in the study was collected at the first hour of the dialysis (also the first time) of every week using a high-flux or low-flux dialyzer, through a T-pipe equipment which is modified from a method reported before [17,18], and immediately stored at −80 °C. Pooled samples were prepared by mixing aliquots of the hemodialysate samples (4.5 ml each) collected in the study.

2.3. Extraction procedure

2.3.1. The use of urease

Hemodialysate contains a high level of urea, which generates a wide peak that often masks the adjacent peaks of other metabolites in total ion chromatograms (TIC) (Fig. 1A). In order to visualize the adjacent peaks in the chromatogram, the urea peak requires attenuation by urease degradation. The amount of urease (0, 50, 100, 150, 200 U) and reaction time (10, 20, 30, 40, 50 min) at 37 °C in 2 ml hemodialysate were extensively investigated according to an orthogonal experimental design, resulting in a total of 25 conditions (Table S1). A series of experiments were performed to evaluate the effect on the peak area of urea in the chromatograms.

2.3.2. Selection of solvents for reconstitution

After the incubation with urease at 37 °C, a 2 ml aliquot of the pooled hemodialysate sample was placed in a screw-top plastic tube, and lyophilized at −80 °C for 4 h. The lyophilized dry sample was reconstituted in different solvents for subsequent GC/TOF/MS analysis. The four solvents, methanol, ethanol, acetonitrile and water, were compared for their extraction efficiency of metabolites in the lyophilized sample with different proportions at a total volume of 500 μl (Table 1). The volume of each solvent varied from 0 to 500 μl. Each of the 19 conditions designed in the study was evaluated in triplicates.

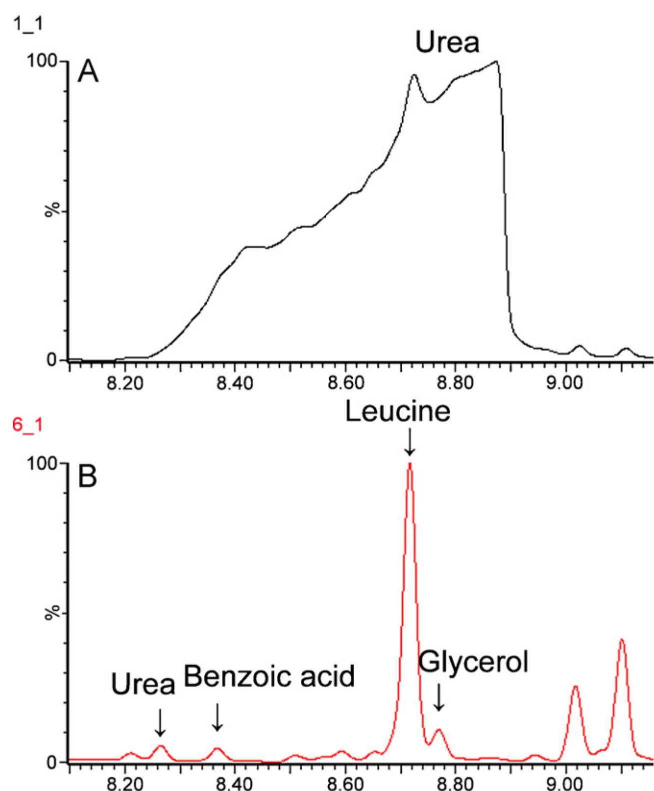

Fig. 1. A representative TIC of a hemodialysate sample showing the peak of urea (A) before and (B) after incubation with urease. Hydrolysis with 50 U urease for 10 min at 37 °C efficiently attenuated the broad urea peak, which obscured adjacent metabolites peaks.

Table 1
Experimental design for the investigation of the extraction efficiency of different solvents.

No.	Run order[a]	Solvent (μl)[b]				
		Water	Methanol	Acetonitrile	Acetone	Peak numbers
N1	14,20,52	500	0	0	0	650
N2	11,36,53	0	500	0	0	619
N3	3,37,54	0	0	500	0	223
N4	12,38,57	0	0	0	500	242
N5	17,23,39	250	250	0	0	660
N6	19,30,55	250	0	250	0	656
N7	4,25,56	250	0	0	250	572
N8	8,29,40	0	250	250	0	661
N9	9,24,49	0	250	0	250	512
N10	16,27,41	0	0	250	250	212
N11	15,31,47	167	167	167	0	586
N12	18,21,51	167	167	0	167	675
N13	5,33,42	167	0	167	167	644
N14	10,28,50	0	167	167	167	557
N15	13,32,45	125	125	125	125	655
N16	7,35,48	313	63	63	63	645
N17	6,22,43	63	313	63	63	668
N18	2,26,46	63	63	313	63	573
N19	1,34,44	63	63	63	313	573

[a] Each experiment and derivatization was carried out in triplicate prior to analysis by GC/TOF/MS, which was randomized.
[b] The total solvent volume for each experiment was fixed at 500 μl.

The effect of ultrasonication on the extraction efficiency of metabolites under 40 Hz from the freeze-dried hemodialysate was also investigated. Temperature and duration were evaluated, resulting in eight conditions (Table S2), and each condition was repeated in triplicates.

2.4. Trimethylsilane (TMS) derivatization procedure

After vortexing for 30 s, the reconstituted sample was centrifuged at 664 × *g* for 10 min. An aliquot of 300 μl of the supernatant was transferred to a GC vial, to which two internal standards (IS) used to evaluate batch reproducibility were added: 10 μl L-2-chlorophenylalanine at 0.3 mg/ml with working concentration of 9.4 μg/ml and 10 μl heptadecanoic acid at 1.0 mg/ml with working concentration of 31.3 μg/ml, which were both within standard linear calibration range from 0.15 to 150 μg/ml. Then the supernatant was dried in a vacuum centrifuge concentrator prior to the derivatization. The derivatization procedure was conducted with minor modifications to our previously published method [19]. Briefly, 80 μl of methoxyamine (at a concentration of 15 mg/ml in pyridine, dried with anhydrous sodium sulfate) was added to the sample vials containing the dried supernatant and vortexed for 30 s. Methoxymation was carried out at 30 °C for 1.5 h. The resultant methoxymation product was then silylated using 80 μl BSTFA (containing 1% TMCS) at 70 °C for 1 h followed by mixing for 30 s. The obtained derivatized samples were allowed to stand for 1 h at room temperature prior to injection into the GC/TOF/MS analysis with controlled room humidity less than 35%.

2.5. Method validation

21 standard compounds which resemble main chemical classes and the retention times of the test metabolites in the hemodialysate including amino acids, organic acids and fatty acids, were chosen for method validation. The linearity of these 21 standard compounds in concentrations ranging from 0.15 to 150 μg/ml was evaluated. These standards were prepared from 150 μg/ml stock solutions by dilution with water or methanol, at dilution factors of 0.001, 0.002, 0.005, 0.010, 0.020, 0.050, 0.100, 0.200, 0.500 and 1.000. The limits of detection (LOD) were measured as the lowest concentrations of every standard with a signal-to-noise (S/N) ratio of 3. The limits of quantification (LOQ) were calculated as the concentrations with S/N ratio of 10. The analytical reproducibility was evaluated as relative standard deviation (R.S.D.) from 21 standard compounds at a concentration of 7.5 μg/ml and the pooled dialysis sample respectively, with six replicates for each sample. The stability of the metabolites identified was estimated by analyzing the same derivatized hemodialysate samples at intervals 0, 6, 12, 24, 36, 48 h, respectively. The recoveries of standard compounds were evaluated by spiking 15 μl of the 21 standard compounds water/methanol solutions at three different concentrations (75, 150, 300 μg/ml) to 2 ml hemodialysates, triplicates for each concentration. Then the hemodialysates were degraded with urease at 37 °C, the resultant solutions were lyophilized at −80 °C for 4 h. The lyophilized solid was dissolved with 500 μl optimized solvent. 300 μl of the dissolution for subsequent derivatization was added to two IS before dried in a vacuum centrifuge concentrator. The dried samples were derivatized with TMS which were treated as detailed in Section 2.4 prior to GC/TOF/MS analysis.

2.6. GC/TOF/MS instrumentation

An aliquot of 1 μl of the derivatized sample was injected into an Agilent 6890N gas chromatograph via an Agilent 7683 autosampler using the splitless mode. The injector temperature was 270 °C. Separation was achieved on a DB-5 MS capillary column (30 m × 250 μm i.d., 0.25 μm film thickness; Agilent J&W Scientific, Folsom, CA, USA) using helium as the carrier gas, at a constant flow rate of 1.0 ml/min. The column temperature was held at 80 °C for 2 min, and then increased to 180 °C by 10 °C/min, held 0 min, to 230 °C by 6 °C/min, follow by 40 °C/min to 295 °C and held for 8 min. The Pegasus HT time-of-flight mass spectrometer (Pegasus HT, Leco Co., CA, USA) was used. Transfer line and ion source temperatures were set at 270 °C and 220 °C, respectively. The mass spectra were obtained with electron impact ionization (70 eV) at full scan mode (*m*/*z* 30–600), and 20 spectra/s were recorded.

2.7. Data analysis

Acquired data files were converted to CDF format by ChromaTOF software (v3.34, Leco Co., CA, USA) and then processed by using custom scripts (toolbox HDA, developed by Jonsson et al. [20,21]) in MATLAB 7.0 (The Math Works Inc., USA). Data pretreatment procedures such as baseline correction, de-noising, smoothing, alignment, time-window splitting, and peak feature extraction were carried out sequentially [21]. The peaks which only existed in a small amount of samples (<20% samples) were excluded in this procedure. The resulting peak intensity data set comprised retention time, sample names, and fragment information. After normalization to the areas of internal standards, some false peaks caused by noise, column bleed and derivatization procedure, which almost exits in every sample, were removed from the data set. Followed by mean centering and unit variance scaling, the resulting data was introduced into the Simca-P 11.5 software (Umetrics, Umeå, Sweden) for further statistical analysis such as principal components analysis (PCA), partial least squares (PLS) regression and/or orthogonal projection to latent structures (OPLS) in order to visually display the correlation between the experimental parameters and metabolic information. The following statistical models are used throughout this article. R^2X is the cumulative modelled variation in the X matrix; R^2Y is the cumulative modelled variation in the Y matrix; and Q^2Y is the cumulative predicted variation in the Y variable or matrix, according to a sevenfold cross-validation. The range of these parameters is 0-1, where 1 indicates a perfect fit. Meanwhile, all the metabolites were identified by comparing their mass spectra with NIST 05 Standard Mass Spectral Databases in NIST MS search 2.0 (NIST, Gaithersburg, MD) software and in-housed library when the similarities were more than 85%. About 2/3 of identified metabolites were further confirmed by our reference standards.

3. Results and discussion

3.1. Optimization of the extraction procedure

We found that 10 min incubation with urease at an amount of greater than 50 U in 2 ml hemodialysate did efficiently minimize the urea peak in the full scan TIC (Fig. S1). As a result, the peaks of leucine, glycerol and benzoic acid were “uncovered” in the TIC spectrum (Fig. 1B). Thus, incubation with 50 U of urease was selected as the preferred method for hydrolysis.

After GC/TOF/MS analysis and data preprocessing, 258 peaks were obtained in over 90% of the experiments and 59 of them were annotated based on the NIST database. Regarding the different solvent conditions for water and/or methanol, the numbers of peaks ranged from 512 to 707, significantly more than those (212–294 peaks) resulted from the acetonitrile and/or acetone solvents. Therefore, the performance of water and methanol seemed to be superior to the other two solvents. A two-component PLS model was generated based on the resolved peak areas (matrix X) and the solvents used in the experiment (matrix Y). The R^2X, R^2Y

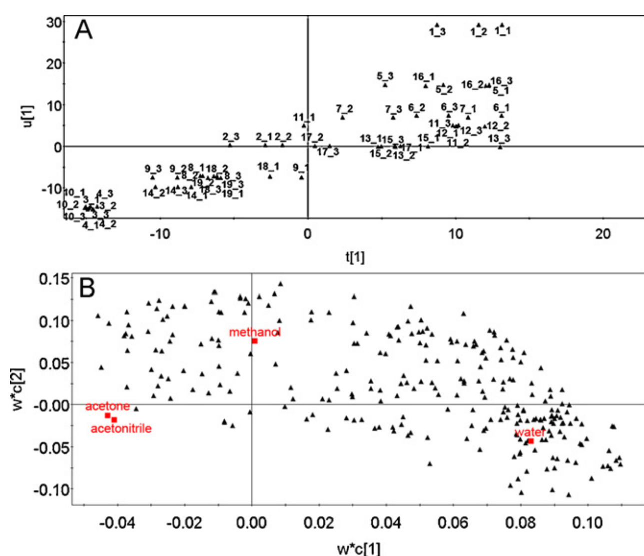

Fig. 2. PLS model (four components, $R^2X = 0.725$, $R^2Y = 0.565$, $Q^2Y = 0.44$) of optional solvents for extraction procedure. (A) The PLS scores plot ($t1 - u1$) shows the correlation between the X matrix (the peak areas of the resolved metabolite peaks) and the Y matrix (the different solvent systems; dots labeled according to Table 1, such as 4_1 i.e. solvent system no. 4, replicate no. 1). The lower left quadrant of the plot describes solvent conditions that produced the lowest overall peak areas, while the upper right quadrant describes those that yielded the best results in terms of peak areas. (B) The PLS loading plot summarizes the influence and correlation between the X matrix (▲, the areas of the resolved GC/TOF/MS peaks) and the Y matrix (■, the different solvents). Methanol and water were strongly correlated with a majority of the resolved peak areas.

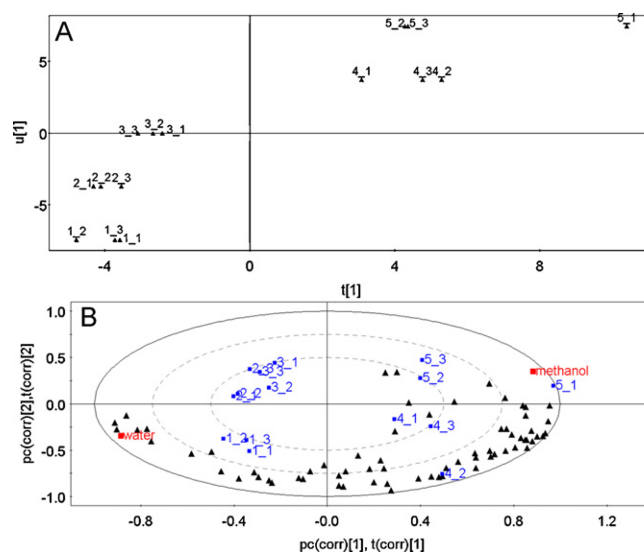

Fig. 3. PLS model (three components, $R^2X = 0.802$, $R^2Y = 0.983$, $Q^2 = 0.949$) of the ratio of methanol and water for extraction procedure. (A) The PLS scores plot ($t1 - u1$) shows the correlation between the X matrix (the peak areas of the resolved identified metabolites) and the Y matrix (the solvent conditions, as in Table S2, such as 5_1 i.e. solvent system no. 5, replicate no. 1). The upper right quadrant shows the experimental conditions that yielded the highest overall peak areas. (B) The PLS bi-plot shows the correlation between the peak areas of 68 endogenous metabolites and the experimental conditions (black triangles, identified metabolites; red squares, methanol and water; blue squares, different methanol: water compositions, with labeling according to Table S2). Solvent composition 4, i.e. the mixture of methanol: water (3:1, v/v) was able to extract most of these compounds. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

and Q^2 parameters of the model are 0.667, 0.325 and 0.271, respectively. In Fig. 2A, solvent systems N3, N4, N8, N9, N10, N14, N18 and N19, using acetone and acetonitrile as solvents, are located in the lower left quadrant based on their relative lower peak intensities. In contrast, solvent systems N1, N5, and N16 yielded higher peak intensities since they are located in the upper right quadrant of the plot. In terms of extraction efficiency and peak intensity, water and methanol seemed to be better than acetone and acetonitrile. The PLS loading plot (Fig. 2B) showed that the majority of the resolved peaks were strongly correlated with methanol and water. Only a few peaks were efficiently extracted by acetonitrile and acetone. This result implied that a solvent system containing water and methanol was most suitable for maximum peak number and intensity.

An experiment was designed to optimize the methanol to water ratio in the solvent system, in which each condition was repeated in triplicates (Table S3). A total of 520 peaks were detected and 68

Table 2
Linearity, LOD, LOQ, stability, precision and recovery for method validation ($n = 6$) with 21 standard compounds.

Compounds	Linear range (μg/ml)	Correlation coefficients	LOD pg on column	LOQ pg on column	Stability (%)	Precision (%)		Recovery (%)
					Samples	Standards	Samples	Average[a]
2-Aminobutyric acid	0.15–150	0.999	8.4	28.1	11.4	1.0	8.5	79.3
Isoleucine	0.15–75	0.997	4.2	14.1	9.4	0.9	3.0	82.5
Leucine	0.15–150	0.999	4.2	14.1	10.3	2.1	3.1	87.5
Lysine	0.15–75	0.998	16.9	56.3	5.1	1.6	7.7	105.8
Proline	0.15–150	0.998	8.4	28.1	12.8	1.2	4.9	85.1
Threonine	0.15–150	0.998	42.3	141.0	8.4	1.6	3.2	92.6
Tryptophan	0.75–150	0.997	33.8	112.5	8.5	1.9	3.2	109.8
Valine	0.15–150	0.999	8.4	28.1	13.3	1.6	4.4	87.3
3-Hydroxybutyric acid	0.15–150	0.994	42.3	141.0	6.5	1.0	5.1	105.3
Citric acid	7.5–150	0.993	42.3	141.0	3.3	1.9	3.1	74.0
Malic acid	0.15–150	0.997	8.4	28.1	5.7	2.7	3.9	95.9
Pyruvic acid	0.15–150	0.995	42.3	141.0	11.5	9.1	2.5	110.1
Hippuric acid	1.5–150	0.996	42.2	140.5	14.5	7.7	9.9	94.4
Indole-3-acetic acid	0.15–150	0.998	16.9	56.2	8.0	3.1	3.7	86.2
Arabitol	0.15–150	0.999	8.4	28.1	5.2	2.0	3.2	102.3
Glycerol	0.15–75	0.999	4.2	14.1	7.2	0.8	2.5	98.5
Threitol	0.15–150	0.998	8.4	28.1	5.0	1.1	3.7	101.2
Myo-inositol	0.15–150	0.997	8.4	28.1	4.6	2.4	3.0	115.8
Sorbitol	0.15–150	0.997	16.9	56.2	3.1	1.3	3.9	71.8
Hexadecanoic acid	0.15–150	0.999	8.4	28.1	14.1	3.5	5.6	80.2
Oleic acid	0.15–150	0.999	84.3	281.0	10.7	3.5	3.2	92.3

[a] Average recovery was obtained by nine samples (three parallel samples at three different concentrations).

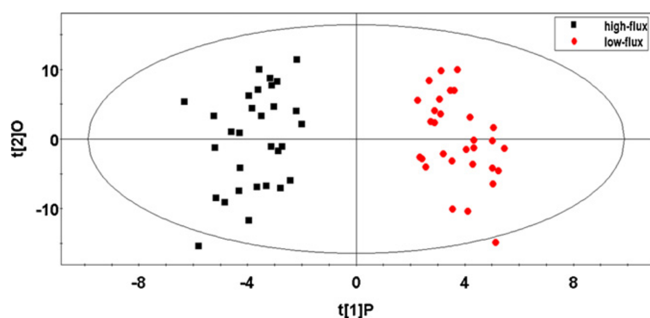

Fig. 4. OPLS scores plot ($1P+3O$, $R^2X=0.269$, $R^2Y=0.93$, $Q^2Y=0.369$) of the metabolite profiles from high-flux treated patients (black squares) and low-flux treated patients (red dots). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

of which were annotated. We used 68 known metabolites and the 15 solvent conditions to construct a three-component PLS model, which yielded good model parameters, $R^2X=0.802$, $R^2Y=0.983$, and $Q^2=0.949$; we also used 520 peaks as variables to construct another three-component PLS model, which also yielded good parameters, $R^2X=0.472$, $R^2Y=0.986$, $Q^2=0.652$. The modelling results from the two sets of variables were similar to each other (see Fig. S2). The $t1$ vs. $u1$ plot (Fig. 3A) showed that solvent systems 4 and 5 resulted in the highest overall peak areas. The bi-plot (Fig. 3B) showed that a higher ratio of methanol was preferable for hydrophobic compounds, but less so for hydrophilic compounds. The result suggested that an acceptable compromise in extraction efficiency between polar and non-polar metabolites could be reached at a 3:1 ratio of methanol/water (experiment 4).

Regarding the ultrasonic influence kept at a constant frequency of 40 Hz during reconstitution, the metabolites were annotated in line with the NIST database after GC/TOF/MS analysis and data processing. The R.S.D.s of most of these metabolites were less than 50%, which suggested that the ultrasound procedure did not significantly enhance the extraction efficiency, and therefore, was not necessary.

3.2. Validation of the optimized method

3.2.1. Linearity, LODs and LOQs

Each calibration curve was constructed by the ratio of standard peak area to IS peak area plotting against nominal standard concentrations. The correlation coefficients (r^2) for all 21 standard compounds exceeded 0.99, indicating good linearity of the method. The LODs and LOQs of standards which showed in pg on column were relatively low (Table 2).

3.2.2. Precision, stability and recovery

The R.S.D.s of six replicates ranged from 0.8 to 9.1% for standards, 2.5–9.9% for pooled hemodialysate samples (Table 2). The results demonstrated high repeatability by different individual operator in our laboratory. Additionally, the stability was found to be acceptable with R.S.D.s ranging from 3.1 to 14.5% within 48 h at room temperature. This result indicates that TMS derivatized samples are chemically stable for at least 48 h, thereby allowing a high-throughput GC/TOF/MS analysis of a large sample size. The recovery of the proposed method ranged from 71.8% to 115.8% for the 21 standard compounds, demonstrating a good reliability of the optimized method.

3.3. Application

The method was used to measure the metabolites in hemodialysate samples from 60 patients, 30 treated with high-flux dialysis and 30 with low-flux dialysis. The GC/TOF/MS data was normalized by peak areas of heptadecanoic acid, one of the internal standards, and then analyzed by uni- and multi-variate statistical methods. A clear separation was achieved between these two groups, indicating significant inter-group metabolic differences. An OPLS model ($R^2X=0.269$, $R^2Y=0.93$, $Q^2Y=0.369$; Fig. 4) was constructed to identify the differential metabolites contributing to the separation of these two groups. A total of 59 variables with VIP > 1 and $P<0.05$ (t-test) were selected as markers. A total of 21 of them were annotated by means of NIST and 15 were confirmed by our in-house library established using reference standards. The VIP scores, significance levels, and fold changes of these metabolite markers are illustrated in the bar graph (Fig. 5). It can be readily observed that the concentrations of metabolites from high-flux treated patients were higher than the other group, including some uremic toxins such as hippuric acid, indole-3-acetic acid and arabitol, in particular. The result suggests that the high-flux hemodialysis may be advantageous over the low-flux dialysis in removing more uremic toxins from the blood stream [22].

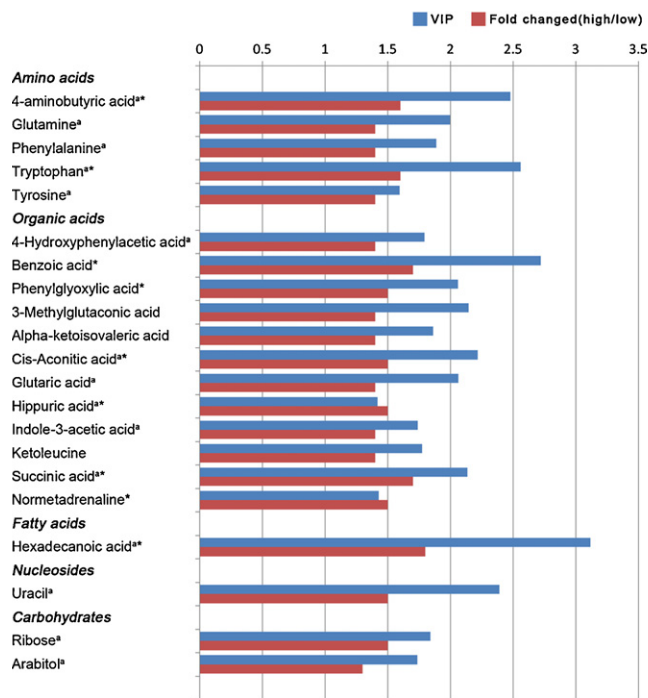

Fig. 5. Comparison of 21 metabolites from two different dialysis methods using GC/TOF/MS. Variables with VIP > 1 and P value < 0.05 (t-test) were selected as differential signals. A fold change with a positive value indicated a relatively higher concentration present in hemodialysate from high-flux treated patients. [a]Metabolites were confirmed by comparison with reference standards, others were directly identified by comparison library database. *Highly significant at $P<0.01$.

4. Conclusion

In this study, a new method for profiling the hemodialysate metabolites has been developed using GC/TOF/MS. The optimized procedure was concluded as following: 20 μl urease (no less than 50 U) was added to 2 ml hemodialysate sample to minimize the influence of the urea before lyophilization. A 500 μl aliquot of mixed solvents (methanol:water = 3:1, v/v) was added to dissolve the lyophilized solid with the maximum amount of metabolites detected for GC/TOF/MS analysis after TMS derivatization. The developed method is sensitive, rapid and reproducible. The method was successfully applied to a metabolomic analysis of hemodialysate samples from 60 patients who were under high-flux or low-flux dialysis. The results demonstrated that the

high-flux dialysis resulted in a relative higher concentration of URS in hemodialysate samples than the low-flux dialysis. The results also suggest that the high-flux dialysis is more beneficial in the removal of uremic toxins from the blood stream.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2011.04.001.

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