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Journal of Chromatography B, 854 (2007) 20–25
JOURNAL OF CHROMATOGRAPHY B
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# GC/MS analysis of the rat urine for metabonomic research

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Received 24 September 2006; accepted 26 March 2007
Available online 8 April 2007

**Abstract**

In this paper, an optimized protocol was established and validated for the metabonomic profiling in rat urine using GC/MS. The urine samples were extracted by methanol after treatment with urease to remove excessive urea, then the resulted supernatant was dried, methoximated, trimethylsilylated, and analyzed by GC/MS. Forty-nine endogenous metabolites were separated and identified in GC/MS chromatogram, of which 26 identified compounds were selected for quantitative analysis to evaluate the linearity, precision, and sensitivity of the method. It showed good linearity between mass spectrometry responses and relative concentrations of the 26 endogenous compounds over the range from 0.063 to 1.000 (v/v, urine/urine + water) and satisfactory reproducibility with intra-day and inter-days precision values all below 15%. The metabonomic profiling method based on GC/MS was successfully applied to urine samples from hyperlipidemia model rats. Obviously, separated clustering of model rats and the control rats were shown by principal components analysis (PCA); time-dependent metabonomic modification was detected as well. It was suggested that metabonomic profiling based on GC/MS be a robust method for urine samples.


*Keywords:* Metabonomics; Urine; GC/MS

## 1. Introduction

Metabonomics is a systematic approach to study in vivo metabonomic profile, which is defined as the quantitative measurement of the time-related multiparametric metabonomic response of living systems to pathophysiological stimuli or genetic modification [1]. Metabonomics has recently demonstrated its potentials in many fields, such as biomedical sciences [2–13] and toxicity [14,15], which had been extensively reviewed by Robertson [16]. In biomedical research, samples for metabonomic study are mostly derived from peripheral tissues such as blood, urine, spinal fluids, saliva, and CSF as well as tissues at the site of pathology. Blood and urine are the most frequently used samples for exploring the systematic modification of metabolome. Compared with blood plasma, urine samples enable non-invasive monitoring of metabonomic modification and should be the first choice. GC/MS has been demonstrated to be a very sensitive and reproducible method[17,18], which is thought the ideal tool for metabonomic profiling since most metabolites in urine are of polar groups, which will facilitate derivatization and analysis in GC/MS.

GC/MS has been applied to metabonomic profiling of urine samples from hydrocortisone-induced animal model [10], where acids or alkali were added for extraction step, and acidic treatments pose severe problems for many analytical methods that follow. A recent publication on metabonomic profiling of urine based on GC/MS [19] utilized acetone treatment and BSTFA as the derivatization agent. While in another paper, only five targeted compounds were analyzed in urine by GC/MS [11]. Till today, no thoroughly optimized method for analyzing urine by GC/MS in metabonomic research has been presented. In this paper, we developed and validated an optimized protocol of extraction and derivation for analyzing the rat urine using GC/MS.

## 2. Experimental

### 2.1. Chemicals and reagents

[$^2$H3]-Myristic acid (97%) for use as internal standard was purchased from Cambridge Isotope Laboratories Inc.

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doi:10.1016/j.jchromb.2007.03.048

(Andover, MA, USA). Alkane series (C8–C40), *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA), trimethylchlorosilane (TMCS) were obtained from Fluka (Buchs, Switzerland), and methoxyamine from Supleco (Bellefonte, PA). Urease was obtained from Sigma (St. Louis, MO, USA). Distilled water was produced by a Milli-Q Reagent Water System (Millipore, MA, USA).

### 2.2. Preparation of standard solutions

Internal standard [$^2$H3]-myristic acid was prepared in methanol at a concentration of 1 mg/ml. Methoxyamine was prepared in pyridine at a concentration of 15 mg/ml.

### 2.3. Sample preparation

Urine samples were collected from Sprague–Dawley rats (190–210 g, male) overnight (12 h) in metabolism cages at ambient temperature. After centrifugation at 4000 × *g* for 10 min to remove residues, urine samples were mixed and stored in aliquots at −80 °C until use.

Two hundred microliter of urine added with 30 units of urease was incubated at 37 °C for 15 min to decompose and remove excess urea present in it. Then 800 μl methanol and 10 μl [$^2$H3]-myristic acid (1 mg/ml) used as internal standard were added to it. The solution was vigorously extracted for 10 min and was centrifuged at 14,000 × *g*, 4 °C for 10 min. The supernatant (200 μl) was transferred to a GC vial, and then evaporated to dryness under nitrogen at room temperature.

Thirty microliter of methoxyamine in pyridine was added to each GC vial. Then the solution was vigorously vortexed for 10 min. After methoximation reaction for 16 h at room temperature, the samples were trimethylsilylated for another 1 h by adding 30 μl of MSTFA with 1% TMCS as catalyst. At last, 40 μl heptane was added to the GC vial, and the solution was vigorously vortexed again for 10 min before GC/MS analysis.

### 2.4. Instruments

One microliter of derivatized sample was injected splitless into a Finigan gas chromatography (Thermofinigan, USA) coupled with mass spectrometry (TRACE DSQ). It is equipped with a 30 m × 0.25 mm ID, fused silica capillary column, which was chemically bonded with 0.25 μm DB1-MS stationary phase (J&W scientific, Folsom, CA, USA). The injector temperature was 270 °C, the septum purge flow rate was 20 ml/min, and the purge was turned on after 60 s. The gas flow rate through the column was 1 ml/min. The column initial temperature was kept at 70 °C for 2 min. Then temperature was increased from 70 to 240 °C at a rate of 20 °C/min, held on for 1 min, and then to the last 320 °C at a rate of 20 °C and held for 1 min. Transfer line temperature was 270 °C and ion source temperature was 200 °C. Ionization was achieved by a 70 eV electron beam at a current of 2.0 mA. Masses were acquired in TIC when the acceleration voltage was turned on after a solvent delay of 240 s.

All data were processed by Xcalibur (Thermofinigan, USA). Peaks whose signal-to-noise (S/N) ratios lower were than 5 were rejected. Retention time correction was done by internal standard in order to minimize run-to-run errors. Retention index for each peak/compounds was calculated by comparing its retention time against those of alkane series (C8-C40, corresponding retention index values from 800–4000). To obtain accurate peak areas for the I.S. and specific peaks/compounds, two quantification masses for each component were specified and the data were re-processed. The area of each peak was normalized against the internal standard, [$^2$H3]-myristic acid, before multivariate data analysis. All compounds were identified by comparing both the MS spectra and retention index with those available in libraries, i.e. NIST, Wiley, and internally compiled spectr libraries.

### 2.5. Validation of assay method

#### 2.5.1. Linearity

Pooled urine pretreated by urease was diluted with water to relative concentrations of 0.063, 0.125, 0.250, 0.500, and 1.000 (v/v, urine/urine + water). A portion (200 μl) of the diluted urine, including 10 μl [$^2$H3]-myristic acid (1 mg/ml) used as internal standard (I.S.), was then mixed with 800 μl of methanol. Extraction and derivatisation were performed according to "Section 2.3". After GC/MS analysis, the peak areas of endogenous metabolites were integrated. The peak-area ratio of each metabolite to I.S. was calculated and linear correlation coefficients were calculated in the analyzed concentration interval.

#### 2.5.2. Precision

Precision was investigated by calculating the relative standard deviation (R.S.D.) at three urine dilutions: 0.063, 0.250, and 1.000 (v/v, urine/urine + water). The intra-day precision values were determined in five replicates at each dilution, and these replicates were processed independently. The inter-day precision values were determined across each dilution on five different days. All the samples were treated as described above.

#### 2.5.3. Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) for urine samples were investigated. Urine dilutions of 0.02, 0.063, 0.125, 0.250, 0.5, and 1.000 (v/v, urine/urine + water) were treated as described above. LOD was defined as that the level giving a signal-to-noise ratio of 3, and LOQ was defined as that the level giving a signal-to-noise ratio of 5.

#### 2.5.4. Stability testing

To evaluate the freeze–thaw stability, urine samples were stored at −80 °C for 7 days, and then thawed at room temperature. The assay parameter for the stored samples was compared with that of freshly prepared samples. Samples were analyzed in five replicates and treated as the described above. To be considered stable, the relative recovery (defined as the agreement between freeze–thawed and fresh samples) should be within 100 ± 15%.

### 2.6. Metabonomics research for urine samples of hyperlipidemia rats

Sprague–Dawley rats (190–210 g, male) were obtained from Sino-British Sippr/BK Lab Animal Ltd. (Shanghai, China). The animals were housed in stainless steel cages in a controlled environment (20 °C, 50% relative humidity, and 12 h light/12 h dark cycle) for at least 6 days prior to the experiment. Animal experiments were carried out in accordance with the Guidelines for Animal Experimental of Nanjing University of Technology (Nanjing, China), and the protocol was approved by the Animal Ethics Committee of this institution.

Twelve rats were randomly divided into two groups, normal and hyperlipidemia, which were induced with standard chow and laboratory chow enriched with 1% cholesterol, 10% lard, 0.2% propylthiouracil, 5% yolk, and 1% sodium tauroglycocholate for 4 weeks, respectively.

Pre-dose and post-dose at days 7, 14, 21, 28 after feeding high lipid diet, all the rats were fasted and urine samples were collected overnight (12 h) in metabolism cages at ambient temperature. After centrifugation at 4000 × *g* for 10 min to remove residues, urine samples were stored in aliquots at −80 °C prior to analysis.

Multivariate data analysis was carried out using SIMCA-P 11 software (Umetrics, Umeå, Sweden). Principal component analysis (PCA) was used to calculate models. PCA is an unsupervised projection method used to visualize the dataset and display the similarity and difference. The data matrix (*X*) can be represented in a *K*-dimensional space (where *K* is equal to the number of variables), and then projected and reduced to a few principal components that describe the maximum variation of different groups or samples. The data matrix was constructed with the observation/samples in columns and the peaks/compounds in rows. Statistically different peaks were calculated with a confidence interval of 0.95 and significance level of 0.05. Cross-validation with seven cross-validation groups was used throughout to determine the number of components in PCA.

## 3. Results and discussion

### 3.1. Metabonomic profiling of urine by GC/MS

The goal of metabonomic research is to analyze as many metabolites as possible in biofluids, and it is much more difficult in contrast with analyzing a limited number of specific compounds. It would be impossible in practice to obtain satisfied extraction efficiency, reproducibility, and accuracy for all of the metabolites contained in urine, which differ widely in physiochemical properties and concentrations. Therefore, our target in this study was to develop a method that is likely to extract as many classes of compounds as possible with high efficiency and reproducibility.

The GC/MS chromatogram of urine samples obtained from normal rat is shown in Fig. 1. 49 compounds were identified in the GC/MS chromatogram. Most of them are organic acids, amines, amino acids, carbohydrates, etc. Since most compounds identified in urine are also presented in blood plasma, the protocol of urine extraction and derivation are based on that of plasma. In the present study, we investigated five common organic solvents (methanol, ethanol, acetonitrile, acetone, chloroform) and found that methanol resulted in overall highest recovery for most compounds investigated (data not shown). Considering that methanol was also preferred in extraction of other biological samples, such as plasma [17,20], cellular solution [21,22], and bacteria [23,24]. It was suggested that methanol be a promising solvent for metabolomic extraction of biological samples.

Because urea concentration in urine is so high that it influences the appearances of other compounds with lower concentration and is easy to contaminate the column and MS detector, so urease was used to degrade excessive urea and did

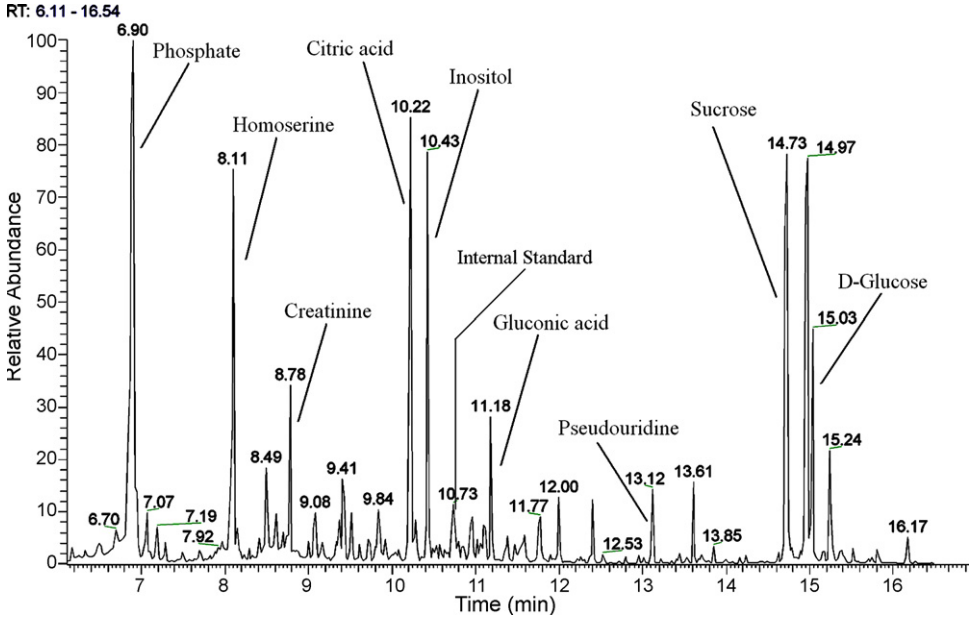


Fig. 1. GC/MS chromatogram of urine samples obtain from normal rat.

Table 1
Linearity of the GC/MS method for the determination of some endogenous compounds in urine

| Compounds | Peak area ratios of urine dilutions | | | | | Correlation coefficient |
|---|---|---|---|---|---|---|
| | 1 | 0.5 | 0.25 | 0.128 | 0.063 | |
| Phosphate | 5.8691 | 3.058 | 1.854 | 0.958 | 0.6580 | 0.9992 |
| Butanoic acid | 0.2893 | 0.1658 | 0.0985 | 0.0621 | 0.0412 | 0.9991 |
| Glycine | 0.2637 | 0.1745 | 0.1247 | 0.1028 | 0.0841 | 0.9990 |
| Uracil | 0.1570 | 0.0954 | 0.0687 | 0.0489 | 0.0411 | 0.9991 |
| L-Homoserine | 2.1600 | 1.3104 | 0.8847 | 0.6321 | 0.5014 | 0.9994 |
| Butanedioic acid | 0.0772 | 0.04995 | 0.0341 | 0.02514 | 0.0203 | 0.9981 |
| L-Proline | 1.4663 | 0.8014 | 0.4658 | 0.3012 | 0.2001 | 0.9999 |
| Creatinine enol | 3.3076 | 2.0147 | 1.3698 | 1.1124 | 0.8541 | 0.9994 |
| Dithioerythritol | 0.2093 | 0.1325 | 0.0958 | 0.07012 | 0.0594 | 0.9989 |
| D-Ribose | 0.2047 | 0.1325 | 0.0865 | 0.07254 | 0.0624 | 0.9988 |
| Xylitol | 0.2395 | 0.1642 | 0.1214 | 0.1003 | 0.0884 | 0.9993 |
| D-Galactose | 0.2138 | 0.1435 | 0.1025 | 0.08541 | 0.0754 | 0.9996 |
| Ribonic acid | 0.6233 | 0.4012 | 0.2745 | 0.2214 | 0.1824 | 0.9995 |
| Citric acid | 6.2719 | 3.3541 | 2.2471 | 1.4578 | 1.0236 | 0.9988 |
| 1H-Indole | 0.0215 | 0.0132 | 0.0096 | 0.0068 | 0.0055 | 0.9982 |
| Glucose-*O*-methoxime | 3.1628 | 1.8541 | 1.2365 | 0.98541 | 0.7412 | 0.9994 |
| Glucitol | 0.0854 | 0.05231 | 0.0311 | 0.0234 | 0.0185 | 0.9989 |
| D-Gluconic acid | 4.0731 | 2.8541 | 2.1472 | 1.7415 | 1.5247 | 0.9980 |
| Hexadecanoic acid | 1.0000 | 0.6234 | 0.4412 | 0.3241 | 0.2847 | 0.9995 |
| Myo-inositol | 2.0439 | 1.3258 | 0.9847 | 0.7421 | 0.6854 | 0.9991 |
| Oleic acid | 0.3004 | 0.1984 | 0.1425 | 0.1211 | 0.0985 | 0.9991 |
| Adenosine | 0.2021 | 0.1325 | 0.0854 | 0.06541 | 0.0511 | 0.9973 |
| Sucrose | 6.0246 | 3.7541 | 2.6587 | 1.9985 | 1.6254 | 0.9994 |
| D-Glucose | 7.1696 | 4.1203 | 2.5871 | 1.4417 | 0.9928 | 0.9977 |
| D-Glucopyranose | 4.3093 | 2.6547 | 1.7451 | 1.3367 | 1.0024 | 0.9992 |
| Melibiose | 0.2602 | 0.1654 | 0.1121 | 0.0857 | 0.0745 | 0.9996 |

not affect other compounds in urine. Most compounds in urine are involatile and have to be derivatized before GC analysis. In order for higher sensitivity and better chromatographic resolution, many factors were investigated to approach the final protocol, such as ratios of sample amount and methanol volume, sample amount for dryness, reaction temperature, time, and amount for both methoximation and trimethylsilylation. GC oven temperature ramping rate was investigated as well. Considering the very much complexity of endogenous compounds in urine, the three stable isotope labeled compounds [$^{13}$C5]-proline, [$^{2}$H6]-salicylic acid, [$^{2}$H3]-myristic acid were investigated. [$^{2}$H3]-Myristic acid was selected since it was eluted in the middle of chromatogram with minimal interference from other metabolites, showed high reproducibility both in quantitatively and qualitatively, e.g. standard deviation, retention time, and mass spectrum.

### 3.2. Validation of assay method

#### 3.2.1. Linearity

Twenty-six of the identified endogenous compounds were selected to investigate the linearity of the method. These compounds covered a wide span of GC retention times, and they belong to various classes of compounds with diverse physicochemical properties, such as organic acids, amino acids, and carbohydrates. The linearity of the response was determined by analyzing urine at five different concentrations and found to be generally high for most of the compounds investigated ($r > 0.999$), as shown in Table 1.

#### 3.2.2. Precision

Twenty-six of the identified endogenous compounds were selected to investigate the precision of the method. The precision of the analysis was calculated as the relative standard deviation of the peak area for each metabolite corrected by the peak area of the I.S. The R.S.D. of most of the 26 compounds was less than 15%, as shown in Table 2. Generally, the lowest concentrations of urine resulted in the highest R.S.D., and the R.S.D. of inter-day was higher than that of intra-day.

#### 3.2.3. Sensitivity

The 26 identified endogenous compounds as described in linearity and precision tests were selected to evaluate LOD and LOQ. These compounds could be detected at S/N greater than or equal to 3 when the urine dilution was 0.02 (v/v, urine/urine + water), and it could be defined as LOD. These compounds could also be detected at S/N greater than or equal to 5 when the urine dilution was 0.063 (v/v, urine/urine + water), and it could be defined as LOQ.

#### 3.2.4. Stability testing

The result of stability testing is shown in Table 3. The 26 identified endogenous compounds as described above were also used to evaluate their stability in urine samples after a freeze–thaw cycle. In general, the freeze–thaw cycles did not result in appreciable differences in the assay parameters and were 90.0–99.6% of those of the corresponding fresh samples.

Table 2
Precision of the GC/MS method for the determination of some endogenous compounds in urine

| Compounds | Intra-day precision | | | Inter-day precision | | |
|---|---|---|---|---|---|---|
| | R.S.D. (%) at dilutions (v/v) of | | | R.S.D. (%)at dilutions (v/v) of | | |
| | 0.063 | 0.25 | 1 | 0.063 | 0.25 | 1 |
| Phosphate | 7.89 | 5.33 | 4.25 | 8.98 | 7.55 | 3.21 |
| Butanoic acid | 8.74 | 8.29 | 6.94 | 13.00 | 10.00 | 7.14 |
| Glycine | 10.05 | 4.12 | 4.92 | 10.88 | 4.59 | 3.76 |
| Uracil | 6.37 | 5.11 | 4.03 | 9.46 | 6.32 | 5.69 |
| L-Homoserine | 8.27 | 4.63 | 2.69 | 9.76 | 7.53 | 4.64 |
| Butanedioic acid | 10.21 | 5.21 | 3.67 | 11.38 | 5.71 | 4.44 |
| L-Proline | 5.89 | 5.14 | 3.27 | 6.06 | 5.60 | 3.45 |
| Creatinine enol | 5.91 | 4.72 | 3.54 | 8.24 | 7.43 | 4.14 |
| Dithioerythritol | 11.15 | 3.78 | 2.49 | 12.58 | 9.72 | 6.99 |
| D-Ribose | 10.89 | 6.48 | 5.54 | 10.59 | 7.70 | 5.65 |
| Xylitol | 7.55 | 5.62 | 4.05 | 8.71 | 5.25 | 3.85 |
| D-Galactose | 7.15 | 7.01 | 4.10 | 7.00 | 6.89 | 5.25 |
| Ribonic acid | 9.54 | 5.84 | 5.40 | 10.09 | 9.29 | 6.31 |
| Citric acid | 14.25 | 8.14 | 5.77 | 16.10 | 8.52 | 9.92 |
| 1H-Indole | 9.84 | 8.44 | 5.24 | 14.20 | 9.70 | 7.80 |
| Glucose-*O*-methoxime | 9.33 | 6.87 | 3.40 | 10.10 | 6.61 | 5.85 |
| Glucitol | 12.47 | 9.55 | 8.24 | 14.90 | 10.81 | 8.37 |
| D-Gluconic acid | 9.87 | 5.58 | 2.88 | 11.70 | 6.13 | 3.35 |
| Hexadecanoic acid | 11.63 | 9.87 | 7.39 | 12.79 | 10.66 | 8.72 |
| Myo-inositol | 7.31 | 4.99 | 2.37 | 8.66 | 6.75 | 5.47 |
| Oleic acid | 12.17 | 9.41 | 5.55 | 13.41 | 11.69 | 6.41 |
| Adenosine | 8.41 | 4.25 | 4.02 | 9.52 | 4.90 | 4.38 |
| Sucrose | 11.54 | 8.64 | 6.11 | 12.95 | 9.36 | 7.48 |
| D-Glucose | 12.47 | 9.36 | 7.14 | 14.51 | 11.12 | 8.11 |
| D-Glucopyranose | 7.41 | 6.28 | 3.54 | 8.53 | 7.71 | 4.82 |
| Melibiose | 8.54 | 7.02 | 4.58 | 9.21 | 8.21 | 5.23 |

### *3.3. Application of the assay to the metabonomics research for urine samples*

The feasibility of using the current assay in the metabonomics research for urine samples was also examined. All of the data from 49 identified compounds were used in calculating PCA models. The score plots of the first two principal components allowed visualization of the data and compared the two group samples. The endogenous compounds can be visualized and positioned in a loading plot corresponding to a score plot. The PCA scores plot of whole dataset is presented in Fig. 2.

For the control group (K), all the samples (K0–4) clustered in the right side of the figure, which suggested a stable status of metabonomics during experiment. For the treatment group (U), the samples at different time points tended to gather in different regions, respectively, displaying some time-dependent movement. Samples of pre-dose (U0) in the treatment group located at the same region as the control group. The samples collected at day 7(U1), 14(U2), 21(U3), 28(U4) after feeding with high lipid diet showed a trend of contraclockwise movement. It was interesting to find that samples of U4 stood far away from those of U1, U2, and U3. Further investigation indicated that there was metabolic disorder during this stage from U1 to U3 (data not shown) and that the rat model of hyperlipidemia was finally available at day 28 of post-dose. It was indicated that the use of GC/MS coupled with PCA has the potential of metabonomic profiling of urine and therefore could provide insight information on metabonomic changes related to hyperlipidemia in rats.

Anyway, the present investigation on the animal model was an attempt and application of the robust GC/MS method. Without any doubt, a massive feed of high lipid food will have great influences on the metabolites, although this model is classic and widely used. However, it is still of great value for the metabonomic profiling, which displayed the time-dependent

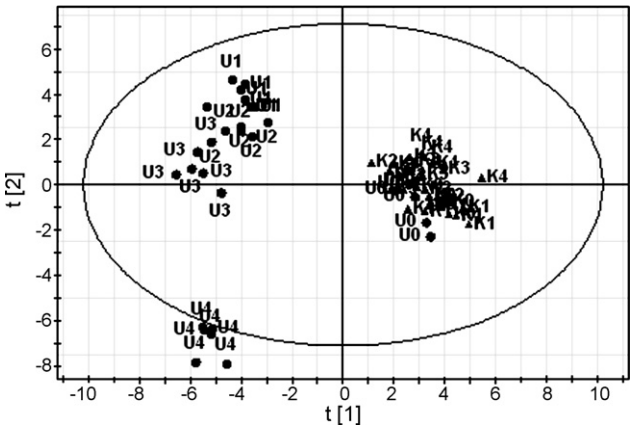


Fig. 2. PCA score plots of rat urine samples from hyperlipidemia and normal control group during four weeks experiment. Obviously separated clustering of model rats and the control rats were shown by PCA, time-dependent metabonomic modification was detected as well. (▲ K) normal control group, (● U) hyperlipidemia group.

Table 3
Stability of the GC/MS method for the determination of some endogenous compounds in urine

| Compounds | Peak area ratios | | Stability (%) |
|---|---|---|---|
| | Before freeze–thaw | After freeze–thaw | |
| Phosphate | 6.251 ± 0.201 | 6.053 ± 0.531 | 96.83 |
| Butanoic acid | 0.352 ± 0.025 | 0.345 ± 0.033 | 98.01 |
| Glycine | 0.292 ± 0.011 | 0.273 ± 0.021 | 93.49 |
| Uracil | 0.150 ± 0.009 | 0.145 ± 0.015 | 96.67 |
| L-Homoserine | 2.283 ± 0.106 | 2.171 ± 0.156 | 95.09 |
| Butanedioic acid | 0.079 ± 0.004 | 0.075 ± 0.012 | 94.94 |
| L-Proline | 1.494 ± 0.051 | 1.487 ± 0.063 | 99.53 |
| Creatinine enol | 3.563 ± 0.147 | 3.471 ± 0.185 | 97.42 |
| Dithioerythritol | 0.182 ± 0.013 | 0.165 ± 0.020 | 90.66 |
| D-Ribose | 0.255 ± 0.014 | 0.238 ± 0.019 | 93.33 |
| Xylitol | 0.264 ± 0.010 | 0.251 ± 0.013 | 95.08 |
| D-Galactose | 0.243 ± 0.013 | 0.234 ± 0.015 | 96.30 |
| Ribonic acid | 0.712 ± 0.045 | 0.701 ± 0.061 | 98.46 |
| Citric acid | 6.381 ± 0.663 | 6.284 ± 0.881 | 98.48 |
| 1H-Indole | 0.020 ± 0.002 | 0.018 ± 0.010 | 90.00 |
| Glucose-*O*-methoxime | 3.282 ± 0.192 | 3.145 ± 0.214 | 95.83 |
| Glucitol | 0.089 ± 0.007 | 0.081 ± 0.009 | 91.01 |
| D-Gluconic acid | 4.511 ± 0.151 | 4.321 ± 0.169 | 95.79 |
| Hexadecanoic acid | 1.202 ± 0.105 | 1.104 ± 0.145 | 91.85 |
| Myo-inositol | 2.472 ± 0.135 | 2.454 ± 0.165 | 99.27 |
| Oleic acid | 0.274 ± 0.018 | 0.263 ± 0.024 | 95.99 |
| Adenosine | 0.215 ± 0.009 | 0.207 ± 0.013 | 96.28 |
| Sucrose | 6.351 ± 0.475 | 6.324 ± 0.631 | 99.57 |
| D-Glucose | 7.042 ± 0.571 | 6.956 ± 0.841 | 98.78 |
| D-Glucopyranose | 4.273 ± 0.206 | 4.011 ± 0.226 | 99.56 |
| Melibiose | 0.215 ± 0.011 | 0.196 ± 0.014 | 91.16 |

biochemical modification of the process during model building and further suggested the right time at day 28 when the model was established. Furthermore, these model animals are supposed to be utilized to evaluate drugs or drug candidates efficacies by investigating the metabonomic/systematic modification after administrating different kind of drug candidates. Both the animal model and the metabonomic profiling method are the groundwork for this purpose.

## 4. Conclusion

An optimized protocol of extraction and derivatization was established and validated for metabonomic profiling of rat urine. Forty-nine endogenous compounds were detected and identified, most of which showed very good linearity and precision. The metabonomic profiling method based on GC/MS and PCA was successfully applied to urine samples from hyperlipidemia model rats. It was suggested that metabonomic profiling based on GC/MS and PCA be a robust method for analyzing urine samples.

## Acknowledgements

The work was supported by Jiangsu Nature Science Fund (BK2005098), National Nature Science Fund (30572228) and Jiangsu international cooperation Fund (BZ2006049).

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