

Application News

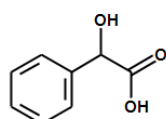
No. AD-0122Ph

Quantitative Bioanalysis / HPLC

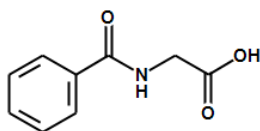
Sensitive HPLC Method for Quantitative Analysis of Mandelic Acid, Hippuric Acid, Methyl Hippuric Acid in Human Urine

Introduction

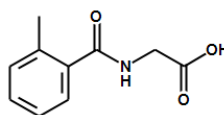
Metabolites in human urine such as hippuric acid (HA), *o*-methyl hippuric acid (M-HA) and mandelic acid (MA) are indicators of exposure to toxic solvents toluene, xylene and styrene, respectively. Frequent exposure to these solvents may cause damage to the human central nervous system¹. To determine the level of exposure, urine samples of subjects are collected and analysed quantitatively for HA, M-HA and MA per creatinine mass of an individual. Normal level of HA for an unexposed subject is 1g/g creatinine, while M-HA should not be present. The threshold limits for the exposed subject are 1.6g HA/g creatinine, 1.5g M-HA/g creatinine² and 0.8g MA/g creatinine³. The HPLC method described in this Application News allows us to determine HA, M-HA, MA and creatinine in urine samples simultaneously without need for sample clean up except filtration.



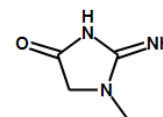
Mandelic Acid (MA)



Hippuric Acid (HA)



o-Methyl Hippuric Acid (M-HA)



Creatinine

Experimental

Preparation of Calibration Standard

Standards were prepared from each 1000 mg/L stock solution of hippuric acid, *o*-methyl-hippuric acid, mandelic acid and creatinine in acetonitrile. An intermediate mixed standard of 100mg/L was prepared using 0.017M of potassium dihydrogen phosphate buffer solution with 0.003M of 1-decane sulfonic acid sodium salt as diluent. The calibration standards used ranged from 1mg/L to 40mg/L. The mixed standards solutions were filtered with 0.45µm Nylon Filter prior to sample injection onto HPLC.

Instrument and Analytical Conditions

Instrument: Shimadzu Prominence HPLC
 Column: Inertsil ODS-80A 5µm (4.6 x 150 mm)
 Column Temp: 40°C
 Mobile Phase: A) 0.017M KH₂PO₄ (pH 3.3) with 0.003M 1-decane sulfonic acid sodium salt, 87%
 B) acetonitrile, 13%
 Elution Mode: Isocratic elution
 Flow Rate: 1.4 mL/min
 Detection: 225nm
 Injection: 10µL

Sample Preparation

200µL of fresh urine was diluted to 10mL with mobile phase solution A. This solution was filtered with 0.45 µm Nylon Filter prior to injection to HPLC.

Results and Discussion

Figures 1 and 2 show that MA, HA, M-HA and creatinine can be effectively separated within 11 minutes both in the calibration standards and actual urine sample, using the set analytical conditions.

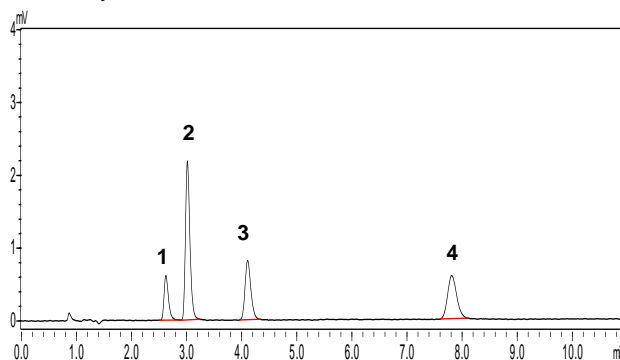


Fig 1. Chromatogram of standard sample, 1 ppm each of MA (1), HA (2), M-HA (3) and Creatinine (4)

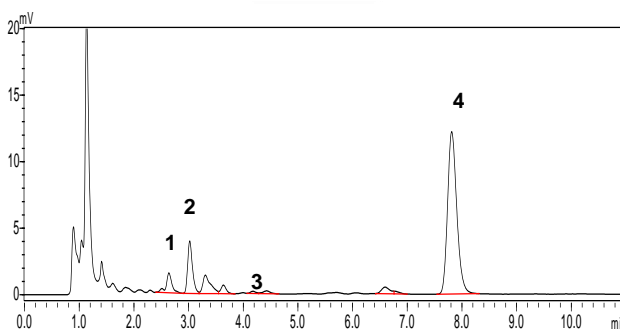


Fig 2. Chromatogram of urine sample, MA (1), HA (2), M-HA (3) and Creatinine (4)

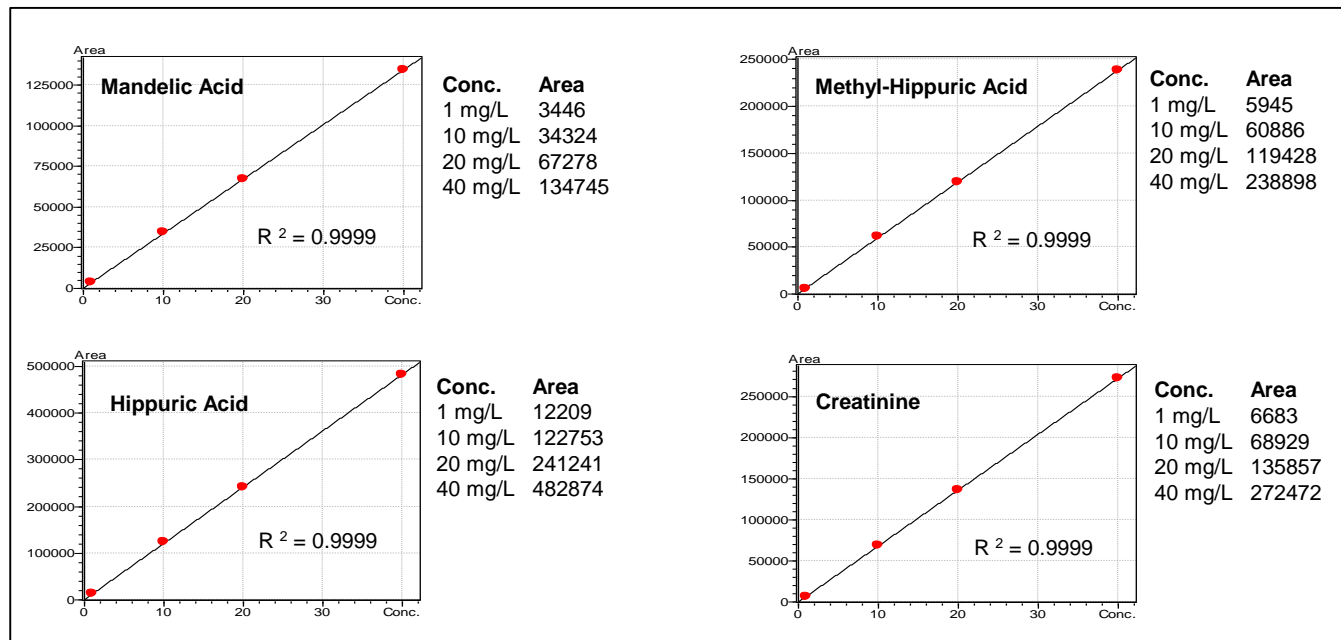


Fig. 3. Calibration Curves and Linearity of MA, HA, M-HA and creatinine

Table 1. Analysis result in a human urine sample by HPLC

Compound	Area	Conc (mg/L)	Conc in Urine (g/g Creatinine)
Mandelic Acid	11,235	3.21	0.15
Hippuric Acid	24,804	1.92	0.09
M-Hippuric Acid	1,142	0.11	0.01
Creatinine	142,976	20.97	1.0

Table 2. Method recovery determined by spiking compounds in urine sample

Compound	Amount of Spike (mg/L)	Measured before spike (mg/L)	Total measured (mg/L)	Recovery (%)
Mandelic Acid	4.00	3.21	7.21	100.1
Hippuric Acid	4.00	1.92	5.97	101.1
M-Hippuric Acid	4.00	0.11	4.36	106.2
Creatinine	4.00	20.97	24.77	94.8

Figure 3 shows the calibration curves of 1, 10, 20 and 40 mg/L mixed standards of mandelic acid, hippuric acid, o-methyl-hippuric acid and creatinine. Good linearity of the HPLC method was achieved with the correlation coefficient (R^2) greater than 0.9999. Table 1 shows the analysis results of MA, HA, M-HA and creatinine of an actual urine sample, indicating that all the targets are below the threshold limits. The limit of detection (LOD) of the method is calculated based on the S/N ratio, which are 0.084, 0.024, 0.063 and 0.087 mg/L for MA, HA, M-HA and creatinine, respectively. The recovery of the method was evaluated by spiking 4.0 mg/L of the targets in the same urine sample above and determining their total amounts. The results are shown in Table 2.

Conclusions

A sensitive HPLC method was established on Shimadzu Prominence HPLC system for quantification of three urine metabolites, namely mandelic acid, hippuric acid and o-methyl hippuric acid, as well as creatinine. The method is

readily used for determining the urine metabolite/creatinine ratios required for monitoring exposure levels of human subjects to toxic solvents of toluene, xylene and styrene.

References

- [1] Rueff J, Teixeira JP, Santos LS, Gaspar JF. (2009) Genetic effects and biotoxicity monitoring of occupational styrene exposure. Clin Chim Acta. 399(1-2):8-23.
- [2] UK (2003) UK Government Information Notes on the Diagnosis of Prescribed Diseases.
- [3] Lauwerys, RR. , Hoet, P. (2001) Industrial Chemical Exposure, Guideline for Biological Monitoring. 3rd Ed. FL, USA. CRC Press.

Disclaimer: The Shimadzu Prominence HPLC system and the data in this Application News are intended for Research Use Only (RUO). Not for use in diagnostic procedures.

