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Application News

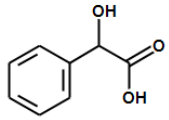
No. AD-0122Ph

Quantitative Bioanalysis / HPLC

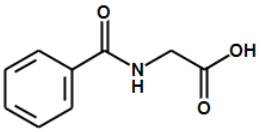
Sensitive HPLC Method for Quantitative Analysis of Mandelic Acid, Hippuric Acid, Methyl Hippuric Acid in Human Urine

❑ Introduction

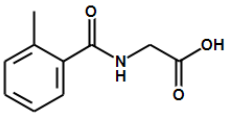
Metabolites in human urine such as hippuric acid (HA), *o*-methyl hippuric acid (M-HA) and mandelic acid (MA) are indicators of exposure to toxic solvents toluene, xylene and styrene, respectively. Frequent exposure to these solvents may cause damage to the human central nervous system[1]. To determine 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 creatinine[2] and 0.8g MA/g creatinine[3]. 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.



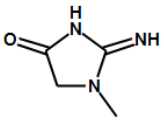
Mandelic Acid (MA)



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 KH_2PO_4 (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.

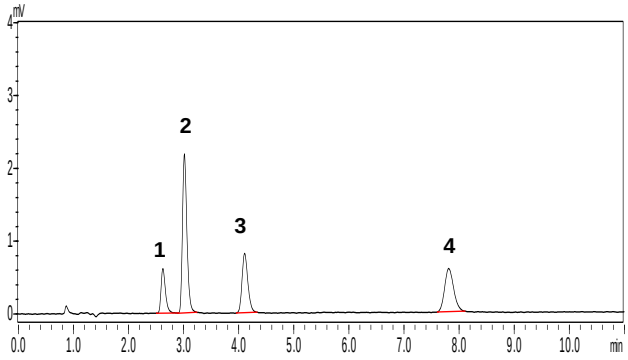


Fig 1. Chromatogram of standard sample, 1 ppm each of MA (1), HA (2), M-HA (3) and Creatinine (4)

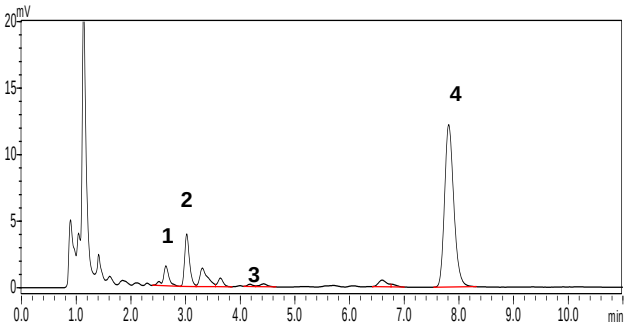


Fig 2. Chromatogram of unine sample, MA (1), HA (2), M-HA (3) and Creatinine (4)

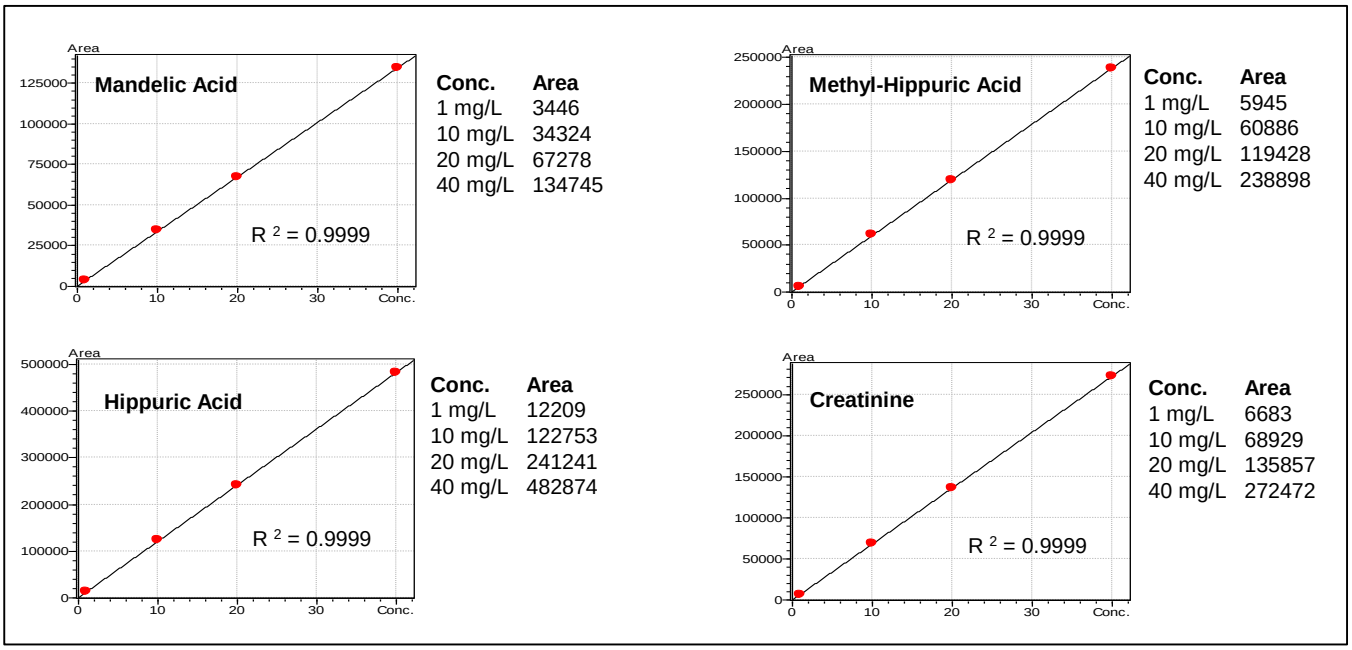


Fig. 3. Calibration Curves and Linearity of MA, HA, M-HA and creatinine

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 Prescirbed 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.

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