

GL Sciences Inc.

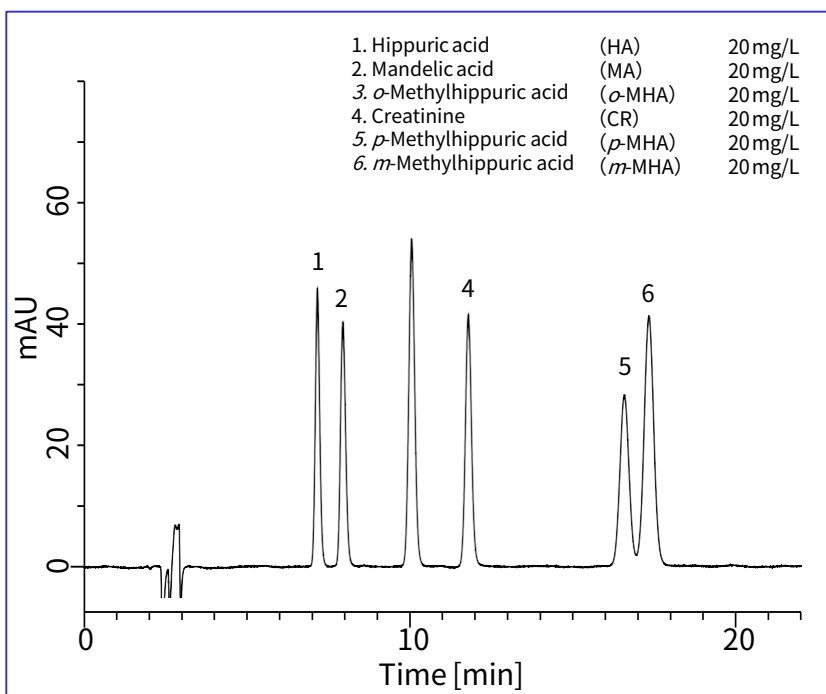
Hippuric acid, methylhippuric acid, and mandelic acid are major urine metabolites of aromatic organic solvents (e.g. toluene, xylene, and styrene). Their amounts in urine are most widely used as an indicator of the organic solvent exposure. This note describes a simultaneous determination method for the urinary metabolites and creatinine.

The separation is usually performed with ODS columns

using ion-pair reagents. However, it is difficult to achieve good separation of meta- and para- isomers of methylhippuric acid.

In this note, Inertsil ODS-80A, which offers excellent performance especially in separation of small molecules, was used. As a result, all the analytes were successfully separated within 20 minutes. (K.Suzuki)

A chromatogram obtained from standard solution



Conditions

System : GL-7400 HPLC system

Column : Inertsil ODS-80A
(5 μ m, 250 x 4.6 mm I.D.)
Cat.No. 5020-01602

Eluent : A) 2-propanol
B) 10 mM phosphate buffer (pH 2.5) containing 2 mM sodium 1-nonanesulfonate A/B = 10/90, v/v
(Mixed by a gradient mixer)

: 1.0 mL/min

Flow rate

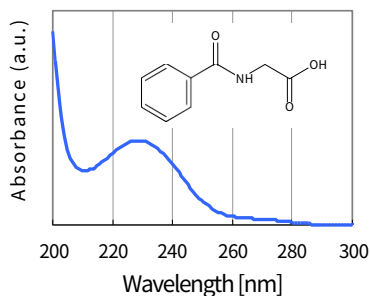
Col. Temp. : 40 $^{\circ}$ C

Detection : PDA 210 nm

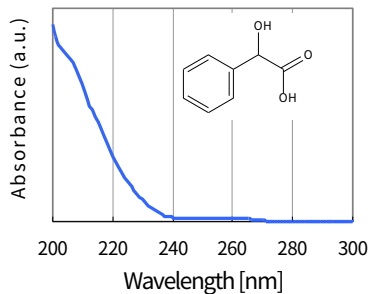
Inj. Vol. : 10 μ L

The chemical structures and the absorption spectra obtained by PDA

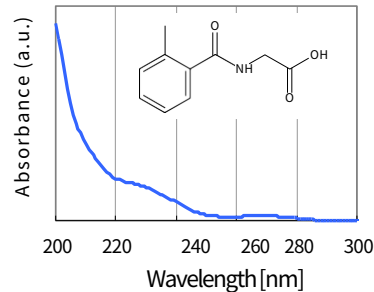
Hippuric acid



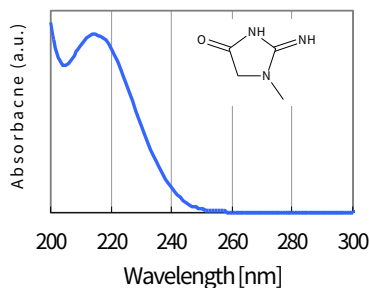
Mandelic acid



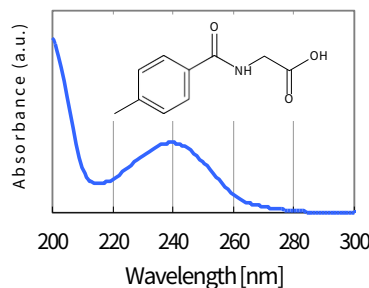
o-Methylhippuric acid



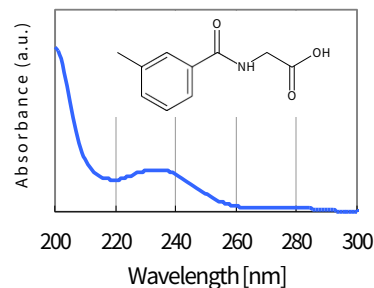
Creatinine



p-Methylhippuric acid



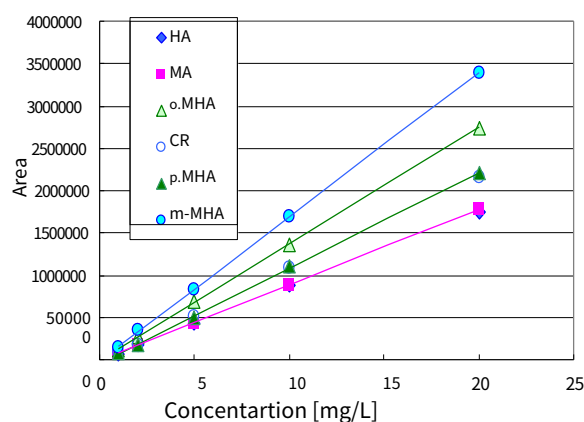
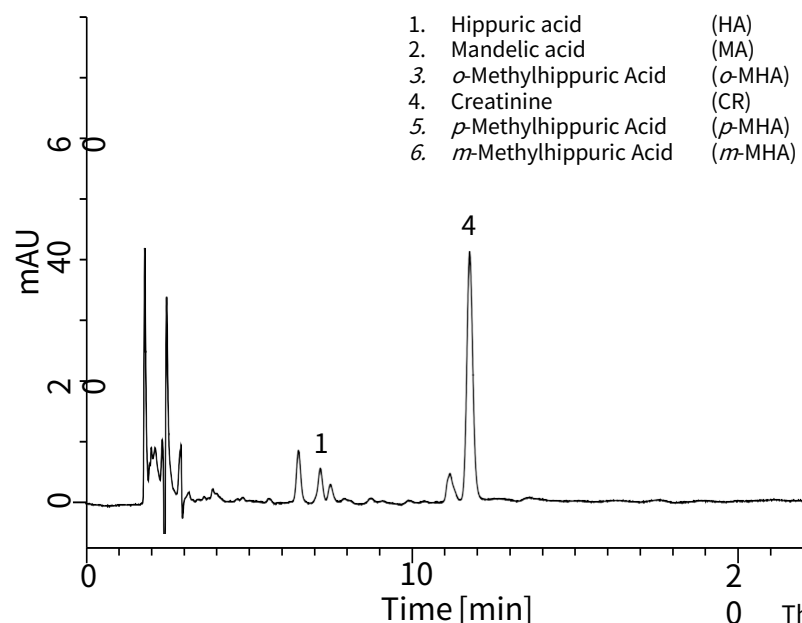
m-Methylhippuric acid



An analysis of human urine sample

After 100-fold dilution with water, the sample was filtrated using a 0.45 μm -membrane filter.

The filtrate was injected into the HPLC system.



	R ²
1. HA	: 0.9999
2. MA	: 1.000
3. <i>o</i> -MHA	: 0.9999
4. CR	: 0.9998
5. <i>p</i> -MHA	: 0.9995
6. <i>m</i> -MHA	: 0.9999

The calibration curves and their correlation coefficients

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