Analysis of Hippuric Acid in Urine by HPLC

GL Sciences Inc.

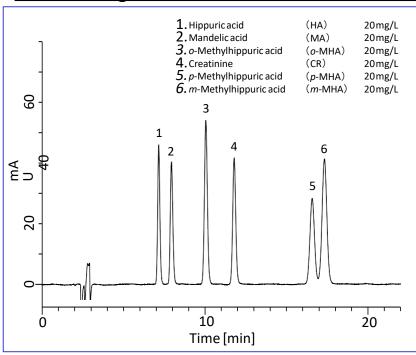
Hippuric acid, methylhippuric acid, and mandelic acid are major urine metabolites of aromatic organic solvents (*e.g.* toluene, xylene, and stylene). Their amounts in urine are most widely used as an indicator of the organic solvent exposure. This note describes a simultaneous determination method for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

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)

The separation is usually performed with ODS columns

A chromatogram obtained from standard solution



Conditions

System : GL-7400 HPLC system
Column : Inertsil ODS-80A

(5 μm, 250 x 4.6 mm l.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)

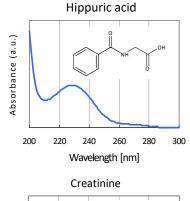
: 1.0 mL/min

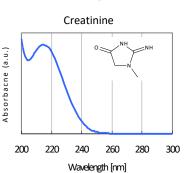
Flow rate

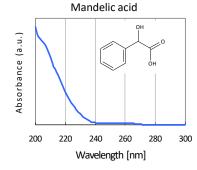
Col. Temp.: $40 \,^{\circ}\text{C}$ Detection: PDA 210 nm

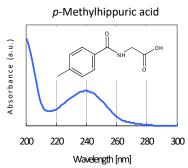
Inj. Vol.: $10 \, \mu\text{L}$

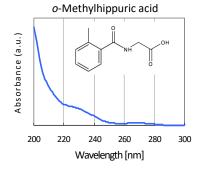
The chemical structures and the absorption spectra obtained by PDA

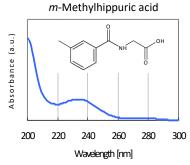










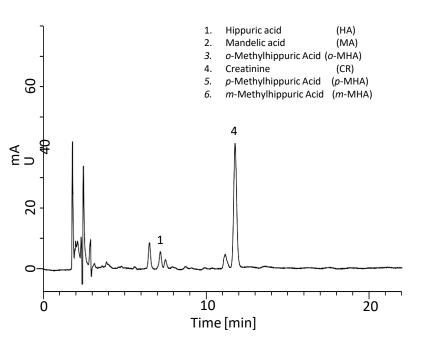


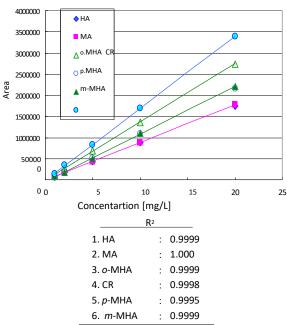


An analysis of human urine sample

After 100-fold dilution with water, the sample was filtrated using a $0.45~\mu m$ -membrane filter.

The filtrate was injected into the HPLC system.





The calibration curves and their correlation coefficients

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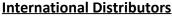
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