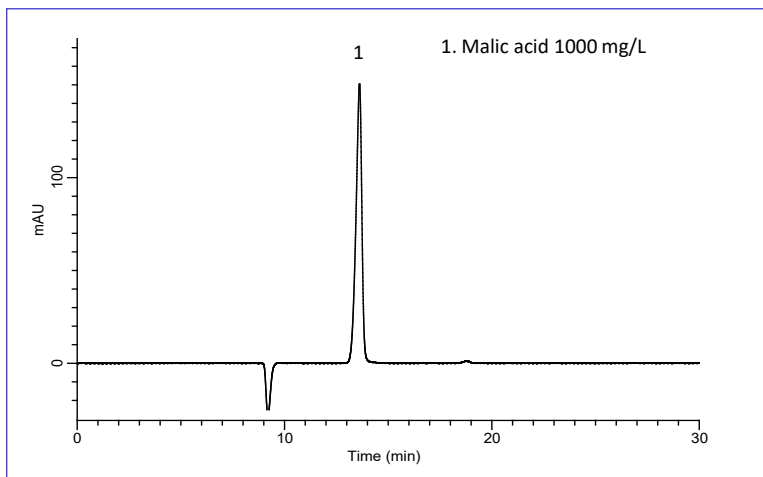


Analysis of malic acid by HPLC

Malic acid is one of the organic acids and is widely present in foods and living organisms. Analysis of the malic acid standard reagent confirmed the phenomenon that two peaks appeared depending on the detection method. This time, we analyzed malic acid using a diode array detector, and it was estimated that the impurity contained in malic acid was fumaric acid. (R. Takahashi)

Example. Analysis of standard (1)

(Post-column used BTB)

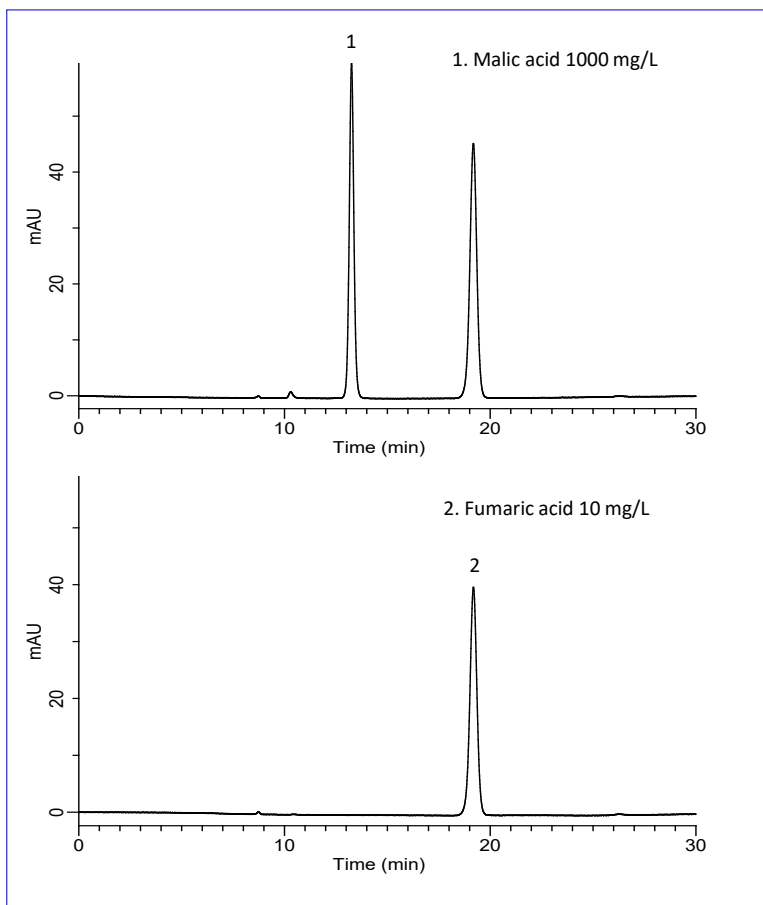


Conditions

Column : InertSphere FA-1
(9 mm, 300 mm × 7.8 mm I.D.)
Guard Column : InertSphere FA-1 Guard
(9 mm, 50 mm × 6.0 mm I.D.)
Eluent : 3 mM HClO₄ in H₂O
Reaction Sol. : 0.1 mM BTB + 30 mM Na₂HPO₄
Flow Rate : 0.5 mL/min
Column Temp. : 35 °C
Detection : VIS 440 nm
Injection. Vol. : 50 μL

Example: Analysis of standard (2)

(UV absorption)



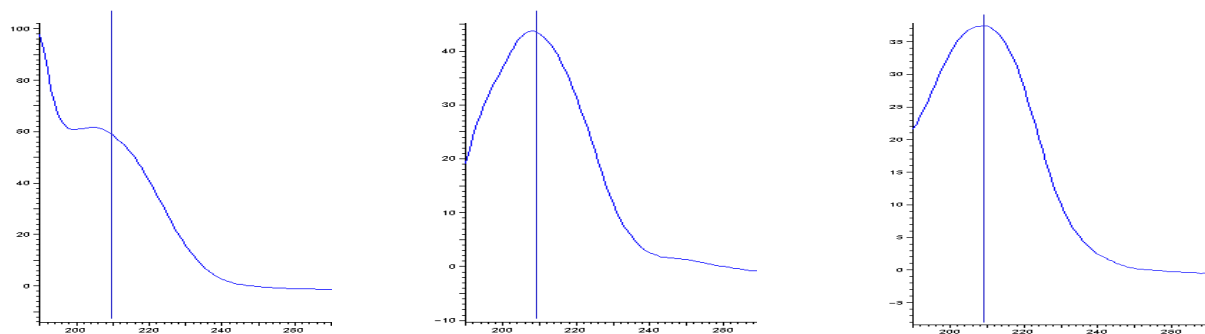
Conditions

Column : InertSphere FA-1
(9 mm, 300 mm × 7.8 mm I.D.)
Guard Column : InertSphere FA-1 Guard
(9 mm, 50 mm × 6.0 mm I.D.)
Eluent : 3mM HClO₄ in H₂O
Flow Rate : 0.5 mL/min
Column Temp. : 40 °C
Detection : UV 210 nm
Injection Vol. : 10 μL

A peak was observed at UV 280 nm at a position that was barely detected by the BTB post-column method.

[>>> Matches the retention time of fumaric acid](#)

UV spectrum of each peak

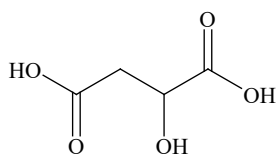


Malic acid

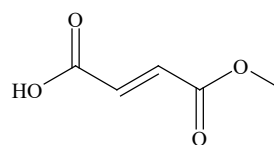
Malic acid impurities

Fumaric acid

Structural formula



Malic acid



Fumaric acid

Structures are created using Chemistry 4-D Draw which is provided by ChemInnovation Software, Inc.

Malic acid and fumaric acid are similar in structure, but fumaric acid has a conjugated double bond, so the UV absorption wavelength is larger than that of malic acid.

>>> A small amount of fumaric acid is hardly detected by the BTB post-column method, but it is detected as a large peak by the UV absorption method.

HPLC Column

●Analytical Column

- InertSphere FA-1 9 mm, 300 mm X 7.8 mm I.D.
Cat.No. 5020-11003

●Guard Column

- InertSphere FA-1 Guard 9 mm, 50 mm X 6.0 mm I.D.
Cat.No. 5020-10998

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