LT192

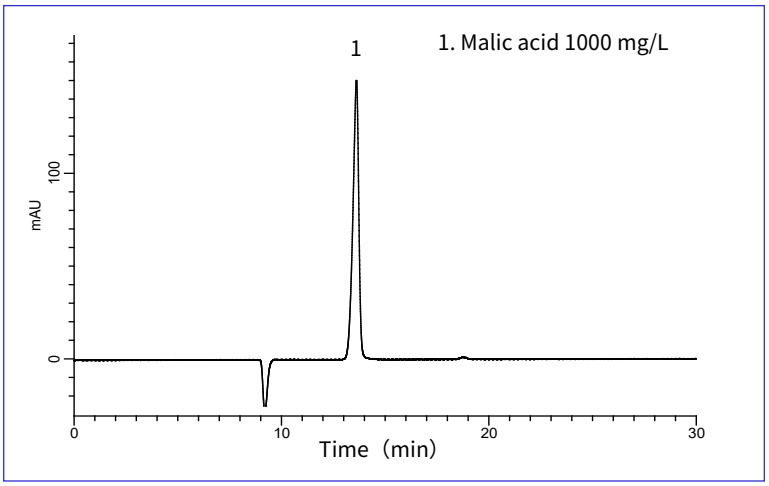
GL Sciences Inc.

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. Analysisof standard (1)

(Post-column used BTB)

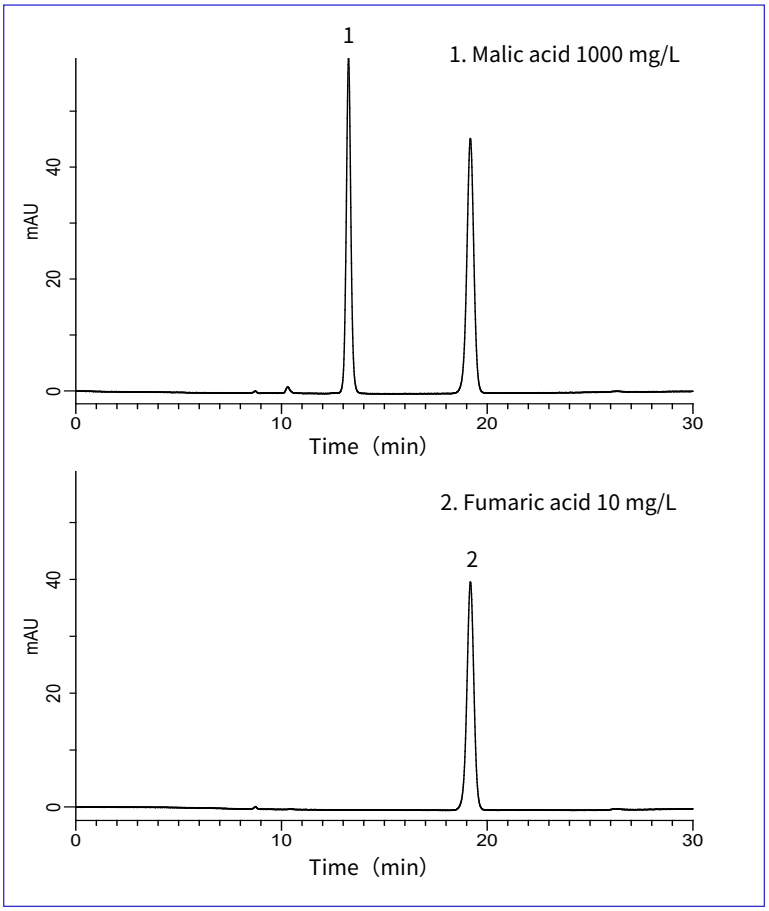


Conditions

Colum	:	InertSphere FA-1 (9 mm, 300 mm×7.8 mm I.D.)
Guard Column	:	InertSphere FA-1 Guard (9 mm, 50 mm × 6.0mm I.D.)
Eluent	:	3 mM $HClO_4$ in H_2O
Reaction Sol.	:	0.1 mM BTB + 30 mM Na_2HPO_4
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)



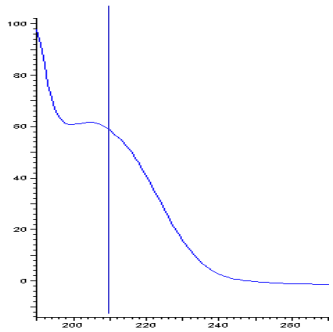
Conditions

Colum	:	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 $HClO_4$ in H_2O
Flow Rate	:	0.5 mL/min
Column Temp.	:	40 °C
Detection	:	UV 210 nm
Injection Vol.	:	10 µL

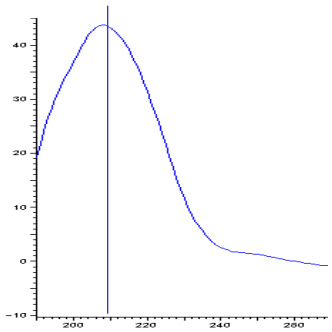
A peak was observed at UV 280 nm at a position that was barely detected by the BTB post-column method.

>>> Matches the retention time of fumaric acid

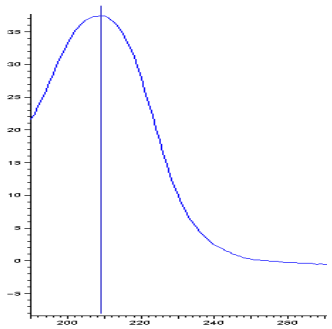
UV spectrum of each peak



Malic acid

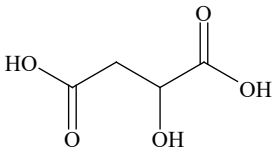


Malic acid impurities

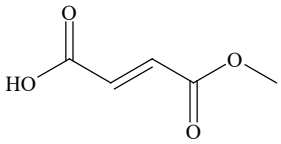


Fumaric acid

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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