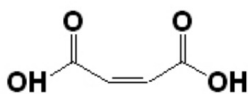
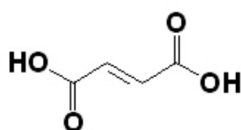


Isobaric Acids Maleic & Fumaric

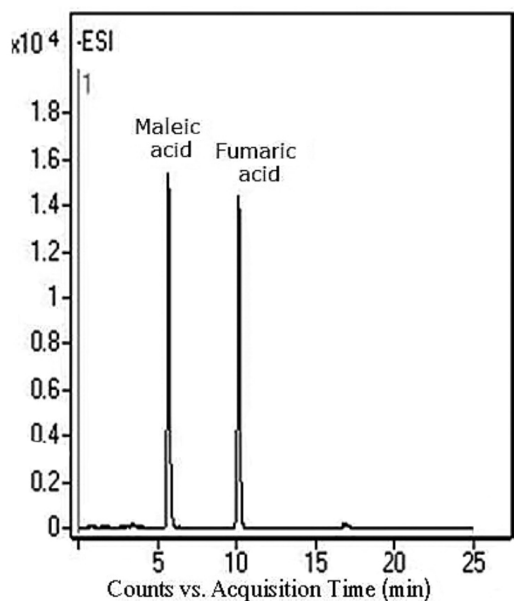
Retention, Separation & Detection



1. Maleic acid



2. Fumaric acid



Note: The gradient used for this application note was a simple linear gradient over 10 minutes. The exact conditions are not provided since they will have to be adjusted depending on individual instrument configuration. It is important to remember not to overload the column or saturate the detector (it is recommended to adjust the sample concentration so that injection is between 1 & 5 μ L).

Method Conditions

Column: Cogent Diamond Hydride™, 4 μ m, 100Å

Catalog No.: 70000-15P-2

Dimensions: 2.1 x150 mm

Solvents: A: 50% Methanol/ 50% DI H₂O/ 0.05% formic acid (strong solvent)

B: 90% Acetonitrile/ 10% H₂O w/10 mM ammonium acetate (weak solvent)

Gradient: Linear gradient over 10 minutes (adjustable per instrument)

Post Time: 5 min

Injection vol.: 1 μ L

Flow rate: 0.4 mL/min

Detection: ESI - neg - Agilent 6210 MSD TOF mass spectrometer

Samples: Sample stock solutions were made in acidified DI H₂O at a concentration of 0.2 mg/mL. Samples for analysis were made by diluting the stock 1:100 in 50:50 solution A and B

Peaks: 1. Maleic Acid, 115 m/z (M-H)⁻
2. Fumaric Acid, 115 m/z (M-H)⁻

Discussion

Two isobaric acids, maleic and fumaric (115 m/z) are separated very well when using the solvents above and a gradient method. Solvent A used in this application note contains 50% methanol which is recommended when biological samples are used. This method is very reproducible and is very fast to equilibrate between gradient runs with 0.08%RSD.

Maleic and fumaric are simple acids and the peak shape of these acids is not affected by the presence of sodium in the system as with more complex acids. When analyzing complex compounds, it is recommended that as much sodium be removed from the system as possible (i.e. replacing glass bottles with Teflon bottles) or the mobile phase should be prepared fresh daily.