

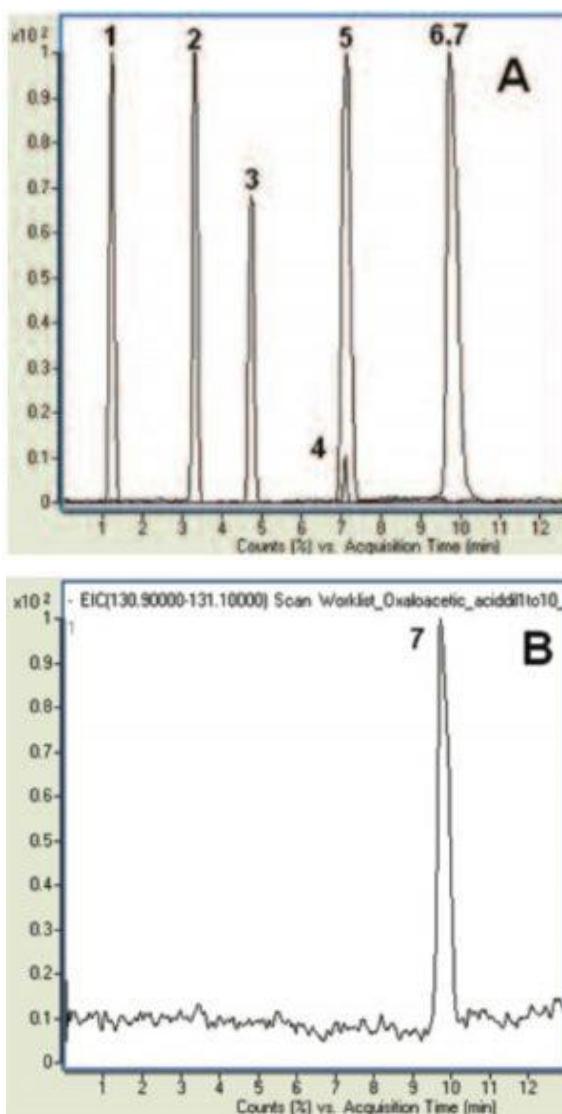


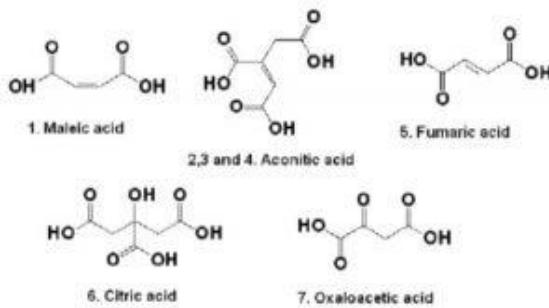
Organic Acids Analyzed with LCMS - AppNote

An Important Class of Compounds

Organic Acids, an important class of Metabolites, are very well Retained and Separated in this Method. *Figure A* shows the combined EICs for five small Organic Acids: Maleic, Aconitic, Fumaric, Citric and Oxaloacetic Acids. Two of these acids are isobaric compounds; Maleic and Fumaric Acids (115 m/z) and are separated very well. There are three Peaks at 173 m/z.

The Peaks were assigned to Trans (Peak 2) and Cis (Peak 3) isomers of the Aconitic Acid and an impurity (Peak 4). Citric and Oxaloacetic Acids were not resolved using the developed Mobile Phase and Gradient, but can be very easily assigned using MS detection. *Figure B* shows the EIC at 131 m/z for Oxaloacetic Acid for integration purpose.





Peaks:

1. Maleic Acid, 115 m/z (M-H)-
2. Trans Aconitic Acid, 173 m/z (M-H)-
3. Aconitic Acid, 173 m/z (M-H)-
4. Impurity 173 m/z (M-H)-
5. Fumaric Acid, 115 m/z ((M-H)-)
6. Citric Acid, 191 m/z (M-H)-
7. Oxaloacetic Acid, 131 m/z (M-H)-

Method Conditions

Column: Cogent Diamond Hydride™, 4µm, 100Å

Catalog No.: [70000-15P-2](#)

Dimensions: 2.1 x 150mm

Mobile Phase:

A: DI Water / 10mM Ammonium Formate

B: 90% Acetonitrile / 10% DI Water / 10mM Ammonium Formate

Gradient:

| Time (minutes) | %B |
|----------------|----|
| 0-3 | 90 |
| 3-6 | 70 |
| 6-7 | 70 |
| 7-7.1 | 30 |
| 7.1-8 | 90 |

Post Time: 5 minutes

Flow rate: 0.4mL / minute

Detection: ESI – neg – Agilent 6210 MSD TOF Mass Spectrometer

Sample Preparation: Sample Stock Solutions were made in acidified DI Water at a concentration of 0.2–0.3mg / mL. Samples for analysis were made by diluting the stock 1:100 in 50:50 Solution A and B.

Notes: Chromatogram presented is adapted from: J.J.Pesek, M.T. Matyska, S. M. Fisher, T. R. Sana, *Journal of Chromatography A*, 1204 (2008) p53.



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