MICROSOLV

Leucine & iso Leucine Separated with LCMS - AppNote

Leucine and Isoleucine, Isobaric Compounds Separated for LCMS Detection

This Application Note presents a Method for the Analysis of two Branch Chain Amino Acids (BCAA) with nearly identical m/z values.

Addition of weak Acetic Acid to the Mobile Phase allows the small difference in the pKas of the two Isobaric Compounds to achieve Resolution. A few microliters of Serum suffices as the Sample to estimate the Leucine and Isoleucine present. The two compounds are Separated and can be easily Quantified.

The Method is especially applicable to the frequent monitoring of Serum while treating patients with Maple-Syrup Disease, and may also be used for rapid diagnosis of the disease in suspected infants.
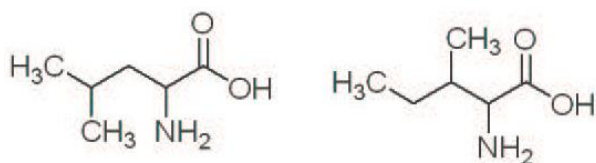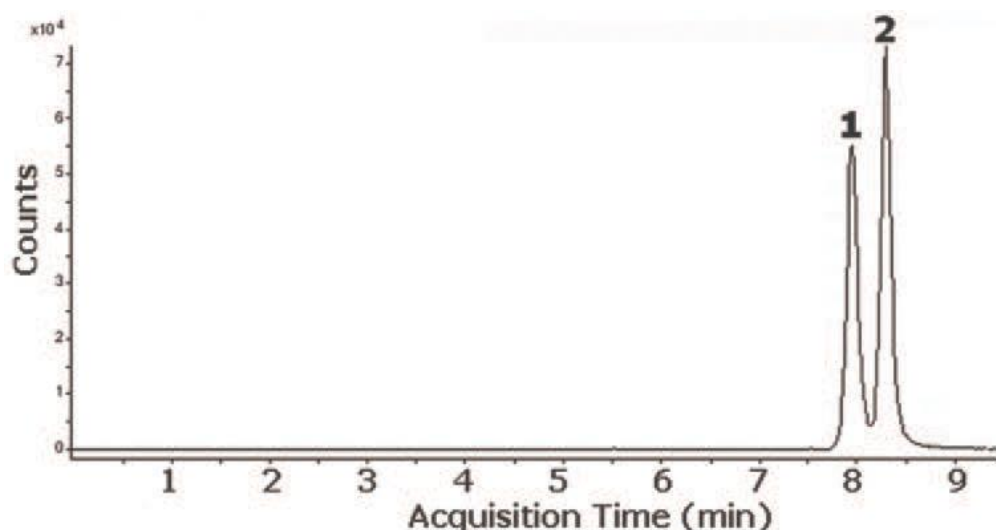
Peaks:

1. D,L - Leucine 132.1025 m/z (M+H)+
2. D,L - Isoleucine 132.1025 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 / 0.1% Acetic Acid

B: Acetonitrile / 0.1% Acetic Acid

Gradient:

Time *(minutes)*	%B
0-2	85
2-3	80
3-5	80
5-6	75
6-7	75
7-8	70
8-9	70
9-10	50
10-11	50
11-11.1	20
11.1-14	20

Post Time: 5 minutes
Flow rate: 0.4mL / minute
Detection: ESI – pos - Agilent 6210 MSD TOF Mass Spectrometer
Sample Preparation: 100ng / mL of each Compound prepared in 50:50 Solvent A / Solvent B and diluted 1:10.

Notes: D,L - Leucine and D,L - Isoleucine are considered essential Amino Acids because human beings cannot survive unless these Amino Acids are present in the diet.

[1] Chromatogram is adapted from: J.J.Pesek, M.T. Matyska, S. M. Fisher, T. R. Sana, Journal of Chromatography A, 1204 (2008) p50.

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Attachment

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