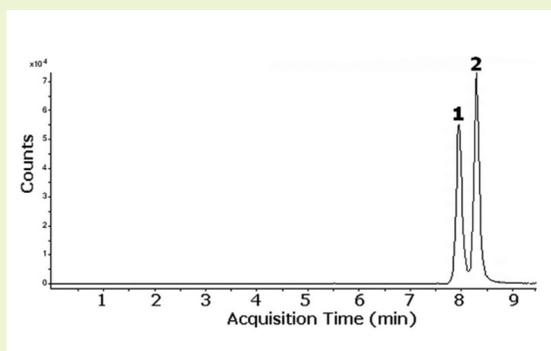
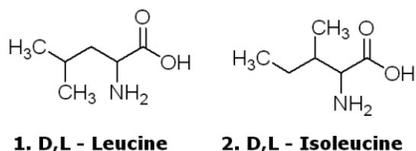


Branched-chain amino acids (BCAAs)

Leucine, Isoleucine



Method Conditions

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

Catalog No.: 70000-15P-2

Dimensions: 2.1 x 150 mm

Solvents: A: DI H₂O/ 0.1% acetic acid

B: Acetonitrile/ 0.1% acetic acid

| Gradient: | time (min.) | %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 min

Flow rate: 0.4 mL/min

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

Sample: 100 ng/mL of each prepared in 50%A/ 50%B and diluted 1:10 before analysis

Peaks: 1. D,L - Leucine 132.1025 m/z (M+H)⁺

2. D,L - Isoleucine 132.1025 m/z (M+H)⁺

Discussion

The chromatogram presented was done using a Cogent Diamond Hydride column and an ANP gradient. The addition of weak acetic acid to the mobile phase allows the small difference in the pK_as of the two compounds to be used to achieve the resolution for this pair of analytes which have identical m/z values. A few microliters of serum suffices to estimate the leucine and isoleucine present. The two branched-chain amino acids are separated and can be easily quantified. The method is especially applicable to the frequent monitoring of serum in treating patients with maple-syrup disease, and may also be used for rapid diagnosis of the disease in suspected infants.

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.

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