



Ultra-Fast Amino Acid Analysis by LC-TOF-MS

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The analysis of amino acids is important in fields ranging from clinical research to food analysis. Because most separation and detection techniques involve lengthy separations and/or lengthy derivatizations, time-of-flight-mass spectrometry (TOF-MS) is the ideal tool for the fast separation and identification of components in complex mixtures. This is due to its fast data-acquisition rate, mass accuracy, wide-dynamic range, and high full-mass-range sensitivity. LECO's Unique® LC-TOFMS was used in the ultra-fast separation and identification of creatine, hydroxyproline, and 17 amino acids as described below.

Experimental Conditions

Instrumentation

Unique® LC-TOFMS with High Flow ESI Source (LECO Corporation):
 Nozzle voltage: 65 V
 Desolvation temperature: 250 °C
 Electrospray voltage: 3000 V
 Desolvation flow (N₂): 7.5 L/min
 Interface temperature: 100 °C
 Nebulization pressure (N₂): 425 kPa
 Split flow: 0.2 mL/min to source; 1.8 mL/min to waste
 Mass calibrants (external): tetramethylammonium chloride and caffeine

1100 Series LC (Agilent Technologies, Wilmington, Delaware):

Flow rate: 2.0 ml/min

Mobile phase A: 1 mM aqueous perfluoroheptanoic acid

Mobile phase B: acetonitrile

Gradient: 100% A to 80% A in 30 s to 60% A in 2 min; 100% A at 2:05 min

Injection volume: 1 µL

Column: Chromolith Performance RP-18e 100 × 4.6 mm (Merck KGaA, Darmstadt, Germany)

Test Solution

Amino acid standard 0.05 mmol/ml (Perce H); hydroxyproline and creatine 0.01 µmol/ml

Results and Conclusions

Separation of the 19 amino acids and related compounds was achieved in less than 3 min using a monolithic column, run at 2 mL/min, with the aid of the ion-pairing reagent, perfluoroheptanoic acid (Figure 1). The Unique LC-TOFMS was used to identify

all components based on accurate-mass measurements and automated-formula calculations were performed using ChromaTOF® software. In most cases, only a single-matching formula was calculated for each peak as a result of the good mass accuracy achieved. The Unique's high-acquisition speeds provide the required-data density for analysis of narrow chromatographic peaks resulting from fast-separation techniques. Accurate mass measurements provide valuable information for compound identification, making the Unique an ideal solution for the fast analysis of small molecules.

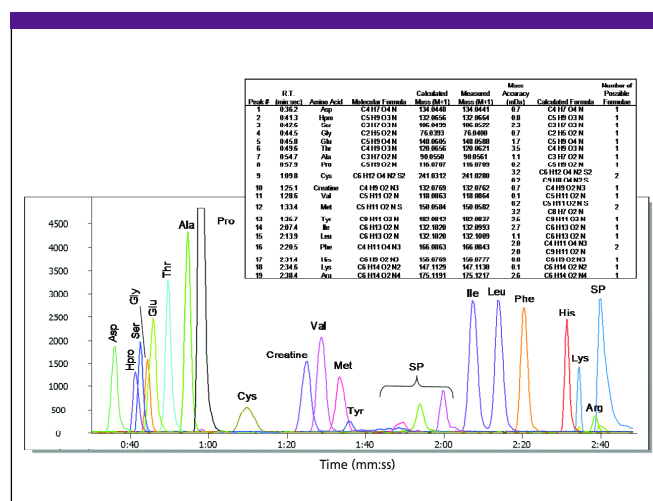


Figure 1: Extracted-ion chromatograms and accurate mass/molecular formula determinations of 17 amino acids, creatine, and hydroxyproline, separated in under 3 min using an ion-pair reagent and a monolithic column. Common isobaric interferences, hydroxyproline and creatine, are easily separated and identified from leucine and isoleucine. Molecular formulas were generated using ChromaTOF software based on accurate mass assignments. Elements C, H, N, O and S were chosen for formula calculation with a threshold of 5-mDa mass accuracy. SP = system peaks

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