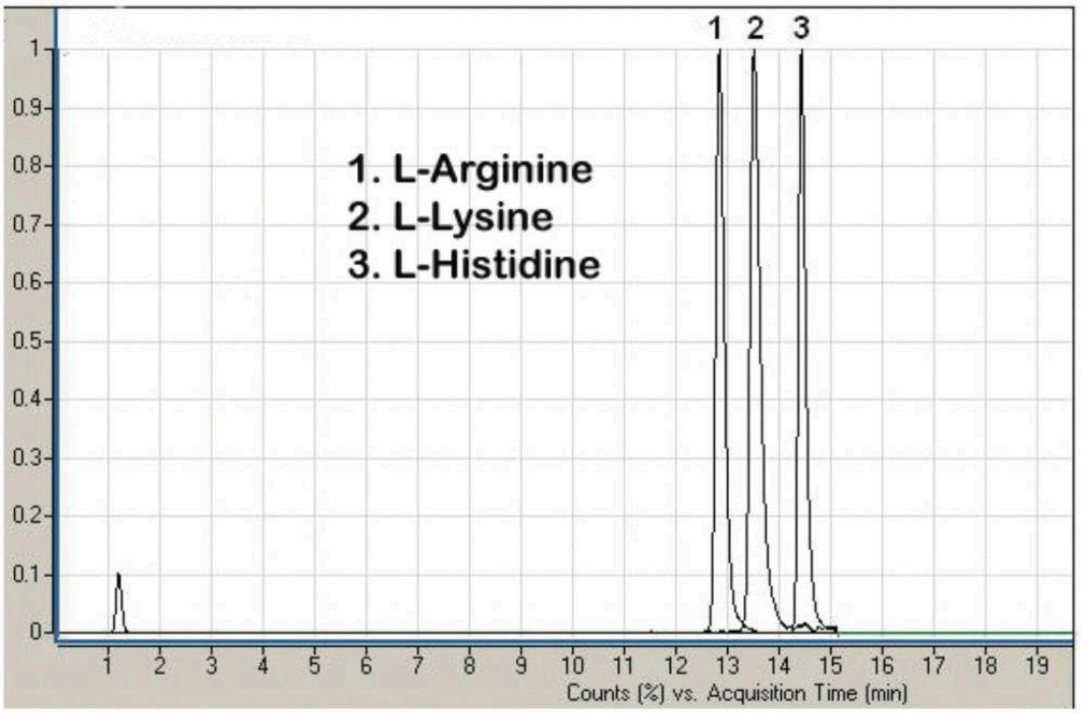
Arginine, Lysine and Histidine Analyzed with LCMS - AppNote

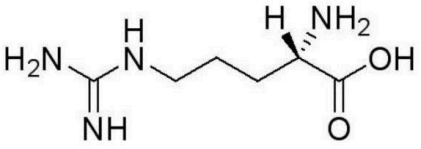
Basic Amino Acids In Synthetic or Human Urine Can be Analyzed

A “Cleanup” procedure for the isolation of the Basic Amino Acids in Urine was developed in this Method and no derivatization procedure was used. Three Basic Amino Acids were Separated using an inverse gradient or Aqueous Normal Phase (ANP) Chromatography.

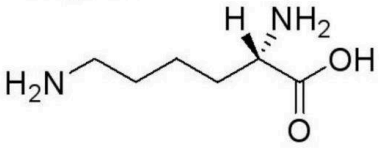
The advantages of this Method are: (1) Isolation and stable recovery (>95%) of the desired Basic Amino Acids, (2) Sensitivity of detection (low pico mole range), (3) Complete resolution of non-derivatized Amino Acids and (4) Low amount of Sample required for Analysis.

The “cleanup” procedure used proved additionally advantageous by eliminating the use of C-18 Solid Phase Extraction Columns normally required by techniques described in the Literature.

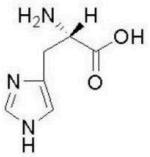




L - Arginine



L - Lysine



L - Histidine

Peaks:

1. L – Arginine *175 m/z RT = 12.83 minutes*
2. L – Lysine *147 m/z RT = 13.49 minutes*
3. L - Histidine *156 m/z 14.42 minutes*

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% Formic Acid
B: 95% Acetonitrile / 0.1% Formic Acid / 0.005% Trifluoroacetic Acid *(TFA)*
Gradient:

Time *(minutes)*	%B
0	100
5	100
6	95
7	95

9	85
10	85
12	70
12.1	100

Post Time: 5 minutes
Flow rate: 0.4mL / minute
Detection: ESI – pos - Agilent 6210 MSD TOF Mass Spectrometer
Sample Preparation: 400µL of Acetonitrile was added to 100µL of synthetic or human urine and the Sample was centrifuged (3000g). Next 20µL of the supernatant was mixed with 10µL of the 50:50 Acetonitrile / DI Water / 0.1% Formic Acid.

Notes: *The level of amino acids in biological fluids can be correlated with several neurological (Alzheimer's Disease, Ischemic Stroke and others) and Metabolic disorders (Argininemia, Phenyloketonuria, Maple Syrup Urine Disease and others).*

COGENT HPLC Columns™

Attachment

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