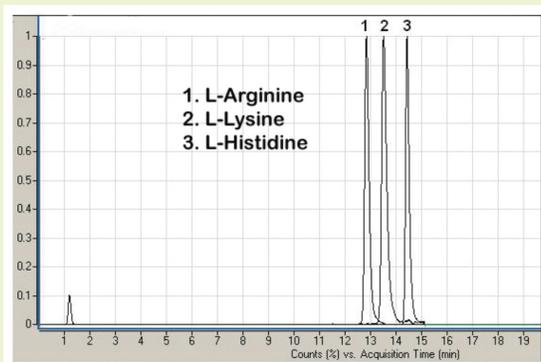
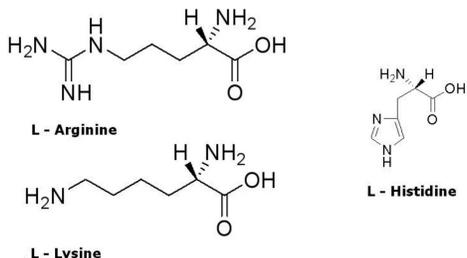


Basic Amino Acids

In Synthetic or Human Urine



Notes: The “cleanup” procedure used proved additionally advantageous by eliminating the use of C-18 solid phase extraction columns required by techniques described in the literature. 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, phenylketonuria, maple syrup urine disease and others).

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% formic acid

B: 95% acetonitrile/ 0.1% formic acid/ 0.005% TFA

Gradient:	time (min.)	%B
	0	100
	5	100
	6	95
	7	95
	9	85
	10	85
	12	70
	12.1	100

Post Time: 5 min

Flow rate: 0.4 mL/min

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

Sample: 400µL of acetonitrile was added to 100µL of synthetic or human urine and the sample was centrifuged (3000 g). Next 20µL of the supernatant was mixed with 10µL of the 50% acetonitrile/ 50% DI H₂O/ 0.1% formic acid

Peaks: 1. L - Arginine 175 m/z RT = 12.83 min
 2. L - Lysine 147 m/z RT = 13.49 min
 3. L - Histidine 156 m/z 14.42 min

Discussion

A “cleanup” procedure for the isolation of the basic amino acids was used. No derivatization procedure was used. Three basic amino acids were separated using gradient 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 pmol range), (3) complete resolution of non-derivatized amino acids via ANP LCMS and (4) limited amount of sample required for analysis.