HPLC Application

ID No.: **1312**

Amino Acids

Products used in this application:

LUNA®

Column: Luna® 5 µm C18(2) 100 Å, LC Column 250 x 4.6 mm, Ea

Dimensions: 250 x 4.6 mm ID

Order No: 00G-4252-E0

Elution Type: Gradient

Eluent A: Water

Eluent B: Acetonitrile

Gradient Profile:

Step No.	Time (min)	Pct A	Pct B
1	0	100	0
1	20	20	80

Flow Rate: 1 mL/min

Col. Temp.: ambient

Detection: UV-Vis Abs.-Variable Wave.(UV) @ 225 nm (ambient)

Analyst Note: Description of Sample Prep and HPLC:

Do not use pipette tips or microcentrifuge tubes that have been autoclaved. The water that may collect from the steam and then dry may contaminate the samples.

Collection:

- Collect sample (3.5-4 ml unheparizied hematocrit tubes of peripheral blood or minimum of 100 mL culture media)
- Centrifuge @ 3000 rpm, 4°C, 10 min
- Transfer 75mL* of serum or culture media to a new microcentrifuge tube
  - Sample can now be stored @ -20 °C until ready to perform extraction

Extraction:

- Add 1.4 mL (2 x 0.7 mL) HPLC-grade methanol, vortex
- Spin @ 15,800 g, 0 °C, 15 min
- Transfer 1.2mL (2 x 0.6 mL) of supernatant to a new microcentrifuge tube
- Dry completely in Speedvac on low heat (2-3 hr)
- Store @ -20 °C until ready to run HPLC or proceed directly to next step

HPLC:

- Warm samples to room temp; resuspend dry extract in 100 mL distilled-deionized water (ddH2O), let sit at RT for about 30 min then vortex
- Transfer 95 mL to HPLC sample vials (we use inserts inside vials); no bubbles
- HPLC
  - run on a Luna C18(2) column (Phenomenex, 250mm x 4.6 mm)
  - 20mL injection volume with a linear water:acetonitrile gradient from 100:0 to 20:80 over 20 min; 1 mL/min
  - absorbance is read at 225 nm and integrated peaks are calculated by computer
  - Beckman System Gold 166 detector

Standards: 75mL* of each triple standard is extracted as above. Each standard is run in duplicate with the average AUC used to construct a standard curve. Use distilled-deionized water (ddH2O) to make up standards.

- Prepare standard stock solutions of kynuerinine, L-tryptophan and 1-methyl-DL-tryptophan (each = 1mg/ml). Protect from light and store @ -70 °C.
- Triple standard is a mix of 50ml each of kynuerinine, L-tryptophan and 1-methyl-DL-tryptophan. When brought to a volume of 1ml, the concentration of each = 50 mg/mL - this dilution is used as the most concentrated standard
- From the above dilution, make 4 additional serial dilutions (25, 12.5, 6.25 and 1.25 mg/mL)

*A 150 mL sample will give a much more readable result if you suspect low amounts of kynuerinine.

Directions for dissolving 1-methyl-DL-tryptophan (1 mg/mlL ~4.6mM)

total volume	100mL	
1-MeTrp		100mg
1N NaOH	1mL	
distilled H2O	98ml	
HCl		~1mL

Weigh 1-MeTrp and place in bottle. Add 1N NaOH and mix until majority of powder is dissolved. Add water and stir for ~ 1hr. Titrate to pH=7 using 1N HCl. Aliquot and wrap in foil and store @ -70°C.

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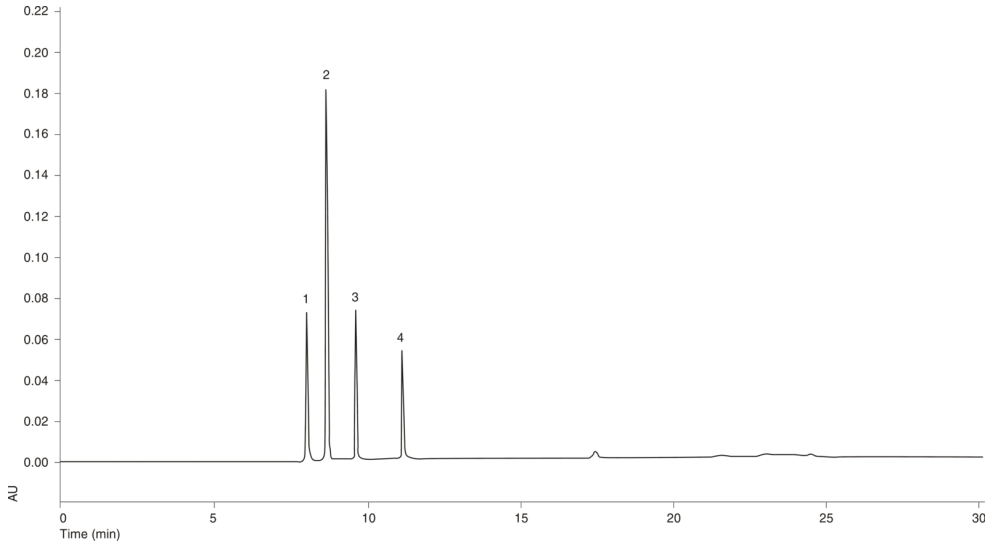

ANALYTES:

1 5-Hydroxytryptophan

2 Kynurenine

3 Tryptophan

4 N-Methyltryptophan