HPLC Application

ID No.: **20684**

PLP and PA in Human sera/plasma on Gemini-NX 3u, C18, 100x4.6 by HPLC w Fluorescence Detection

Column: Gemini® 3 µm NX-C18 110 Å, LC Column 100 x 4.6 mm, Ea
Dimensions: 100 x 4.6 mm ID
Order No: 00D-4453-E0
Elution Type: Gradient
Eluent A: 20mmol/L Na2HPO4 + 1mL acetic acid in 1L DI Water
Eluent B: ACN : MeOH (70:30)

Gradient Profile:

Step No.	Time (min)	Pct A	Pct B
1	0	95	5
2	5	40	60
3	5.1	5	95
4	6	95	5
5	7	95	5

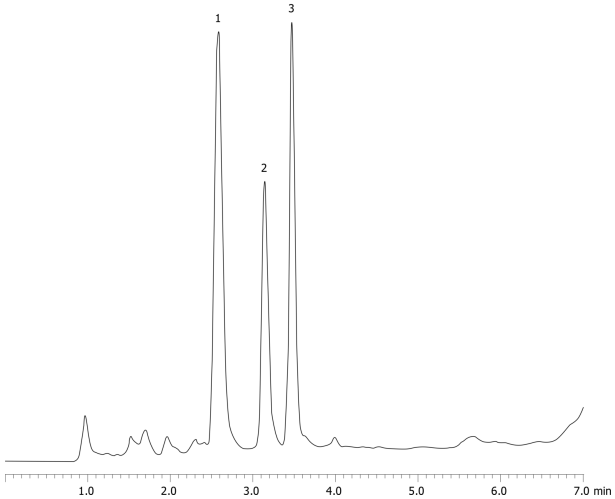
Flow Rate: 1 mL/min
Col. Temp.: 35 °C
Detection: Fluorescence (FLUOR) @ Ex 360 nm (ambient)
Detector Info: Shimadzu RF-20A

Products used in this application:

Analyst Note: SAMPLE PREPARATION

1. Thaw patient plasma samples and sera/plasma spiked calibrators or pre-manufactured calibration standards and controls at ambient temperature. Protect from light.
2. Pipette 400 µL of the blank (Water), calibration standards, controls and plasma specimens into the appropriate labeled 1.5 mL Eppendorf microcentrifuge tubes.
i. Briefly vortex the calibrators and controls immediately prior to sampling.
ii. Mix the plasma samples by gentle inversion immediately prior to sampling.
iii. Protect the tubes from light
3. Add 30 µL of 250 mg/mL semicarbazide/glycine solution in rapid succession into all the tubes containing samples; cap the tubes, vortex for 15 seconds.
4. Incubate in the dark at room temperature for 30 minutes.
5. Uncap the tubes; add 50 µL of 20 % meta-Phosphoric acid to the controls and patient samples.
6. Recap the tubes and vortex for 30 seconds at room temperature.
7. Centrifuge for 5 minutes at 14,000 rpm. Note: The relative centrifugal force (RCF)=16,000 g.
8. Transfer 350 µL of supernatant to a 2 mL amber vial.
9. Cover the vial with lid and place in the autosampler (RT).
10. Inject 30 µL

20684



For more information contact your Phenomenex Representative at support@phenomenex.com

Phenomenex products are available worldwide.

PLP and PA in Human sera/plasma on Gemini-NX 3u, C18, 100x4.6 by HPLC w Fluorescence Detection

ANALYTES:

1 Pyridoxal 5'-phosphate

2 4-Pyridoxic acid

3 Pyridoxal