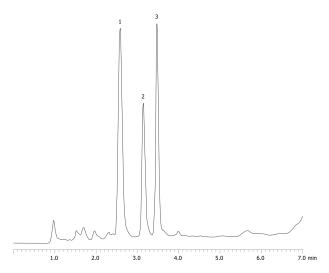
## HPLC Application

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

PLP and PA in human seta/ plasma on Gemmi-AX 50, C16, 100X4.0 by hPLC w Platfescence Detection					
Column: Dimensions: Order No: Elution Type: Eluent A: Eluent B:	Gemini® 3 μm NX-C18 110 Å, LC Column 100 x 4.6 mm, Ea 100 x 4.6 mm ID 00D-4453-E0 Gradient 20mmol/L Na2HPO4 + 1mL acetic acid in 1L DI Water ACN : MeOH (70:30)				Gemini
Gradient	Step No.	Time (min)	Pct A	Pct B	
Profile:	1	0	95	5	
	2	5	40	60	Products used in this application:
	3	5.1	5	95	Products used in this application:
	4	6	95	5	
	5	7	95	5	Gemini
Flow Rate:	1 mL/min				
Col. Temp.:	35 °C				
Detection:	Fluorescence (FLUOR) @ Ex 360 nm (ambient)				
Detector Info:	Shimadzu RF-20A				
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. Plette 400 µL of the blank (Water), calibration standards, controls and plasma specimens into the appropriate labeled 1.5 mL Eppendorf microcentrifuge tubes. 1. 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 $\mu$ L of supernatant to a 2 mL amber vial.				
	9. Cover the vial with lid and place in the autosampler (RT).				
	10 Inject 20 ul				

10. Inject 30 µL

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## ANALYTES:

- Pyridoxal 5'-phosphate 1
- 2 4-Pyridoxic acid
- Pyridoxal 3

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