

HPLC Application

ID No.: 20881

TMP and Thiamine Standard 5 nmol/L by Impact PPT on Kinetex 5u C18 100x4.6mm

Column: Kinetex® 5µm C18 100 Å, LC Column 100 x 4.6 mm, Ea

Dimensions: 100 x 4.6 mm ID

Order No: 00D-4601-E0

Elution Type: Gradient

Eluent A: 25mM Na₂HPO₄, 10% Methanol, pH 7.0

Eluent B: 25mM Na₂HPO₄, 70% Methanol, pH 7.0

Gradient Profile:	Step No.	Time (min)	Pct A	Pct B
	1	0	97	3
	2	0.25	75	25
	3	0.75	75	25
	4	3	65	35
	5	4	40	60
	6	4.5	97	3
	7	5	97	3

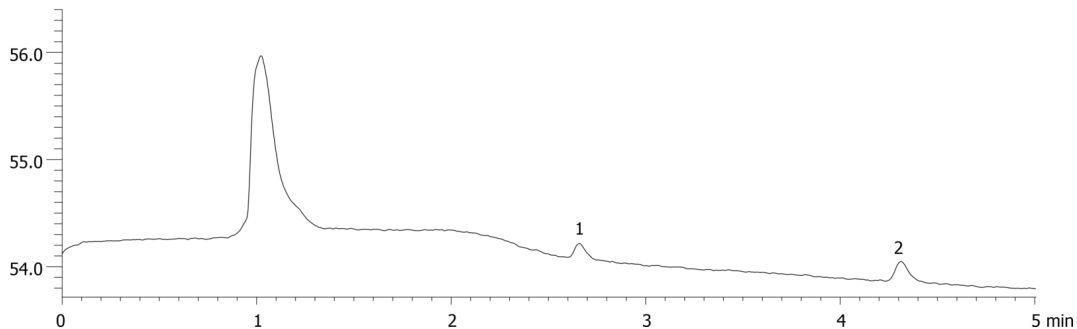
Flow Rate: 1 mL/min

Col. Temp.: 25 °C

Detection: Fluorescence (FLUOR) @ Ex. 375 nm (ambient)

Analyst Note: Using Impact Protein Precipitation Plate (Cat Log #CE0-7565) for sample preparation

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Products used in this application:



ANALYTES:

1 Thiamine monophosphate

Retention Time: 2.658 min

2 Thiamine

Retention Time: 4.312 min



Sample Preparation Details

for HPLC Application ID No.: 20881

TMP and Thiamine Standard 5 nmol/L by Impact PPT on Kinetex 5u C18 100x4.6mm

PRODUCT DESCRIPTION:

Impact™ Protein Precipitation, 2mL Square Well Filter Plate, 2/Pk

Order No.: CE0-7565

SOLID PHASE EXTRACTION (SPE) PROCEDURE:

Note: The solvent volumes shown below are for a Proprietary bed mass.

The solvent volumes will need to be adjusted for a smaller or larger bed mass.

Condition:

Load:

Wash:

Dry:

Elute:

Final Prep and Analysis:

Add 400µL of methanol to a well of a Protein Precipitation Plate; Add 100µL plasma to the well. Mix 3 times with pipette tip (or vortex the whole plate briefly); Wait for 5 minutes; Filter through

Inject: 20 µL on HPLC Fluorescence (FLUOR) @ Ex. 375 nm (ambient)

ANALYTES:	Spiked Conc. (ng/mL)	Log P	pKa	% Rec	%RSC (n=0)
1 Thiamine monophosphate	5				
2 Thiamine	5				

Note: This method is designed as a convenient starting point for further investigation and can be tailored to meet your extraction goals. Call your local Phenomenex Representative for assistance in method development and optimization techniques.

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



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