

## TMP and Thiamine Standard 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 Na<sub>2</sub>HPO<sub>4</sub>, 10% Methanol, pH 7.0

**Eluent B:** 25mM Na<sub>2</sub>HPO<sub>4</sub>, 70% Methanol, pH 7.0

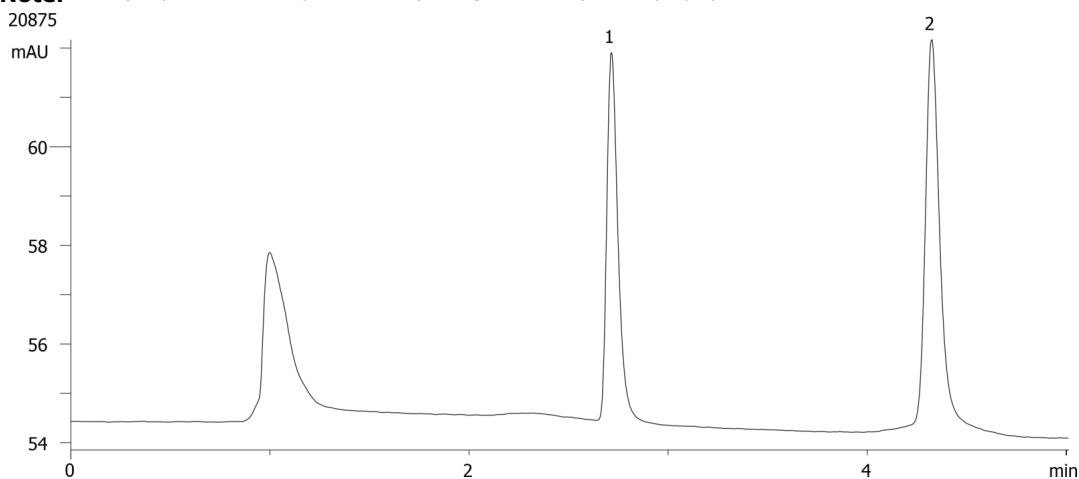
Gradient Profile:	Step No.	Time (min)	Pct A	Pct B
	1	0	97	3
	2	0.25	75	25
	3	0.75	75	25
	4	3	65	35
	5	4	40	60
	6	4.5	97	3
	7	5	97	3

**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



### ANALYTES:

1 Thiamine monophosphate

Retention Time: 2.715 min

2 Thiamine

Retention Time: 4.323 min



Products used in this application:



# Sample Preparation Details

for HPLC Application ID No.: 20875

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

### PRODUCT DESCRIPTION:

Impact<sup>™</sup> 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:

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#### Load:

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#### Wash:

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#### Dry:

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#### Elute:

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### Final Prep and Analysis:

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

Inject: 20  $\mu$ L on HPLC Fluorescence (FLUOR) @ Ex. 375 nm (ambient)

ANALYTES:	Spiked Conc. (ng/mL)	Log P	pKa	% Rec	%RSC (n=0)
1 Thiamine monophosphate	200				
2 Thiamine	200				

**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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