HPLC Application ID No.: **21878**



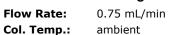
Human Breast Milk TMP & Thiamine by Gemini-NX 3u C18 100x3mm with Impact Plate_1

Gemini® 3 µm NX-C18 110 Å, LC Column 100 x 3 mm, Ea

100 x 3 mm ID **Dimensions:** Order No: 00D-4453-Y0 Elution Type: Gradient

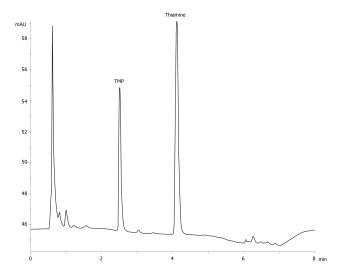
25nM Na2HPO4, 10% methanol Eluent A: Eluent B: 25mM Na2HPO4, 70% methanol

Gradient	Step No.	Time (min)	Pct A	Pct B
Profile:	1	0	3	97
	2	0.25	25	75
	3	0.75	25	75
	4	3	35	65
	5	4	100	0
	6	5	100	0
	7	5.1	3	97
	8	8	3	97



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

Detector Info: Shimadzu ELSD



ANALYTES:

TMP

Retention Time: 2.517 min

Thiamine

Retention Time: 4.128 min



Products used in this application:



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Sample Preparation Details

for HPLC Application ID No.: 21878



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PRODUCT DESCRIPTION:

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

Order No.: CE0-7565

SOLID PHASE EXTRACTION (SPE) PRODCEDURE:

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

ANALYTES:	Spiked Conc.	Log P	рКа	% Rec	%RSC
	(ng/mL)				(n=0)
1 TMP	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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