# HPLC Application ID No.: 21879

## Human Breast Milk TMP & Thiamine by Gemini-NX 3u C18 100x3mm with Impact Plate\_5

| Column:<br>Dimensions:<br>Order No:<br>Elution Type:<br>Eluent A:<br>Eluent B: | Gemini® 3 µm NX-C18 110 Å, LC Column 100 x 3 mm, Ea<br>100 x 3 mm ID<br>00D-4453-Y0<br>Gradient<br>25nM Na2HPO4, 10% methanol<br>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    |  |  |  |
| Flow Rate:   | 0.75 mL/min   |                 |               |       |  |  |  |
| Col. Temp.:  | ambient   |                 |               |       |  |  |  |
| Detection:   | Fluorescence  | (FLUOR) @ Ex 32 | 75 nm (ambier | it)   |  |  |  |
| <b>Detector Info:</b>  | Shimadzu ELSD   |                 |               |       |  |  |  |
| 21879  |   |                 |               |       |  |  |  |
| mAU  |   |                 |               |       |  |  |  |
| 58 —   |   |                 |               |       |  |  |  |
| -  |   |                 |               |       |  |  |  |
| 58 -   |   |                 |               |       |  |  |  |
| -  |   |                 |               |       |  |  |  |
| 56 -   |   |                 |               |       |  |  |  |
| 54 -   |   |                 |               |       |  |  |  |
| -  |   |                 |               |       |  |  |  |
| 52 -   |   |                 |               |       |  |  |  |
| -  |   |                 |               |       |  |  |  |
| 50 -   | тм  | Thiamine        |               |       |  |  |  |
| -  |   |                 |               |       |  |  |  |
| 48 -   |   |                 |               |       |  |  |  |
| 46 -   |   |                 |               |       |  |  |  |
| -  |   |                 |               |       |  |  |  |
| 0  | 2   | 4               | 6             | 8 min |  |  |  |
|  | -   |                 |               |       |  |  |  |



**ANALYTES:** 

Retention Time: 2.517 min

#### 2 Thiamine

Retention Time: 4.128 min

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Gemini

Products used in this application:



for HPLC Application ID No.: 21879



### Human Breast Milk TMP & Thiamine by Gemini-NX 3u C18 100x3mm with Impact Plate\_5

#### **PRODUCT DESCRIPTION:**

Impact<sup>™</sup> 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) |
| <b>1</b> 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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