HPLC Separation of Thiamine Pyrophosphate and Pyridoxal Phosphate

**Column:** Primesep SB  
**Part number:** SB-46.050.0510  
**Column size:** 4.6 x 50 mm, 5 µm, 100A  
**Mobile phase:**  
- A: 5% ACN with 0.05% formic acid  
- B: 20% ACN with 0.8% formic acid  
**Gradient:** From 100% A to 100% B in 3 min, 3 min hold  
**Flow rate:** 1 ml/min  
**Detection:** 290 nm

### Application Comments

It has been discovered that the level of thiamine pyrophosphate and pyridoxal phosphate in blood is linked to Alzheimer’s Disease [1, 2]. LC/MS analysis of these two compounds in blood is a challenging task, due to the complex sample preparation as well as a lack of retention on traditional C18 columns. Methods with ion-pairing reagents are not compatible with mass-spectrometry detection.

SIELC has developed a LC/MS method with good retention and separation for these two compounds using SIELC’s Primesep SB reversed-phase anion-exchange column paired with a simple mobile-phase gradient. Other polar acidic compounds in bio fluids can now also be analyzed by this approach.


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