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Application Note

Enhancing the LC-MS/MS Analysis of B-group Vitamins with MaxPeak High Performance Surface Technology

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Strong interactions between certain analytes and metal surfaces in a liquid chromatography flow path may result in poor chromatographic peak shapes, severe analyte losses, inconsistent peak responses, and other issues in LC, which lead to inaccurate results. To reduce the impact of these interactions, workarounds or additional steps in the methods are often required, which adds complexity to the methods and reduces laboratory productivity. To address these issues, Waters has developed a family of technologies, MaxPeak High Performance Surfaces (HPS), which provide an effective solution to mitigate analyte interactions with metal surfaces. This application brief highlights the improvements seen when applying HPS technologies to the analysis of B-group vitamins by liquid chromatography, coupled with tandem quadrupole mass spectrometry (LC-MS/MS). The ACQUITY PREMIER System and ACQUITY PREMIER BEH C_{18} Column showed performance enhancements when compared to a conventional system and column. Better peak shapes, increased response, higher sensitivity, and no analyte carry-over were observed using the HPS technologies. The extent of the improvements for B vitamins varies from compound to compound. Dramatic improvements in response were found for flavin mononucleotide, thiamine, thiamine pyrophosphate, pyridoxal 5′-phosphate, and pantothenic acid. No negative impact was observed for any B-group vitamin.

Benefits

- MaxPeak HPS Technology greatly improves the LC-MS analysis of B vitamins
- Higher response, better peak shapes, and no carry-over were achieved using the ACQUITY PREMIER Solution
- Demonstrated higher sensitivity, higher accuracy and precision, and enhanced robustness for the analysis of B vitamins

Introduction

The inner surface in the flow path in liquid chromatography equipment and columns has a relatively small surface area as compared to the surface area of the packing material in the chromatography column. Despite the small surface area, the inner surface in the flow path should not be overlooked as a potential source of unwanted interactions with target analytes. Strong interactions between the metallic surfaces and analytes can lead to surface adsorption of analytes, which may result in poor peak shape, reduced or no peak response, and inaccurate results.[1,2] There are workarounds to address these issues, such as replacing the stainless-steel material with other materials, using additives in the mobile phases to disturb the interactions, or coating the surface with strong adsorbents prior to the analysis. These workarounds have their limitations[3] and may negatively impact the chromatographic performance and analytical productivity.

Waters has developed a family of technologies, MaxPeak High Performance Surfaces (HPS), which provide an effective solution to mitigate analyte interactions with metal surfaces. The MaxPeak HPS are comprised of a highly crosslinked layer containing ethylene-bridged siloxane material that is similar to that of BEH particles.[3] Dramatic improvements have been observed in LC analyses for organic acids, organophosphates, oligonucleotides, peptide, glycans, and phospholipids.[4–7]

B vitamins are water-soluble vitamins that are essential for normal human metabolic and physiological functions. The routine testing of B vitamins is an important procedure for food, beverage, and nutraceutical quality control, as well as for nutritional research. This application brief highlights the key benefits of the MaxPeak HPS Technology in the LC-MS/MS analysis of B vitamins. The structures of the B vitamins and their bioactive vitamers are shown in Figure 1.

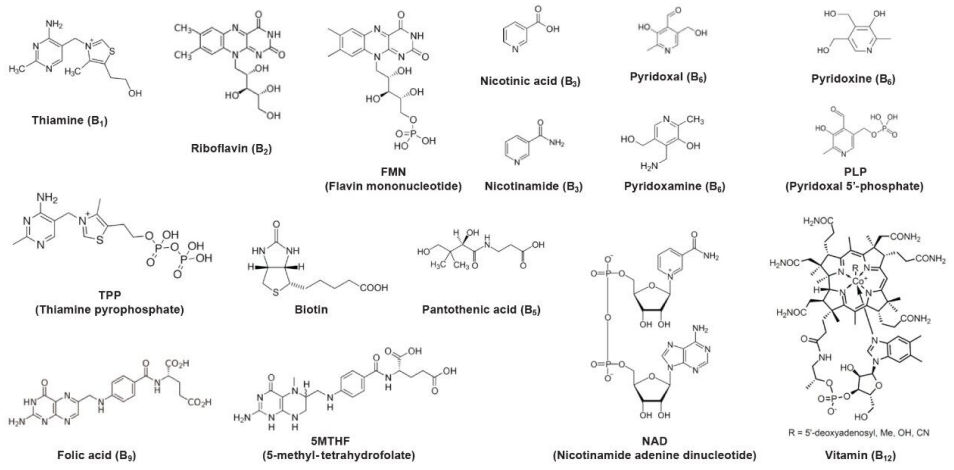

Figure 1. Structures of B vitamins and their vitamers.

Results and Discussion

The LC conditions in the AOAC Official Method 2015.14 for simultaneous determination of total vitamins B_1, B_2, B_3, and B_6 in infant formula and related nutritionals[8] were used in this study. The MRM transitions and MS detection parameters for 18 vitamins, including the additional vitamins B_5, B_9, B_{12}, and biotin were optimized for the Xevo TQ-S micro MS System. Two LC system setups were used for comparison, one comprised of an ACQUITY PREMIER System and an ACQUITY PREMIER BEH C_{18} (1.7 µm, 2.1 x 100 mm) Column (referred to as the HPS setup), and the other one comprised of an ACQUITY UPLC H-Class PLUS System and an ACQUITY UPLC BEH C_{18} (1.7 µm, 2.1 x 100 mm) Column (referred to as the SOP setup). The MaxPeak HPS has been implemented in the HPS setup while the conventional stainless-steel parts were used in the SOP setup. These two systems were coupled to the same Xevo TQ-S micro MS System sequentially to minimize instrumental variables in the comparison study.

Increased Response

Higher peak intensities and larger peak areas were observed for majority of the 18 vitamins using the MaxPeak HPS Technology. Figure 2 shows comparison plots of the vitamin peak areas from seven replicate injections of a standard mix solution (at a concentration of 1 µg/mL) in the SOP and the HPS setups. The data were obtained when the systems and columns were new (no B vitamins have ever been injected onto the systems). One can see that greater or the same responses (peak areas) were obtained in the HPS setup for all 18 vitamins. Among these compounds, the flavin mononucleotide (FMN), thiamine, thiamine pyrophosphate (TPP), pyridoxal 5′-phosphate (PLP), and pantothenic acid showed significant increases in peak areas in the HPS setup (Figure 2). Figure 3 shows a comparison of the chromatograms of the FMN, thiamine, PLP, and pantothenic acid that were obtained from the first injections of the standard mix solution on both LC setups. The peak intensities of these compounds were significantly larger on the HPS setup. Also, narrower peaks and less peak tailing was observed for the thiamine and PLP peaks in the HPS setup. The increase in response in LC-MS/MS analysis of B vitamins with the HPS setup was still evident after extended use of the LC-MS system (data not shown). There was no decrease in response was observed for any of the B vitamins.

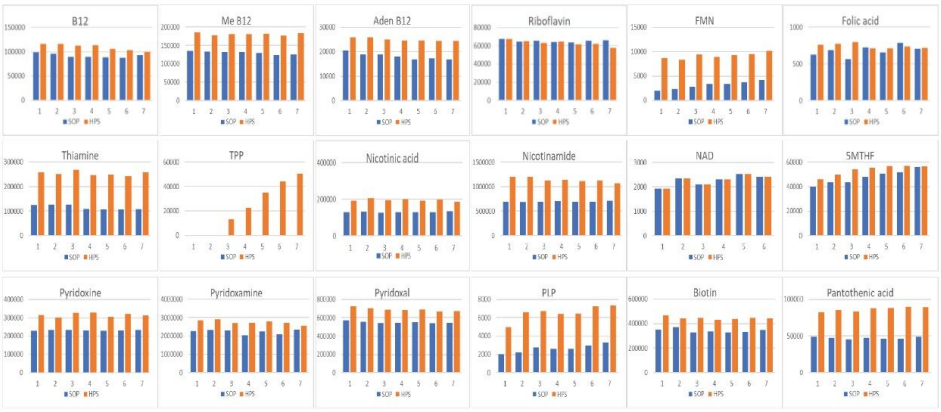

Figure 2. Comparison of LC-MS peak areas of B vitamins and their vitamers with the HPS setup and the SOP setup. Peak areas from standard surface (SOP, blue bar) are plotted side by side with those from HPS surface (HPS, orange bar).

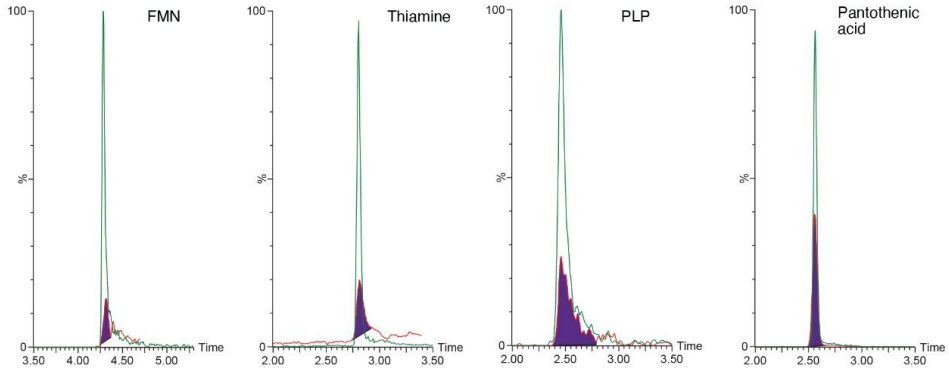

Figure 3. Peak intensities were significantly increased using the MaxPeak HPS hardware. Comparison of LC-MS chromatograms of FMN, thiamine, PLP, and pantothenic acid with the HPS and the SOP setups. Peak intensities from standard surface (solid filled peaks) are normalized to the peak intensities (not filled peak) from HPS.

Higher Sensitivity

The increased responses lead to higher sensitivities in the LC-MS analysis of the B vitamins. Right after the seven replicate injections of a standard mix solution (as shown in Figure 2), a series of standard mix solutions at concentrations ranging from 3 ng/mL to 10 µg/mL were injected onto the HPS and the SOP system setups. The limit of quantification (LOQ) in both LC system setups was estimated using the results from these injections of standard solutions. Large improvements in LOQ (3–10 times improvement) were achieved for FMN, thiamine, PLP, and pantothenic acid in the HPS system setup (Table 1).

Compound	LOQ (ng/mL)		LOQ improvement in HPS than SOP setup
	SOP setup	HPS setup	
FMN	1000	100	10 times
Thiamine	1000	300	3 times
PLP	1000	300	3 times
Pantothenic acid	30	10	3 times

No Carryover

The HPS technology minimizes interactions between the B vitamins and the stainless-steel inner surface of the flow path in LC system and column and reduces the risk of carry-over that often occurs in the LC analysis of B-group vitamins. Figure 4 shows a comparison of the B vitamins' LC-MS chromatograms obtained with the HPS and the SOP system setups in a carry-over study. Residual peaks were found in the blank injection on the SOP system setup for riboflavin, pyridoxine, 5-methyl-tetrahydrofolate, and methylcobalamin while no residual peak was found on the HPS system setup. The MaxPeak HPS Technology reduces the risk of carry-over in the LC-MS analysis for B vitamins.

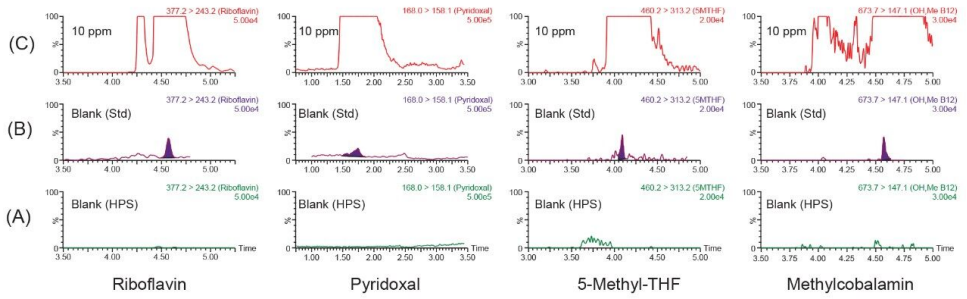

Figure 4. Comparison of LC-MS chromatograms of blank injections obtained with HPS setup (A) and with SOP setup (B) right after the injections of a 10 ppm standard solution (C) for riboflavin, pyridoxine, 5-methyl-THF, and methylcobalamin. Small residual peaks were observed in the SOP system setup (B) for these four vitamins at 0.03–0.1% level of the 10 ppm peaks (C). No residual peak was observed with the HPS system (A).

Conclusion

This application brief highlights the key improvements in chromatography using the ACQUITY PREMIER System and the ACQUITY PREMIER BEH C_{18} Column for the LC-MS/MS analysis of B-group vitamins. The key improvements include sharper and more symmetric peak shapes, greater peak areas, higher sensitivity, and reduced risk of carryover. These improvements help analysts to quantify B vitamins at lower concentrations, with higher accuracy and precision, and enhanced reliability. The ACQUITY PREMIER Solution, featuring the MaxPeak HPS Technology, offers a better solution for the analysis of B-group vitamins.

References

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