ENHANCING LC-MS/MS ANALYSIS OF B-GROUP VITAMINS WITH HYBRID SURFACE TECHNOLOGY

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INTRODUCTION

New vitamin B vitamers, such as flavin mononucleotide (FMN) and pyridoxal 5'-phosphate (PLP), have been formulated in dietary supplements and beverage. These phosphate containing B vitamers are often called "native" or coenzymated B vitamins and are believed to be readily absorbed by human body. Phosphate containing compounds are known to have issues in liquid chromatography, such as severe peak tailing, reduced peak height, and carry-over, which lead to inaccurate and unreliable results in HPLC analysis. The main reasons for those issues are the interactions between phosphate functional groups and metal ions at wettable surfaces in HPLC system [1-2].

Waters MaxPeak High Performance Surfaces (HPS) technology, which provides a hybrid organic-inorganic surface on metal components in Waters ACQUITY Premier Solution (system and columns), is an effective way to mitigate the analyte metal interactions. Dramatic improvements have been observed using the MaxPeak HPS in HPLC analyses for organic acids, oligonucleotides, peptide, glycans, and phospholipids [3 - 7]. We investigated the effects of the MaxPeak HPS on the analysis of B-group vitamins using liquid chromatography with tandem mass spectrometry (LC-MS/MS). The advantages of applying the MaxPeak HPS to the analysis of B vitamins, including those non-phosphate containing B vitamins, are highlighted in the simultaneous LC-MS/MS analysis of B-group vitamins in energy drink and dietary supplement samples.

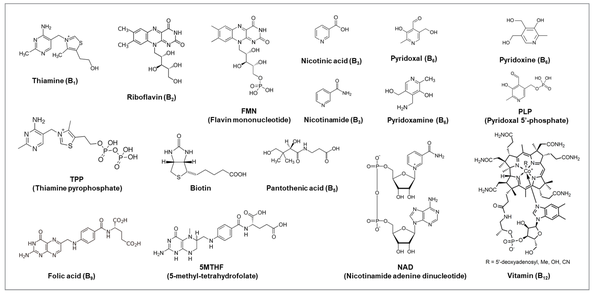

Figure 1. Structures of B vitamins and their vitamers

METHODS

System setups for comparison study:

Two UPLC-MS/MS system setups were configured for the comparison study. One comprised of a Waters ACQUITY Premier System, a Xevo TQ-S micro mass spectrometer, and an ACQUITY Premier BEH C18 Column (1.7 µm, 2.1 × 100 mm). The ACQUITY Premier System and the ACQITY Premier BEH C18 Column were manufactured with metal components incorporate the HPS. This UPLC-MS/MS system setup is hereon referred as the HPS Setup. The other UPLC-MS/MS system setup comprised of a Waters ACQUITY H Plus System, a Xevo TQ-S micro mass spectrometer, and an ACQUITY UPLC BEH C18 Column (1.7 µm, 2.1 × 100 mm). Conventional stainless-steel components were used in this UPLC-MS/MS system setup. This setup is hereon referred as SOP Setup. These two UPLC-MS/MS instrument setups were comparable to each other in mechanical and fluidic designs. The main difference between the HPS and the SOP Setups was that HPS components were used in the HPS Setup while conventional stainless-steel components were used in the SOP Setup.

METHODS

LC conditions

LC System:	ACQUITY Premier System
MS system:	Xevo™ TQ-S micro system
Run time:	9.0 min
Column:	ACQUITY Premier BEH™ C18 Column, 1.7 µm, 2.1 × 100 mm (186009453)
Vial:	LCMS Certified Amber Glass Max Recovery Vial (p/n 600000755CV)
Temp:	40 °C
Mobile phases:	A: 20 mM ammonium formate in water (pH 5.0). B: Methanol
Flow rate:	0.35 mL/min
Injection volume:	2 µL

Gradient program:

Time (min)	Flow (mL/min)	A %	B %
Initial	0.35	99	1
0.50	0.35	99	1
2.50	0.35	92	8
5.00	0.35	10	90
6.00	0.35	10	90
6.10	0.35	99	1
9.00	0.35	99	1

MS system settings:

Polarity:	ES+
Capillary Voltage:	1.4 kV
Cone Voltage:	70V
Source Temp.:	150 °C
Desolvation Temp.:	350 °C
Cone Gas Flow:	350 L/Hr
Desolvation Gas Flow:	650 L/Hr

RESULTS

1) Comparison of peak area

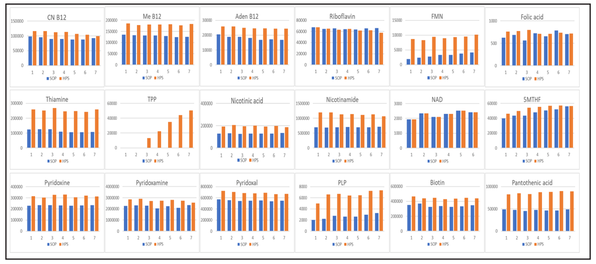

Figure 2. Comparison of LC-MS/MS peak areas of B vitamins and their vitamers with the HPS setup and the SOP setup. Peak areas from stainless steel surfaces (SOP, blue bar) are plotted side by side with those from MaxPeak HPS (HPS, orange bar).

RESULTS

2) Comparison of peak shape and peak intensity

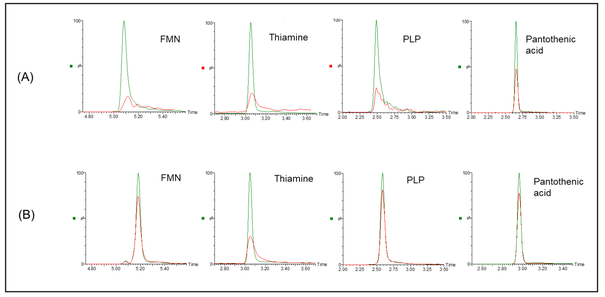

Figure 3. Comparison of LC-MS chromatograms of FMN, Thiamine, PLP and Pantothenic acid obtained on the HPS setup (green traces) and the SOP setup (red traces). (A) Observed during the initial injections of the same standard mix on fresh LC systems. (B) Observed during the B vitamin analysis of a DS sample on LC systems that have been extensively used.

3) Carry-over study

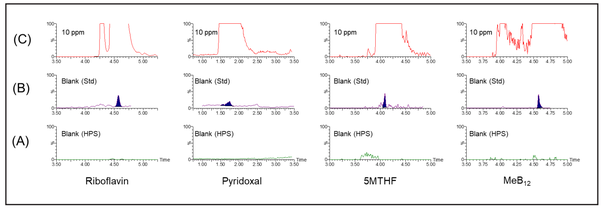

Figure 4. Carry-over study. Comparison of LC-MS/MS chromatograms of blank injections obtained with HPS setup (A) and with SOP setup (B) right after the injections of a 10 ppm standard solution (C) for riboflavin, pyridoxal, 5MTHF, and MeB12. Small residual peaks were observed with the SOP system setup (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).

Key results:

- **Increased peak area and improved peak shape with the MaxPeak HPS**
- **Improved sensitivity with the MaxPeak HPS**
- **No carry-over with the MaxPeak HPS**

RESULTS

4) Comparison of sensitivity

Vitamins	LOQ (ng/mL)	Range (ng/mL)	R^2	LOQ (ng/mL)	Range (ng/mL)	R^2	Order of polymomial fitting	I.S.
	HPS			SOP				
Cyanocobalamin	10	10 - 3,000	0.99	10	10 - 3,000	0.97	1st	None
Methylcobalamin	10	10 - 10,000	0.995	10	10 - 10,000	0.99	1st	None
Riboflavin	3	3 - 10,000	0.9993	10	10 - 10,000	0.998	1st	$^{13}C_4$ $^{15}N_2$ - Riboflavin
Thiamine	3	3 - 1,000	0.9991	10	10 - 1,000	0.99	1st	$^{13}C_4$ - Thiamine
Biotin	3	3 - 3,000	0.9991	3	3 - 3,000	0.99	1st	None
Pantothenic acid	10	10 - 10,000	0.99	10	10 - 10,000	0.99	1st	$^{13}C_3$ ^{15}N - Pantothenic acid
Pyridoxine	1	1 - 1,000	0.998	1	1 - 1,000	0.998	1st	$^{2}H_3$ - Pyridoxine
Nicotinic acid	30	30 - 10,000	0.993	30	30 - 10,000	0.99	2nd	$^{2}H_4$ - Nicotinic acid
Nicotinamide	10	10 - 3,000	0.997	30	30 - 10,000	0.99	2nd	$^{2}H_4$ - Nicotinic acid
Folic acid	100	100 - 10,000	0.99	100	100 - 10,000	0.93	1st	$^{13}C_5$ ^{15}N - Folic acid
FMN	100	100 - 100,000	0.995	300	300 - 30,000	0.95	1st	None
PLP	100	100 - 30,000	0.991	300	300 - 3,000	0.96	2nd	$^{2}H_3$ - Pyridoxine
Pyridoxal	3	3 - 300	0.9993	3	3 - 300	0.99	2nd	$^{2}H_3$ - Pyridoxine
5-M-THF	10	10 - 10,000	0.9991	100	100 - 3,000	0.98	1st	None

CONCLUSION

Comparison study of the LC-MS/MS analysis of 18 B-group vitamins on two UPLC setups showed lasting benefits of using MaxPeak HPS over conventional stainless-steel surfaces for majority of the B vitamins. The main benefits include:

- **Higher peak area**
- **Less peak tailing**
- **Improved analysis sensitivity**
- **Less carry-over**

The Waters ACQUITY Premier Solution exhibited clear advantages over conventional LC solution in the sensitivity, accuracy, and precision for the analysis of B-group vitamins.

References

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