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Queued and Ready to Run? Testing the Stability of Vitamin Extracts While in Queue Using Triple Quadrupole LC/MS/MS Analysis

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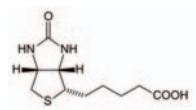
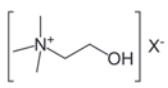
1. Introduction

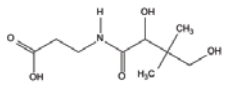
Many Americans include vitamin supplements in their diets, making reliable measurement of these complex mixtures essential for quality assessment and accurate labeling. Though many AOAC Official Methods for vitamins employ microbiological assays or LC with UV fluorescence detection, use of LC/MS/MS techniques in this field is rising. While LCMS techniques offer advantages including specificity, speed, ability to detect impurities, and broad applicability, they are not impervious to chemical challenges posed by these molecules. Sensitivity to light, heat, oxidation, and other formulation components can cause vitamins to degrade over time, potentially leading to inaccurate analyses. This study of 8 water soluble vitamins seeks to determine the stability of a vitamin extract over a 12 hour period.



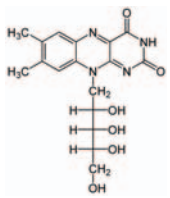
Biotin $C_{10}H_{16}N_2O_3S$
MW 244.31



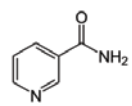
Choline $C_5H_{14}NO$
MW 104.17



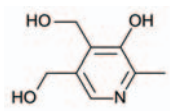
D – Calcium Pantothenate
$C_9H_{17}NO_5$ MW 219.23



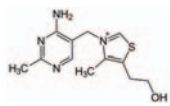
Ribo avin $C_{17}H_{20}N_8O_6$
MW 376.36



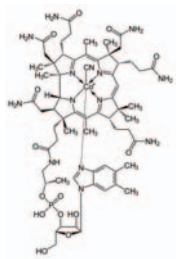
Nicotinamide $C_6H_6N_2O$
MW 122.12



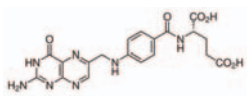
Pyridoxine $C_8H_{11}NO_3$
MW 169.18



Thiamine $C_{12}H_{17}N_4OS$
MW 265.36



Vitamin B12 $C_{63}H_{88}CoN_{14}O_{14}P$
MW 1355.38



Folic Acid $C_{19}H_{19}N_7O_6$
MW 441.4

Fig. 1 Vitamin structures and molecular weights

2. Method

To determine the rate of analyte degredation, all standards and vitamin tablet extracts were prepared simultaneosly and vials for each time point were placed at time zero in the autosampler sample rack cooled to 4°C. At each time point beginning at zero, an aliquot of Calcium Pantothenate-[$^{13}C_3$, ^{15}N] was spiked into each sample. It was expected that this internal standard would not degrade due to lack of interaction with other vitamins and matrix materials prior to analysis, yielding a consistent signal intensity at each time point for comparison with other signals in the mixture. Additionally, a calibration curve was acquired spanning from 1 ppb - 40 ppm over twelve concentrations to serve as external standards.

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Water soluble vitamin standards including choline, thiamine, riboflavin, niacinamide, d-calcium pantothenate, pyridoxine, biotin, and folic acid are each accurately measured, dissolved and combined to make a stock solution with equal concentration per vitamin, which is incrementally diluted to produce the external calibration standards. Unknowns are prepared by weighing and grinding over the counter prenatal vitamin capsules to powder, adding appropriate extraction solvent (94/5/1 Water/Acetonitrile/Acetic Acid), vortexing, sonicating, centrifuging, and filtering.

These analyses are conducted on a Shimadzu LCMS-8040 using a Shimpack XR-ODS-II C18 (100 × 2.0 mm) column heated to 40°C. The mobile phases used in this separation are (A) 5 mM Ammonium formate, 0.1% formic acid in water and (B) Acetonitrile.

Table 1 Optimized MRM parameter

Analyte	Transition	Dwell Time (ms)	Q1 (V)	CE (eV)	Q3 (V)
Choline	104>60, 104>58	20	-24, -24	-21, -33	-23, -23
Thiamine	265>122, 265>144	20	-30, -30	-16, -14	-11, -25
Riboflavin	377>243, 377>198	20	-29, -29	-26, -42	-23, -19
Niacinamide	123>80, 123>53	20	-28, -28	-22, -31	-30, -21
Pantothenic acid	220>90, 220>202	20	-15, -15	-15, -12	-30, -19
Pyridoxine	170>152, 170>134	20	-17, -17	-16, -21	-26, -23
Biotin	245>227, 245>97	20	-17, -17	-16, -34	-23, -16
Folic acid	440>175, 440>132	20	21, 21	36, 49	30, 28
IS Pantothenic acid	224>94, 224>206	20	-12, -12	-16, -12	-19, -16

Compound Name	Range (ng/mL)	Correlation coefficient (R^2)
Biotin	5 – 20000	0.998
Choline	2 – 1250	0.994
Folic acid	100 – 20000	0.992
Nicotinamide	10 – 20000	0.997
Pantothenic acid	25 – 20000	0.999
Pyridoxine	5 – 1250	0.999
Riboflavin	5 – 20000	0.999
Thiamine	10 – 5000	0.997
IS Pantothenic acid	5 – 20000	0.999

LCMS Instrument Parameters

Column temp. : 40°C
Drying Gas : 10 L / min
DL Temp. : 250°C
Flow Rate : 0.200 mL/min
Heating Block : 400°C
Nebulizing Gas : 1.5 L / min
Flow Program : 0% B (0 min) – 40% B (7 min) – 0% B (7.01 min) – 0% B (9 min)

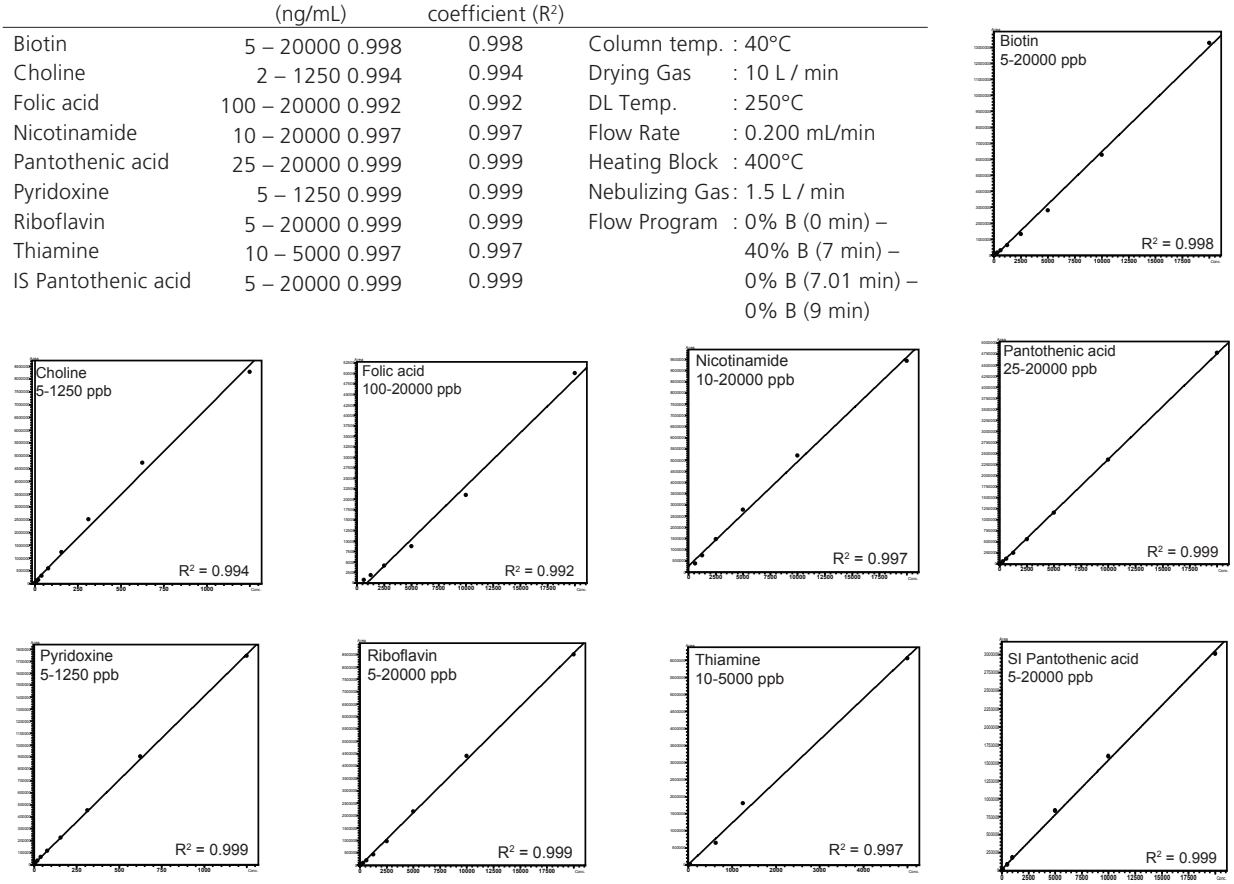

Fig. 2 Water soluble vitamin ranges of linearity, LCMS acquisition parameters, and analyte calibration curves

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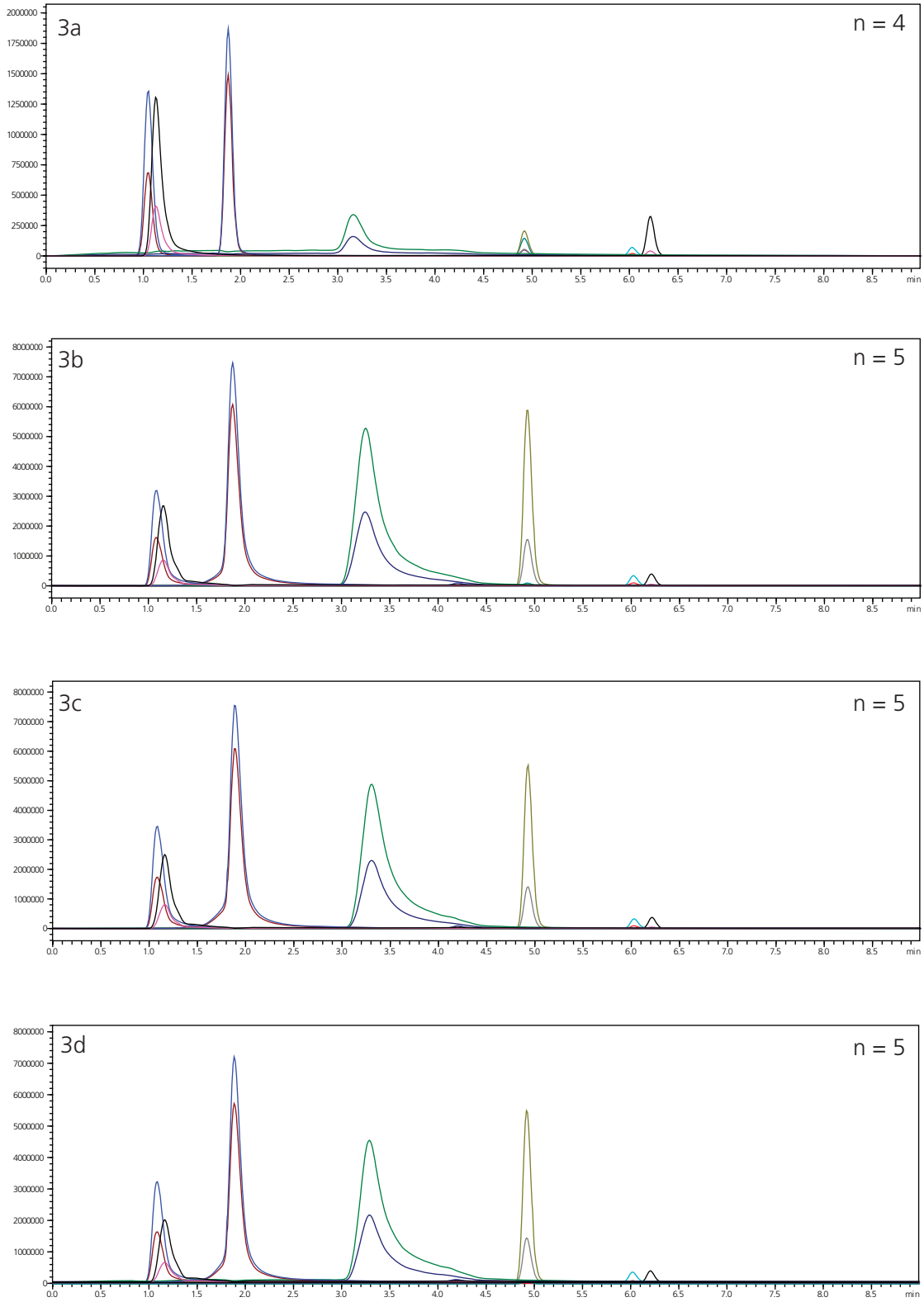

Fig. 3 (a) calibration curve at 5 ppm, (b, c, d) LCMS Chromatograms at Time 0, 6 hours, and 12 hours respectively

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Table 2 Calculated concentrations of analytes from vitamin capsule at times 0, 6 hours, and 12 hours

	Concentration at Time 0 (ppb)	Concentration at 6 Hours (ppb)	% Decrease at 6 Hours	Concentration at 12 Hours (ppb)	% Decrease at 12 Hours
Biotin	5923	5564	-6.0	5353	-9.6
Choline	42005	40523	-3.5	38544	-8.2
Folic acid	4297	4175	-2.8	3424	-20.3
Nicotinamide	172353	156804	-9.0	148007	-14.1
Pantothenic acid	192542	175558	-8.8	169355	-12.0
IS Pantothenic acid	3827			3608	-5.7
Pyridoxine	23755	21668	-8.8	20465	-13.3
Ribo avin	28098	27403	-2.5	25354	-9.8
Thiamine	15519	12836	-17.3	10412	-32.9

3. Results and Discussion

Many verified methods for water soluble vitamin analysis quantitate just one analyte, as might be expected with such significant structural diversity (Fig. 1). Using microbiological assays, or LC with UV or fluorescence detection, places greater stringency on extraction methods and molecular stabilization strategies than do the more versatile capabilities of LC/MS/MS approaches, which can be adapted to a variety of extraction solvents at a range of pHs. Additionally, because LC/MS/MS is fast, selective and sensitive it is an excellent platform for multi-vitamin analysis.

Fig. 2 demonstrates that linearity is observed for these species across a varying and sometimes large dynamic range, spanning from less than 10 ppb through 20 ppm.

Nontheless, separate from these many advantages, mass spectrometric measurements of vitamins, as with all analytes, must account for sample degredation prior to measurement. As vitamins are especially sensitive to degradation, the time from sample preparation to LC injection and MS measurement must be kept to a minimum. Fig. 3a demonstrates an LCMS Chromatogram for a 5 ppm calibration standard. Fig. 3b-d shows the time course progression from time 0-12 hours, illustrating the decrease in signal that occurs over time for each analyte. Additionally, Table 2 provides the percent decrease of each analyte over time from the calculated concentration at time 0.

4. Conclusions

Each of these vitamin species demonstrate a loss of signal which continues to diminish with time. This trend complicates the practice of extracting samples in bulk and setting vials in an autosampler rack for an extended duration prior to analysis. Reducing sample run time using UHPLC online with an ultrafast mass spectrometer will prove crucial to minimizing intra- and interday results when making quality control analyses for these analytes.

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