

Poster Reprint

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Detection and Accurate Quantitation of 14 Water Soluble Vitamins and 14 Fat Soluble Vitamins in Supplements by LC-MS/MS Triple-Quadrupole

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Introduction

The water-soluble B vitamins and the fat-soluble vitamins are essential dietary nutrients. Vitamin supplements are often consumed through various forms – such as tablets, capsules, gummies, softgels, and drinks. Accurate quantitative measurements for water-soluble B vitamins and fat-soluble vitamins are required to ensure product quality and regulatory compliance.

Two fast and sensitive LC-MS/MS methods were developed respectively for the simultaneous determination of 14 water-soluble B vitamins and 14 fat soluble vitamins on Agilent 1290 Infinity II LC coupled to an Agilent 6470 triple quadrupole LC/MS system in positive electrospray ionization mode. The approaches of improving the accuracy of mass spectrometry quantitation results including matrix matched standard/standard addition were introduced. Method criteria for data acceptance were established.

The methods were applied to quantify the water-soluble B vitamins and fat-soluble vitamins in a highly complex multivitamin tablets matrix. All tested water-soluble B vitamins and fat-soluble vitamins met the claims. It was concluded that the methods can be utilized for quality control and establishment of the nutrition labels for water-soluble vitamins/fat-soluble vitamins-containing supplement products.

Analytes List

Water-soluble B vitamins

Thiamine	B1	Biotin	B7
Riboflavin	B2	Folic Acid	B9
Niacin	B3	5-Methyltetrahydrofolic Acid	B9
Niacin amide	B3	Cyanocobalamin	B12
Pantothenic Acid	B5	Methylcobalamin	B12
Pyridoxine	B6	Hydroxycobalamin	B12
Pyridoxal 5'-phosphate	B6	Adenosylcobalamin	B12

Fat-soluble vitamins

Retinol	A	Alpha-Tocopherol succinate	E
Retinol Acetate	A	Phytonadione	K1
Retinyl Palmitate	A	Menaquinone, MK2-4	K2
Ergocalciferol	D2	Menaquinone, MK2-7	K2
Cholecalciferol	D3	β -Carotene	Carotenoid, A
Alpha-Tocopherol	E	Lutein	Carotenoid
Alpha-Tocopherol Acetate	E	Lycopene	Carotenoid

Experimental



Agilent 1290 Infinity II LC with 6470 Triple Quadrupole LC/MS System.

Chromatographic Conditions

Water-Soluble B Vitamins

UHPLC: Agilent 1290 Infinity II
Column: Agilent Poroshell 120 Phenyl-Hexyl, 2.7 μ m, 3.0 x 100mm
pn: 695975-312

Column oven temperature: 30 \pm 2 $^{\circ}$ C

Injection volume: 1 μ L

Autosampler: 5 \pm 2 $^{\circ}$ C

Flow rate: 0.50 mL/min

Mobile Phase A: *5 mM Ammonium Formate/0.1% Formic Acid in Water

Mobile Phase B: 0.1% Formic Acid in Methanol

Gradient:

Time, min	%A	%B
0	97	3
1.0	94	6
4.5	55	45
5.5	10	90
6.5	10	90
6.6	97	3
9.0	97	3

*In order to achieve the best peak shape, the column needs a relatively long time to equilibrate; or up to 20 mM ammonium formate can be used

Fat-Soluble Vitamins

UHPLC: Agilent 1290 Infinity II

Column: Agilent Poroshell 120 SB-AQ, 2.7 μ m, 2.1 x 150mm
pn: 683775-914

Column oven temperature: 45 \pm 2 $^{\circ}$ C

Injection volume: 1 μ L

Autosampler: 5 \pm 2 $^{\circ}$ C

Flow rate: 0.25 mL/min

Mobile Phase A: 0.1% Formic Acid in Water in Water

Mobile Phase B: 0.1% Formic Acid in Methanol

Gradient:

Time, min	%A	%B
0	20	80
7.0	0	100
9.5	0	100
10	20	80
12	20	80

Experimental

MS Conditions - Agilent 6470 Triple-Quadrupole LC/MS

Parameter	Water-Soluble B Vitamins
MS acquisition	Dynamic MRM
Stop time	6.5 min
Ion source	Agilent Jet Stream electrospray ionization (AJS ESI positive)
Drying gas temperature	270 °C
Drying gas flow	13 L/min
Nebulizer	40 psi
Sheath gas heater	375 °C
Sheath gas flow	11 L/min
Capillary	2500 V
Nozzle voltage	0 V

Parameter	Fat-Soluble Vitamins
MS acquisition	Dynamic MRM
Stop time	9.5 min
Ion source	Agilent Jet Stream electrospray ionization (AJS ESI positive)
Drying gas temperature	250 °C
Drying gas flow	9 L/min
Nebulizer	40 psi
Sheath gas heater	350 °C
Sheath gas flow	12 L/min
Capillary	4000 V
Nozzle voltage	1000 V

Sample Preparation

Water-Soluble B Vitamins

- ✓ Determine the average weight
- ✓ Extraction
 - Add extraction solvent (0.1% H₃PO₄ + 5% ACN + 0.5% EDTA + 0.5% vitamin C in water)
 - Heat the bottle at 90-95 °C water bath for 25 mins for releasing riboflavin

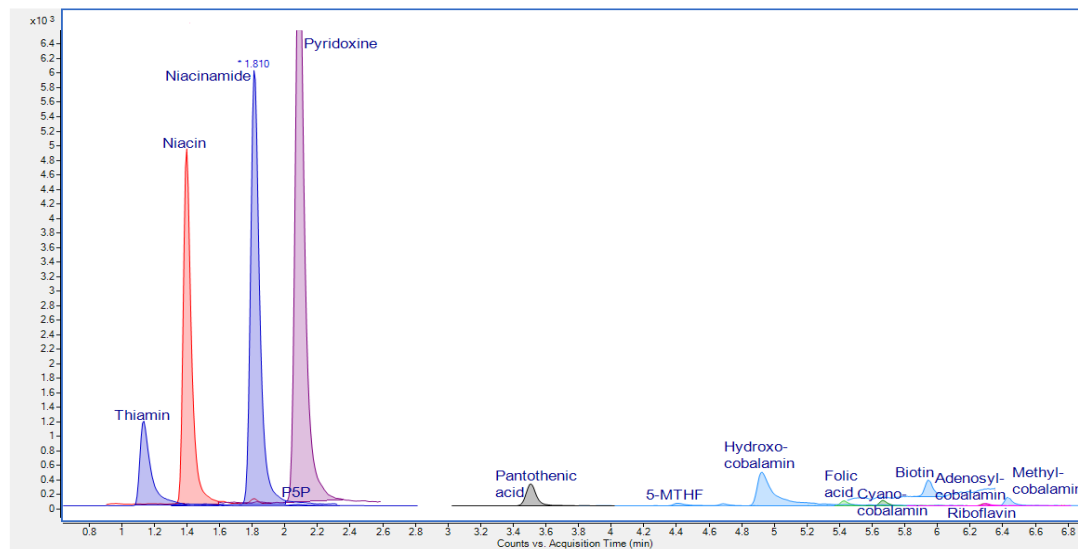
- ✓ Dilution

Fat-Soluble Vitamins

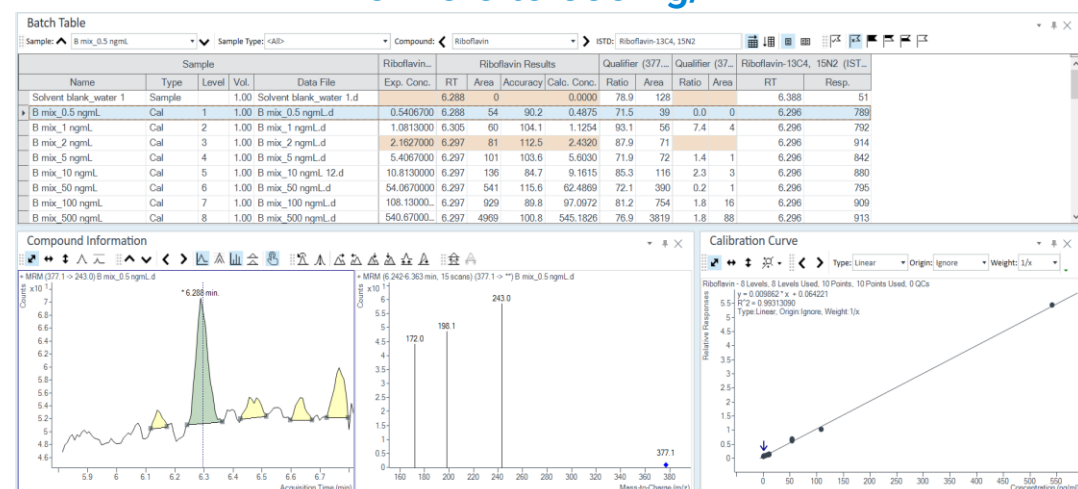
- ✓ Determine the average weight
- ✓ Extraction
 - Add DMSO, heat at 55-65 °C for ~5 mins
 - Add Ethanol, shake
- ✓ Dilution

Results and Discussion

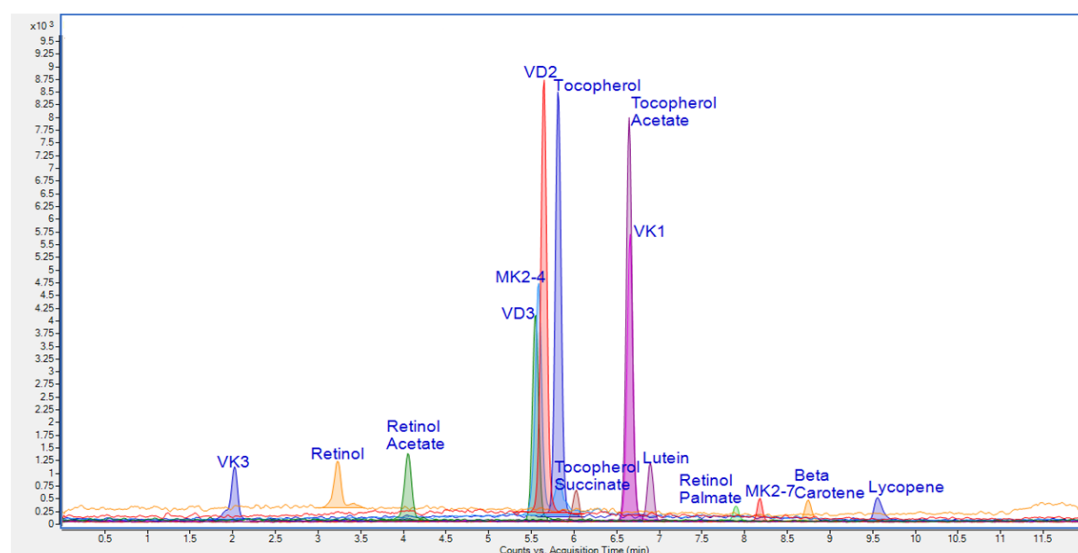
Elution Profile of 14 Water-Soluble B Vitamins



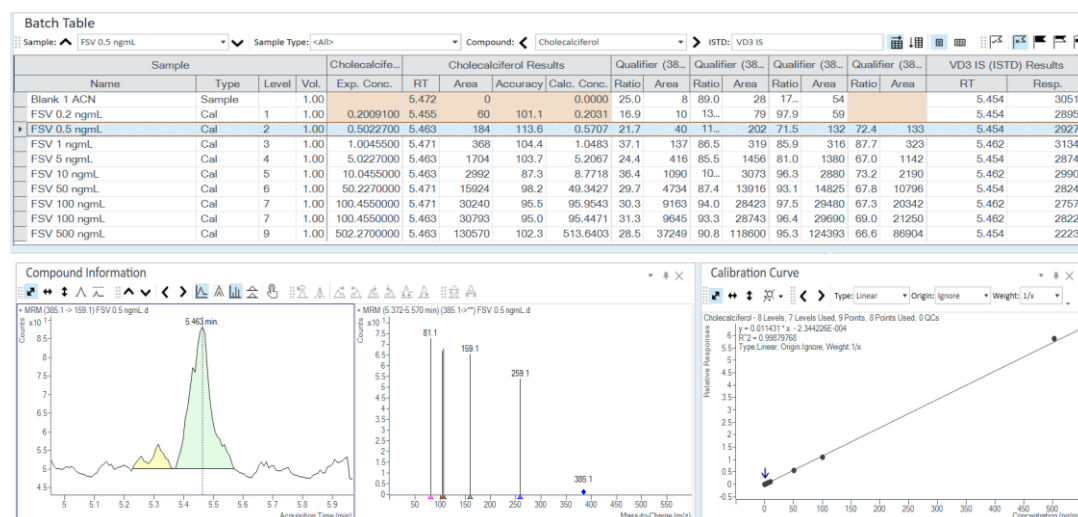
Riboflavin as an Example-Great Linearity of Calibration from 0.5 to 500 ng/mL



Elution Profile of 14 Fat-Soluble Vitamins



Cholecalciferol as an Example-Great Linearity of Calibration from 0.5 to 500 ng/mL



Multivitamin Tablet Sample Testing Results

Compound Name	Vitamins	Serving Size (g)	Claim on Product Label (mg/Serving)	Corrected Results by IS (mg/Serving)	Corrected Results by post-Spike Recovery (mg/Serving)	Post-Spike Recovery (%)
Thiamin	B1	5	40	-	41	102
Riboflavin	B2	5	40	57	57	97.1
Niacinamide	B3	5	80	89	87	108
Pantothenic Acid	B5	5	62.5	-	75.9	97
Total of Pyridoxine and P5P	B6	5	42.5	47.1	43	111
Biotin	B7	5	0.033	0.047	0.05	94.7
5-methyltetrahydrofolate glucosamine salt	B9	5	0.42	-	0.44	By standard addition
Methylcobalamin	B12	5	0.2	-	0.21	70.8
Retinyl Acetate	A	5	0.54	-	0.60	87.8
β -Carotene	A; Carotenoids	5	1.26	-	1.85	103
Cholecalciferol	D3	5	0.05	0.08	0.078	97.1
Alpha-Tocopherol Succinate	E	5	60	-	87	106
Phytonadione	K1	5	0.12	-	0.18	106
Retinol	A	5	-	-	-	86.6
Retinyl Palmitate	A	5	-	-	-	102
Ergocalciferol	D2	5	-	-	-	99.7
Alpha-Tocopherol	E	5	-	-	-	115
Alpha-Tocopherol Acetate	E	5	-	-	-	99.1
Menaquinone, MK2-4	K2	5	-	-	-	91.2
Menaquinone, MK2-7	K2	5	-	-	-	104
Lutein	Carotenoids	5	-	-	-	96.4
Lycopene	Carotenoids	5	-	-	-	105

Criteria to Accept the Quantitation Results

- ✓ Ion ratio for sample matches that of mean of all standards within the range of $\pm 30\%$
- ✓ The retention times of the native analyte and its isotope labelled internal standard should overlap
- ✓ The calibration curve constructed from external points or standard addition has a coefficient of determination (r^2) of ≥ 0.99
- ✓ If the post spike recovery (single point standard addition) or IS recovery is within (e.g., 70% - 130%), a correction will be performed
- ✓ If the post spike recovery (single point standard addition) or IS recovery is out of the established criteria (70% -130%)
 - Dilute the sample extract
 - Improve chromatography resolution
 - A standard addition curve should be generated

Conclusions

- ✓ Rapid and sensitive sample prep and LC-MS/MS methods for water-soluble and fat-soluble vitamins detection and quantification
- ✓ High efficiency, throughput, cost reduction and accurate quantitation comparing to the traditional involvement of multiple assays
- ✓ Post matrix spike used for
 - Correction for matrix effects
 - Good recovery
 - Accurate quantitation
- ✓ Tablet tested meeting the claims for all required ingredients

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