

Poster Reprint

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Comparing LC/MS with LC/MS/MS for the Simultaneous Extraction and Analysis of Water-Soluble Vitamins and Fat-Soluble Vitamins in Multivitamin Supplements

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Introduction

Dietary supplements have become increasingly important in recent years. In the USA, about one in two people consumes dietary supplements. Among those, multivitamin supplements are the most common.

Accurate quantitative measurements for water-soluble vitamins and fat-soluble vitamins are required to ensure product quality and regulatory compliance.

The method includes an easy, fast sample extraction and rapid, sensitive LCMS detection was developed for the simultaneous extraction and determination of 6 water-soluble B vitamins and 3 fat soluble vitamins (vitamin C was included in MSD LC/MS analysis) on Agilent 1290 Infinity II LC coupled to an Agilent 6470 triple quadrupole LC/MS/MS or Agilent iQ MSD LC/MS in positive electrospray ionization mode. The approach of matrix matched standard to improve the accuracy of mass spectrometry quantitation results was introduced. Method criteria for data acceptance were established.

Vitamins in dietary supplements can range in concentrations from low $\mu\text{g/g}$ to mg/g levels. Accurately measuring this wide concentration range can be difficult in complex multivitamin supplements. In this study, LC/MS and LC/MS/MS were compared to evaluate how each system provides selective and sensitive identification and quantification. Single ion monitoring (SIM) LC/MS dramatically improved both selectivity and sensitivity comparing to traditional HPLC-DAD methodology. LC/MS/MS however demonstrated superior selectivity and sensitivity for the multivitamins in matrix.

InfinityLab LC/MSD IQ and 6470 triple quadrupole LC/MS



Experimental

Chromatographic Conditions

UHPLC: Agilent 1290 Infinity II
Column: Agilent Poroshell 120 Phenyl-Hexyl, 2.7 μm , 3.0 x 100mmpn: 695975-312
Column oven temperature: $40 \pm 2^\circ\text{C}$
Injection volume: 0.5 μL
Autosampler: $5 \pm 2^\circ\text{C}$
Flow rate: 0.50 mL/min

Mobile Phase A: 10 mM Ammonium Formate/0.1% Formic Acid in Water

Mobile Phase B: 0.1% Formic Acid in Methanol

Gradient:

Time, min	%A	%B
0	98	2
2.0	98	2
6.0	0	100
9.0	0	100
9.5	98	2
12	98	2

MS Conditions-Agilent MSD IQ Single Quadrupole/Agilent 6470 Triple Quadrupole

Parameter	
MS acquisition	SIM/Dynamic MRM
Ion source	Electrospray ionization/ Agilent Jet Stream electrospray ionization (AJS ESI positive)
Drying gas temperature	270 $^\circ\text{C}$
Drying gas flow	11 L/min
Nebulizer	40 psi
Sheath gas heater	375 $^\circ\text{C}$ (LC/MS/MS only)
Sheath gas flow	12 L/min (LC/MS/MS only)
Capillary	4000 V
Nozzle voltage	0 V (LC/MS/MS only)

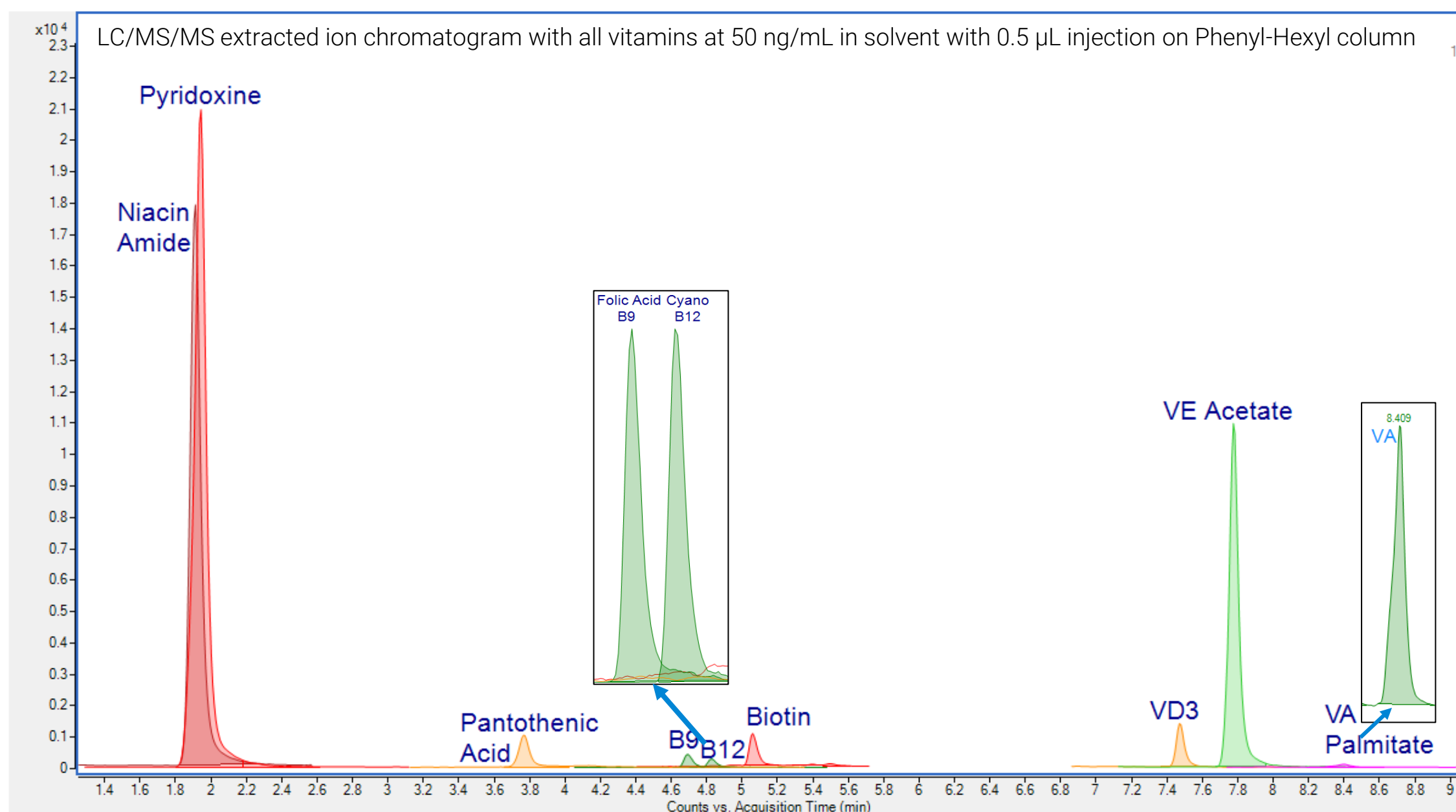
Sample Preparation

- ✓ Determine the average weight
- ✓ Extraction
 - Weigh one gummy sample
 - Add 10 mL 0.1% formic acid + 5% ACN in water
 - Heat at 60-70 $^\circ\text{C}$ and stir with a stir bar to completely dissolve the gummy sample
 - Add 35 mL ethanol, shake for 10 mins
 - Draw ~1.5 mL extract to a microcentrifuge tube and centrifuge at 16000 RPM for 5 mins
- ✓ Dilute as needed

Compound Specific Conditions

Compound Name	Group	Precursor Ion (m/z) SIM	Product Ion (m/z) MRM	Fragmentor (V)	Collision Energy (V)
Water Soluble Vitamins					
Ascorbic Acid	VC	177.1	141.0, 95.0	80	4, 12
Niacin amide	B3	123.1	80.0, 53.0	112	20, 36
Pantothenic Acid	B5	220.1	90.0, 71.9	107	8, 16
Pyridoxine	B6	170.1	134.0, 77.0	92	24, 40
Biotin	B7	245.1	227.1, 97.1	102	8, 32
Folic Acid	B9	442.1	295.0, 176.0	102	20, 44
Cyanocobalamin	B12	678.2	359.0, 456.9	152	25, 30
Fat-Soluble Vitamins					
Retinyl Palmitate	VA	525.5	269.1, 105.1	128	12, 56
Cholecalciferol	VD3	385.1	159.1, 107.1	104	25, 28
Cholecalciferol-D6	VD3	391.1	105.1	128	48
Alpha-Tocopherol Acetate	VE	473.4	207.1, 165.0	172	16, 40

Elution Profile of Water-Soluble and Fat-Soluble Vitamins



Multivitamin Gummy Sample Testing Results*

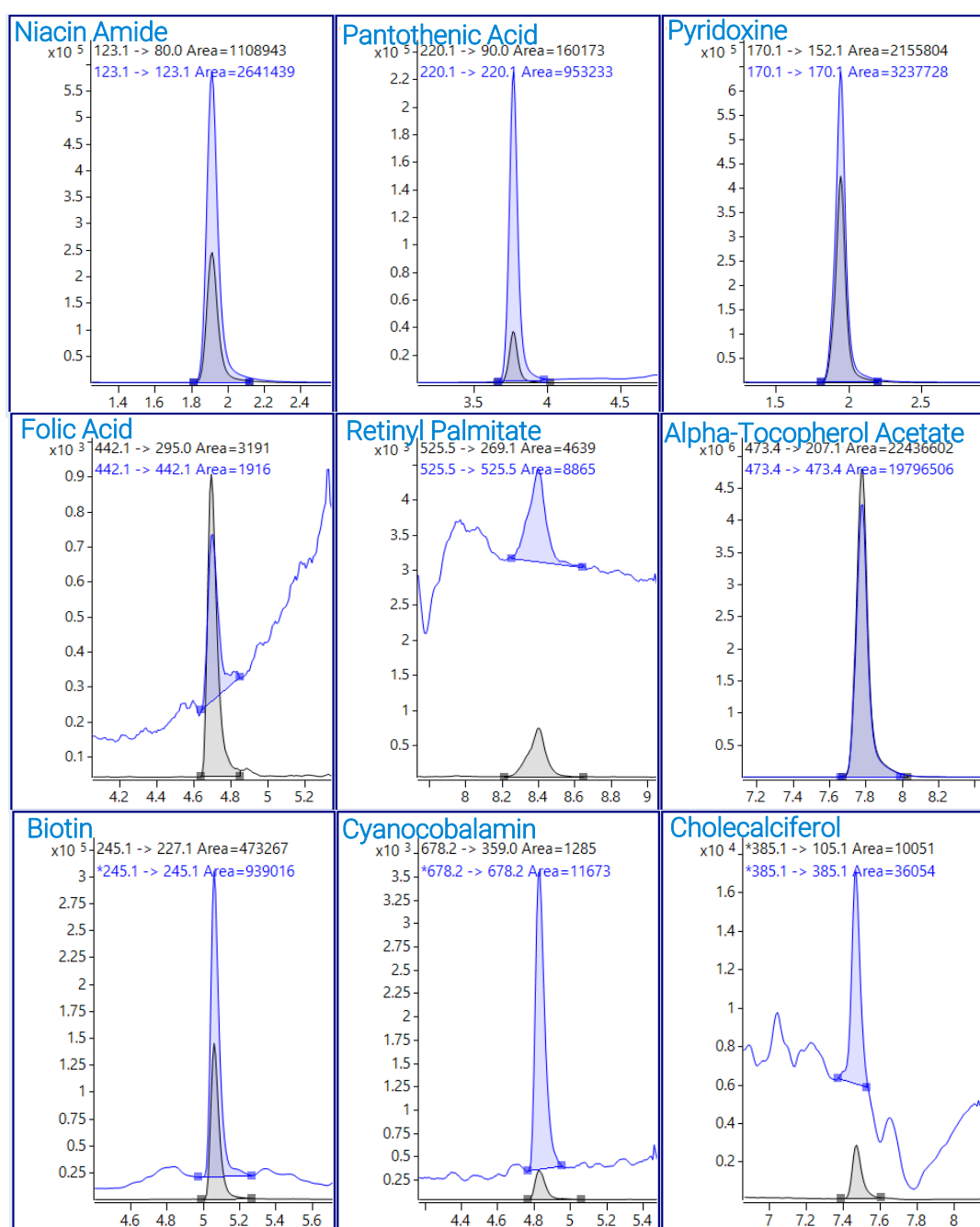
Compound name	Vitamins	Claim, mg (mcg)/gummy	Serving Size, g	LCMSMS Corrected Results by post-Spike Recovery (mg/Serving)	Post-Spike Recovery (%)
Ascorbic acid	VC	15 mg	2.2	19.8**	73
Niacin amide	B3	1.5 mg	2.2	1.92	105
Pantothenic Acid	B5	2.75 mg	2.2	2.98	125
Pyridoxine	B6	0.5 mg	2.2	0.74	92
Folic Acid	B9	140 mcg	2.2	0.160	109
Biotin	B7	33 mcg	2.2	0.0615	91
Cyanocobalamin	B12	2.15 mcg	2.2	0.00294	94
Retinyl Palmitate	VA	560	2.2	0.799	115
Cholecalciferol	VD3	5 mcg	2.2	0.0091***	47
Alpha Tocopherol acetate	VE	8.4 mg	2.2	8.9	93

*The testing results from SIM and MRM mode agree well. Only the results from MRM mode was listed

** Ascorbic acid result was from SIM mode and was included in the same run along with other analytes

***The post-spike recovery for VD3 was out of accepted range of 70-130%. The result was corrected by the isotopically labeled internal standard VD3-D6

SIM/MRM Detection of Vitamins in Gummy*



*: The peak in blue is by SIM mode; the peak in gray is by MRM mode

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 isotopically labeled internal standard should overlap
- ✓ The calibration curve constructed from external points or internal standard calibration has a coefficient of determination (r^2) of ≥ 0.99
- ✓ If the post spike recovery (single point standard addition) is within (e.g., 70% - 130%), a correction will be performed

Conclusions

- ✓ Simultaneous extraction and detection by LC/MS or LC/MS/MS provides high efficiency, throughput, cost reduction and accurate quantitation for water-soluble and fat-soluble vitamins in supplements comparing to the traditional involvement of multiple assays
- ✓ SIM has good sensitivity but limited selectivity
- ✓ MRM has great sensitivity and selectivity
- ✓ Post matrix spike was used for
 - Correction for matrix effects
 - Accurate quantitation
- ✓ Gummy tested meets the claims for all required ingredients

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