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Bringing LC/MS to the Wellness Aisle: Quantitation of Water Soluble Vitamins in Over-the-Counter Supplements

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Introduction

Vitamin supplements are consumed to augment/support general health and wellness and are provided in various forms such as tablets, capsules, gummies, softgels, drinks, and powders. Traditional methods for quality control of these products generally applies multiple assays carried out for each vitamin separately such as: spectrophotometric (which are not suitable for mixtures and have low specificity); microbiological (labor intensive, low throughput, and highly variable); or LC-UV which has poor sensitivity and selectivity in complex matrixes, especially for coeluting analytes.

Leveraging the methodology previous developed with LC/MS triple quadrupole, we propose the use of a single quadrupole mass spectrometer to combine these assays in a single analysis while ensuring all sensitivity, selectivity, and accuracy targets are met.

Pro iQ and Pro iQ Plus with the Infinity III HPLC

Experimental

The study was conducted on a popular, over the counter supplement: Emergen-C Termeric & Ginger Flavored Gummies and Emergen-C Termeric & Ginger Fizzy Drink Mix.

The powdered product was dissolved with 177.4 mL, which is the 6 oz volume recommended dose. The mixture was prepared as Filtered vs Unfiltered to stress the robustness of the mixing, then analyzed using an Infinity III LC Stack containing a Prime Pump, Multisampler, UV-DAD, Pro iQ LC/MS Single Quadrupole, with a MultiMode Ionization source (ESI+APCI composite).

The sample preparation protocol was adapted from a previous application note using LC/TQ[1]

1. Add approximately 70 mL extraction solvent (0.1% H_3PO_4 + 5% ACN + 0.5% EDTA + 0.5% vitamin C in water) to the flask (Vitamin C was added to the solution to prevent oxidation of the target analytes).
2. Shake for 20 minutes.
3. Fill the flask to the 100 mL volume mark and mix well.
4. Draw approximately 1.5 mL of sample extract to a 2 mL microcentrifuge tube.
5. Heat the flask to 90 to 95 °C in a water bath for 25 minutes for releasing vitamin B2.
6. Cool down to room temperature.
7. Draw approximately 1.5 mL of sample extract to a 2 mL microcentrifuge tube.
8. Centrifuge the sample extract from step 5 and step 8 for 5 minutes at 13,000 rpm.
9. Dilute the supernatants with 0.1% H_3PO_4 + 5% ACN in water as needed.
10. Prepare the post-spiked samples along with the diluted samples.
11. Inject samples to LC/MS/MS using positive ESI mode for analysis.

Various sample concentrations and dilutions were analyzed to ensure robust quantification methodology that can accommodate the various vitamin concentration levels present in the sample.

Figure 1 – SIM chromatograms of vitamin B3 (Niacin) from 20-200,000 ppb

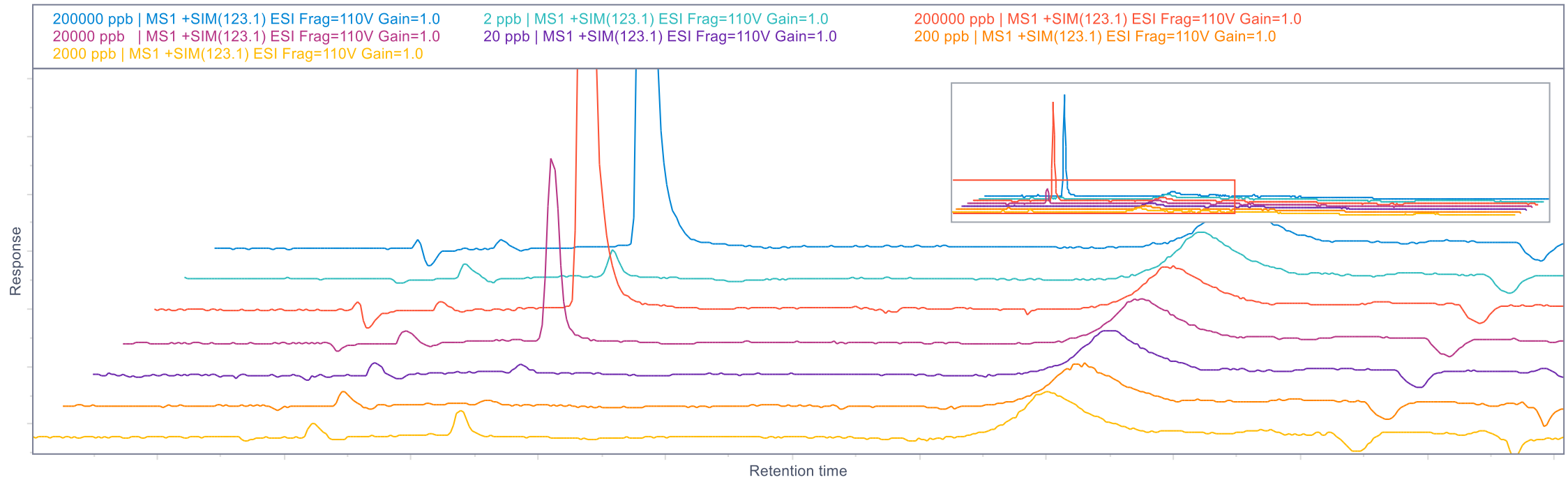


Table 1 – Vitamins analyzed in this method, their LOD, LOQ, and claims on label

Vitamin	Measured as	Amount on label	recovery	SIM m/z	Formula	ppm (9.0 g)	LOD ppb	LOQ ppb
Vitamin C	Acsorbic Acid, Zinc Ascorbate	250 mg	98	177.1	C6H8O6	27777.8	0.1	0.3
Thiamine	Thiamine Hydrocloride (B1)	0.36 mg	101	265.1	C12H17ClN4OS · HCl	40	0.2	0.5
Riboflavin	Rivoflavin 5 phosphate (B2)	0.39 mg	103	377.1	C17H20N4O6	43.3	0.1	0.4
Niacin	Vit (B3)	4 mg	105	124	C6H5NO2	444.4	0.1	0.4
Vitamin B 6	Pyridoxine Hydrocloride	10 mg	95	170.1	C8H11NO3 · HCl	1111.1	0.1	0.5
Folate	Vit B 9	100 mcg Folic Acid	110	442.1	C19H19N7O6	11.1	0.1	4
Vitamin B12	Cyanocobalamin	25 mcg	84	678.1	C63H88CoN14O14P	2.8	0.14	0.5
Pantothenic Acid	Calcium Pantothenate (B5)	2.5 mg	97	220.1	HOCH2C(CH3)2CH(OH)CONHCH2CH2CO2 ·1/2Ca	277.8	0.2	0.6

Figure 2 – Example of samples analyzed of different types

Table 2 – Quantification of vitamin B3 (Niacin) for tests of minimum claims

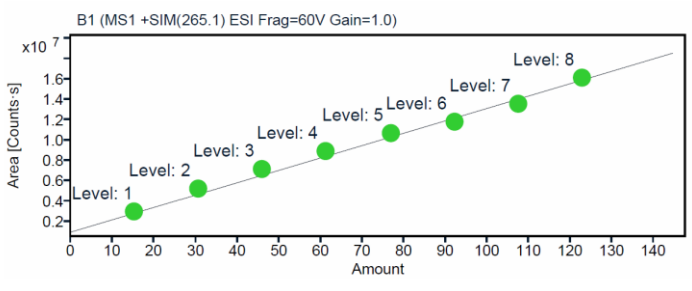
Name	RT	Area	Concentration (ppm)
Filtered Sample	1.43	395907.5	645.7467
Filtered Sample	1.43	396716.7	647.483
Filtered Sample	1.43	388346.9	629.5249
Filtered Sample	1.43	436179.5	732.1534
Filtered Sample	1.43	382276.3	616.5
Filtered Sample	1.43	381180	614.1478
unfiltered sample	1.43	382235.9	616.4134
unfiltered sample	1.43	388753	630.3963
unfiltered sample	1.43	388688.6	630.258
unfiltered sample	1.43	389014.9	630.9582
unfiltered sample	1.43	384794.6	621.9034
unfiltered sample	1.43	388508.3	629.8712

Metric	RT	Area	Concentration (ppm)
Mean		391883.5	637.113
Std.Dev.		14783.33	31.7187
%RSD		3.7724	4.9785
Number of records	12		

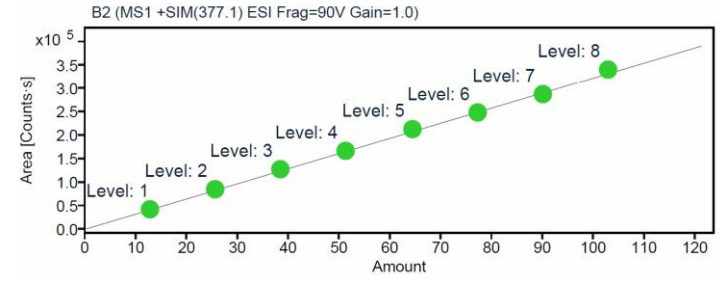
Results and Discussion

Figure 3 – Calibration curves for all vitamins when analyzed near the Limit of Detection (LOD) and Limit of Quantitation (LOQ)

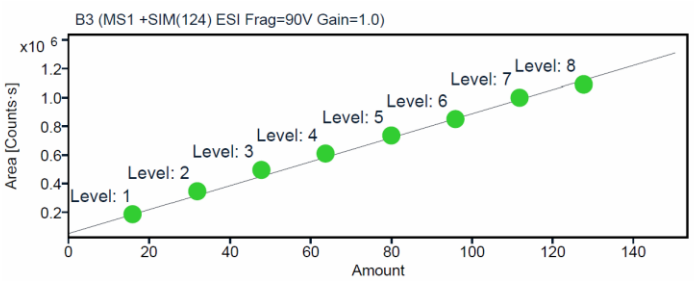
Compound: B1 (MS1 +SIM(265.1) ESI Frag=60V Gain=1.0)
Exp. RT: 1.364
Residual STD: 547352.36451
R: 0.995067
R^2: 0.99016
Formula: y = ax + b
a: 121773.99749
b: 886028.61727
c: 0.00000
d:



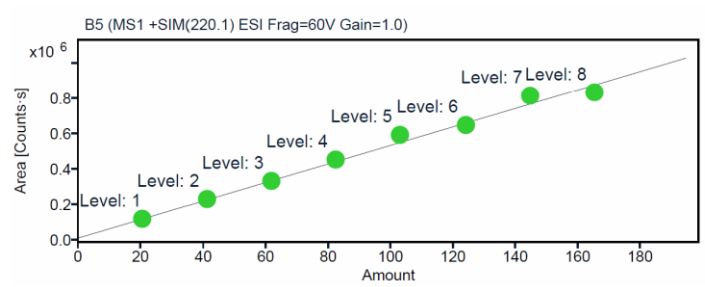
Compound: B2 (MS1 +SIM(377.1) ESI Frag=90V Gain=1.0)
Exp. RT: 5.412
Residual STD: 3373.13350
R: 0.999616
R^2: 0.99923
Formula: y = ax + b
a: 3222.88266
b: -825.72009
c: 0.00000
d:



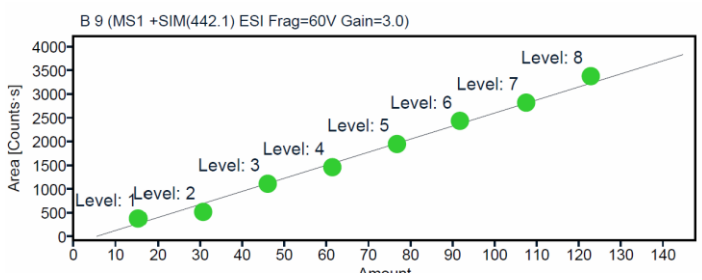
Compound: B3 (MS1 +SIM(124) ESI Frag=90V Gain=1.0)
Exp. RT: 1.443
Residual STD: 29970.90194
R: 0.997089
R^2: 0.99419
Formula: y = ax + b
a: 8367.28949
b: 50737.46683
c: 0.00000
d:



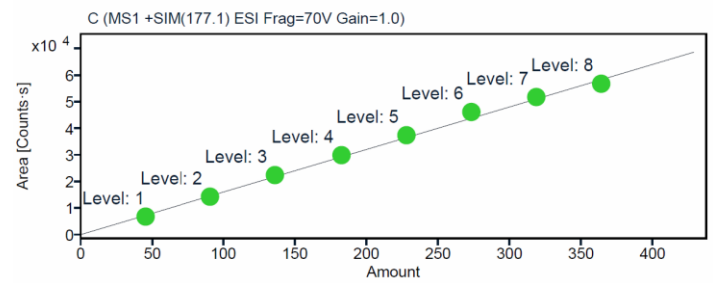
Compound: B5 (MS1 +SIM(220.1) ESI Frag=60V Gain=1.0)
Exp. RT: 3.201
Residual STD: 27922.86074
R: 0.996144
R^2: 0.99230
Formula: y = ax + b
a: 5224.71816
b: 8838.32858
c: 0.00000
d:



Compound: B 9 (MS1 +SIM(442.1) ESI Frag=60V Gain=3.0)
Exp. RT: 4.585
Residual STD: 124.54624
R: 0.994988
R^2: 0.99000
Formula: y = ax + b
a: 27.51139
b: -152.05094
c: 0.00000
d:



Compound: C (MS1 +SIM(177.1) ESI Frag=70V Gain=1.0)
Exp. RT: 1.259
Residual STD: 1165.38942
R: 0.998521
R^2: 0.99704
Formula: y = ax + b
a: 160.12034
b: -34.54976
c: 0.00000
d:



Conclusions

- A rapid, sensitive, and accurate analysis method for the identification and quantitation of water-soluble vitamins in a popular wellness supplement is presented. The method used an Agilent 1290 Infinity III LC system coupled to an Agilent Pro iQ system with a MultiMode ion source (ESI+APCI) in SIM mode. All vitamins were detected simultaneously in one run, providing improved efficiency, throughput, and cost reduction compared to traditional methods that require multiple assays.

- The method demonstrated linearity at the limits of detection, with an R^2 value of 0.99. All vitamins in the supplement product met claimed value specifications .Overall, the evaluation demonstrated that the method achieves the required specificity, linearity, and accuracy for water-soluble vitamin analysis.

References

1. Simultaneous Detection and Quantitation of 14 Water-Soluble Vitamins in Supplement by LC/MS/MS Triple-Quadrupole

https://www.agilent.com/en/promotions/asms

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