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# Profiling of Vitamin B in Fortified Daily Nutritional Supplements Using LC-MS/MS

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## Introduction

### Abstract

This study demonstrates the usage of Agilent 1290 Infinity II LC system coupled with 6470 LC/TQ Mass Spectrometry system to screen components of Vitamin B and their forms in food supplements. The developed method provides quick, easy and cost-effective sample preparation and a short run time is well suitable for analytical as well as manufacturing industries.

### Introduction

Vitamin B, from B1 to B12 along with their derivatives, is a group of water-soluble vitamins, as present in various nutritional supplements. Such products have variable concentrations of vitamin B and their derivatives.

Few screening methods as available for vitamin B in food are suitable for limited number of B-vitamins and does not cover the various other forms [1-4]. Other available methods require usage of some reagents with specific handling requirements [1-4].

The proposed methodology follows easy to use sample preparation method of 30-minute duration and demonstrates successful screening of 12 different forms from vitamin-B. The method is found to be suitable when extended to commercially available malt based and milk solid based food supplements.

Figure 1: 1290 Infinity II, 6470 LC/TQ, Samples in PP tubes (inset)

## Experimental

### Sample Preparation

1g of powdered sample was taken on 50ml PP tube. 10ml of 10mM Ammonium acetate was added and vortexed for 5 minutes followed by sonicating in water bath at room temperature for 15 minutes. Added 10ml of Chloroform and vortexed for 5 minute. The extract was centrifuged at 10,000 RPM for 10minutes at 8°C. The turbid solutions were filtered through 0.2 µ Nylon syringe filter and injected into LCMS for analysis.

### LC/TQ Conditions

Ionization Source = Agilent Jet Stream
Nebulizer Gas = 45psi
Drying Gas = 8L/min at 200° C
Sheath Gas = 11L/min at 225° C
Capillary Voltage = + 4000 V
Nozzle Voltage = + 1250 V

### UHPLC Conditions

Mobile Phase A = 0.1%FA,10mM Amm Acetate, water
Mobile Phase B = 0.1%FA,10mM Amm Acetate, MeOH

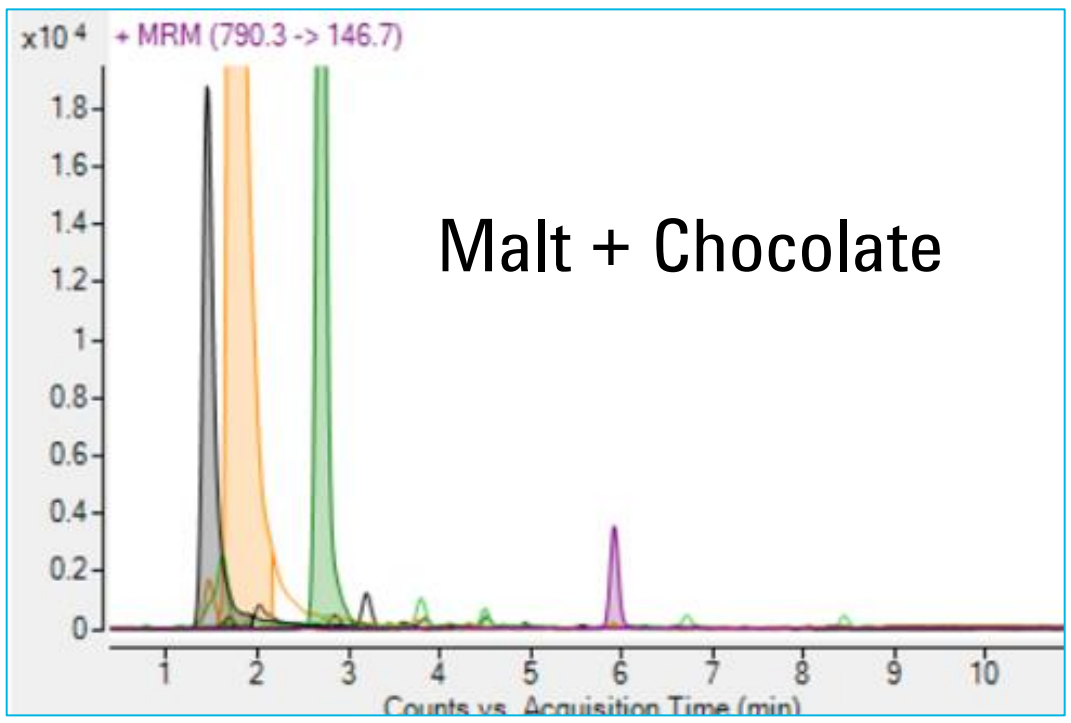
| Parameter | Value |
|---|---|
| Column | Agilent SB-Aq, 2.1mm x 50 mm x 3mm (Agilent P No 857700-314) |
| Flow Rate | 300 µl/min |
| Injection Vol | 10 µL |
| Column Temp. | 300 C |

| Time (min) | % Aqueous | % Organic |
|---|---|---|
| 0.0 | 95 % | 5 % |
| 8.0 | 5 % | 95 % |
| 10.0 | 5 % | 95 % |
| 10.10 | 95 % | 5 % |
| 15.0 | 95 % | 5 % |

Table 1: HPLC parameters and gradient program

## Malt based food supplement

The malt-based samples showed presence of Vitamin B1, B2, B5, B7 and B9. The abundance among forms of B6 was variable especially for Pyridoxal and Pyridoxamine. Similarly, the abundance among 4 forms of vitamin B12 was observed. However, for confirmation and quantity, a quantitative analysis with respective reference standards is useful.



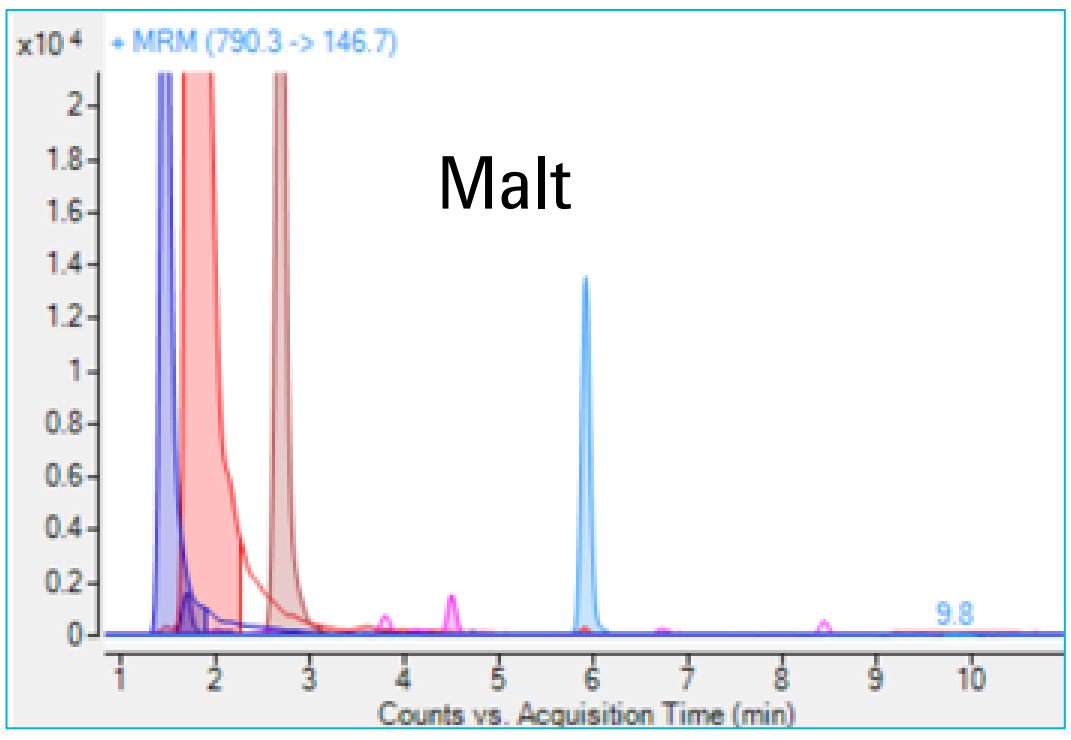
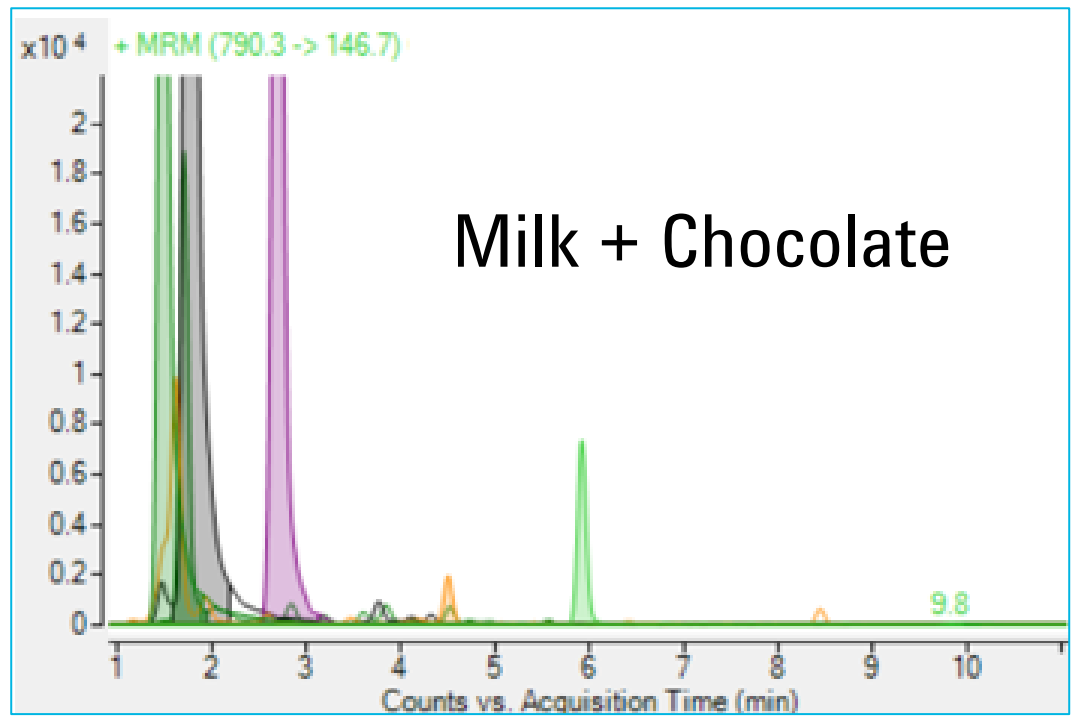




Figure 2: Chromatogram in malt-based samples

## Milk based food supplement

The milk-based samples showed presence of Vitamin B1, B2, B5, B7 and B9. The abundance among forms of B6 was variable especially for Pyridoxamine. Similarly, the abundance among 4 forms of vitamin B12 was observed. However, for confirmation and quantity, a quantitative analysis with respective reference standards is useful.



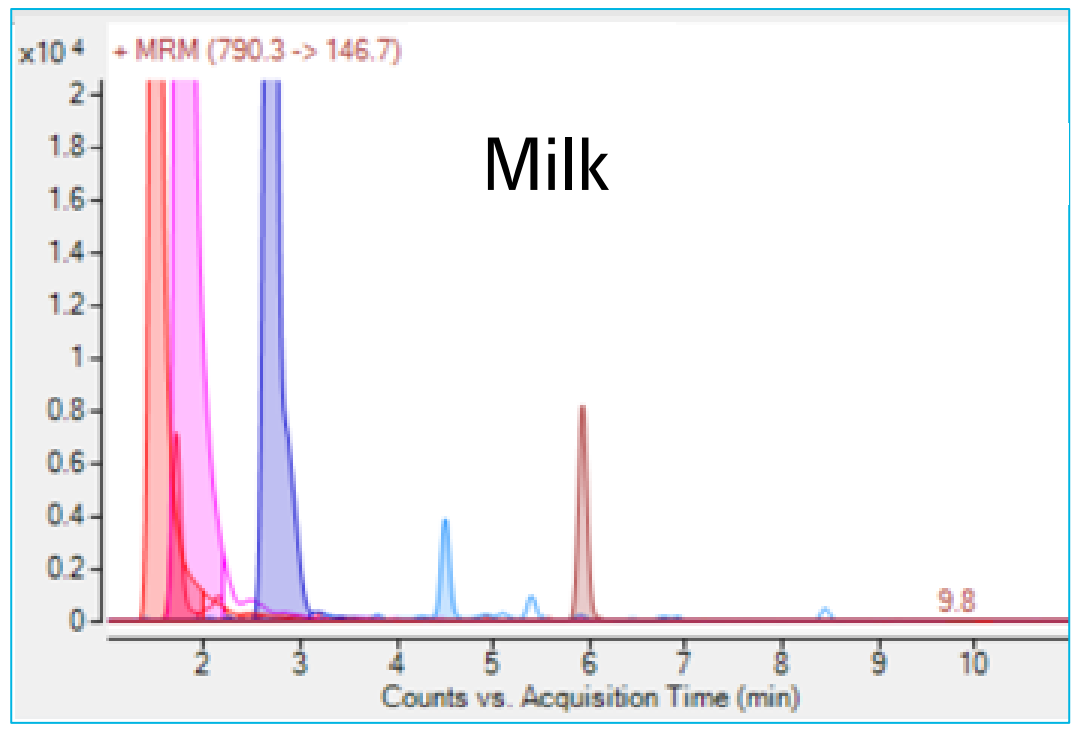




Figure 3: Chromatogram in milk-based samples

## Results and Discussion

The developed method was useful in screening 12 forms among vitamin B. The sample preparation step was fast, easy and cost effective. The MRM method was able to detect various form of vitamin B12, which are generally fortified at very low level among all vitamins. There are variations observed among different food supplements, especially in vitamin B6 and vitamin B12. It is necessary to evaluate the differences using individual certified reference materials (CRMs).

| S. No | Analyte | Malt Chocolate | Malt | Milk Chocolate | Milk |
|---|---|---|---|---|---|
| 1 | Thiamine (B1) | Present | Present | Present | Present |
| 2 | Riboflavin (B2) | Present | Present | Present | Present |
| 3 | Pantothenic Acid (B5) | Present | Present | Present | Present |
| 4 | Pyridoxal (B6) | Present | Low | Present | Present |
| 5 | Pyridoxamine (B6) | Low | Present | ND | Low |
| 6 | Pyridoxine (B6) | Present | Present | Present | Present |
| 7 | Biotin (B7) | Present | Present | ND | ND |
| 8 | Folic Acid (B9) | Present | Present | Present | Present |
| 9 | Adenosyl-cobalamine (B12) | Present | ND | Present | Present |
| 10 | Cyano-cobalamine (B12) | Present | Present | Present | Present |
| 11 | Hydroxy-cobalamine (B12) | ND | Present | ND | Present |
| 12 | Methyl-cobalamine (B12) | ND | Present | ND | ND |

Table 2: Distribution of various form of vitamin-B among food supplements (Present = high response; Low = low abundance; ND = could not detect).

## Conclusions

- Quick and easy sample preparation steps are used.
- 12 different forms of vitamin B food supplements are demonstrated to be analyzed by a single method.
- LC-MS/MS based methods can be useful in screening of Vitamin B among various malt- and milk-based food supplements.

## References


1. Agilent Publication No 5991-0647EN
2. Agilent Publication No 589-7084EN
3. Baiyi Lu et al, Simultaneous Determination of Four Water-Soluble Vitamins in Fortified Foods by Ultra-Performance Liquid Chromatography Coupled with Trip[le Quadrupole Mass Spectrometry; J of Chr. Sc., 46, 2008.
4. AOAC Official Method 2011.10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