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Quantitative analysis of vitamin B complex in Dietary supplement powder by LC-MS/MS

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Introduction

Water-soluble B vitamins are essential nutrients required for the normal functioning of human body. It is important to obtain them from food sources as they are not produced in adequate amounts in human body. Several named vitamin deficiency diseases like Beriberi, Pellagra, Epilepsy etc. may result from the lack of sufficient B vitamins. Since a large percentage of Vitamin B comes from animal sources, many vegetarians and vegans are deficient in Vitamin B, and the only way to meet the recommended daily allowance is through dietary supplements. Hence, simultaneous quantitative analysis of water-soluble vitamins (B complex) with largely different contents is required.

Multiple Reaction Monitoring (MRM) based LC-MS/MS techniques are widely used on triple quadrupole platforms for quantitation. Here, UFMS (Ultra-Fast Mass Spectrometry) technology and the IonFocus™ technology of LCMS-8060NX (Shimadzu Corporation, Japan) has been used for rapid, reliable and sensitive quantitation of Vitamin B complex in complex matrix like supplement powder. Shimadzu Shim-pack GIST C18AQ column was used to retain polar vitamins with reversed phase chromatography.

Figure 1 Supplement powder

Methods and Materials

The B complex standards were procured from Sigma Aldrich. Further all individual standards stock solution were prepared in water. Further mixture of all stock solution was prepared in water. This stock was serially diluted to prepare the calibration levels ranging from 0.1 ppb to 1000 ppb.

Figure 2 LCMS-8060NX triple quadrupole mass spectrometer

LCMS-8060NX coupled with Nexera series by Shimadzu, set a new benchmark in UHPLC coupled with triple quadrupole. Nexera series with its Automated support functions utilizing digital technology, such as M2M, IoT, and Artificial Intelligence (AI), that enable higher productivity and maximum reliability. LCMS-8060NX with an unsurpassed sensitivity (UFsensitivity),ultra fast scanning speed of 30,000 u/sec (UFscanning) and polarity switching speed of 5 msec (UFswitching). This system ensures highest quality of data, with very high degree of reliability.

Sample extraction

Commercially available dietary supplement powder sample with label claim for vitamins was used for analysis. The sample preparation method is as given below.

Take 1 gm of sample into 50 mL tube. Add 10 mL of MS grade water, vortex for 20 min.
Allowed to stand for 5 min.

↓

Add 30 mL 1% acetic acid . Vortex for 20 min.

↓

Centrifuge at 5000 rpm for 5 min. at 15°C.

↓

Draw 1 mL of supernatant layer and dilute it to 5 mL with MS grade water.

↓

Filter the above solution using nylon 0.22 micron filter.
Transfer to HPLC vial and inject.

LC-MS/MS analysis

All vitamins were analysed using ultra high-performance chromatography (UHPLC) Nexera X3 coupled with LCMS-8060NX triple quadrupole system (Shimadzu Corporation Japan). The details of analytical conditions are given below.

Table 1: UHPLC conditions (Nexera X3 system)

Parameter	Value
Column	Shim-pack GIST C18AQ 100mm×4.6mm, 3.0 µm
Mobile Phase	A : 5 mM Ammonium acetate containing 0.1 % Formic acid in water B : 100% MeOH
Flow rate	0.4 mL/min
Injection Vol	20 µL
Column Temp	40 °C
Needle wash	Water: Methanol (1:1 v/v)

Table 2: Gradient Program

Time (mins)	%A Conc.	%B Conc.
0.00-1.5	100	0
1.5-4.5	30	70
4.5-5.5	30	70
5.5-8.0	0	100
8.0-9.0	0	100
9.0-10.0	100	0
10.0-12.0	100	0

Table 3: MS conditions (LCMS-8060NX) Ionization: ESI, Positive MRM mode

Parameter	Value
Nebulizing gas flow	3 L/min
DL Temperature	200 °C
Interface Temperature	350 °C
Heat Block Temperature	200 °C
Drying gas flow	5 L/min

Results

The analysis was performed using aqueous standard. Auto MRM optimization feature was used for optimization for MRM transition. Linearity studies were carried out using external calibration method.

The calibration levels were prepared and injected in full scan MRM mode. The representative calibration curve for few vitamins are shown in Figure 3 and correlation coefficient >0.99 was observed for all vitamins.

Table 4: Sample results

Concentration of Vitamins in Supplement sample (mg/100g)							
Vitamins	B1	B2	B3	B5	B6	B9	B12
Sample-1	1.29	1.49	11.87	4.67	1.77	0.19	0.0014
Label Claim	1.5	1.5	12.0	5.0	1.8	0.20	0.0015

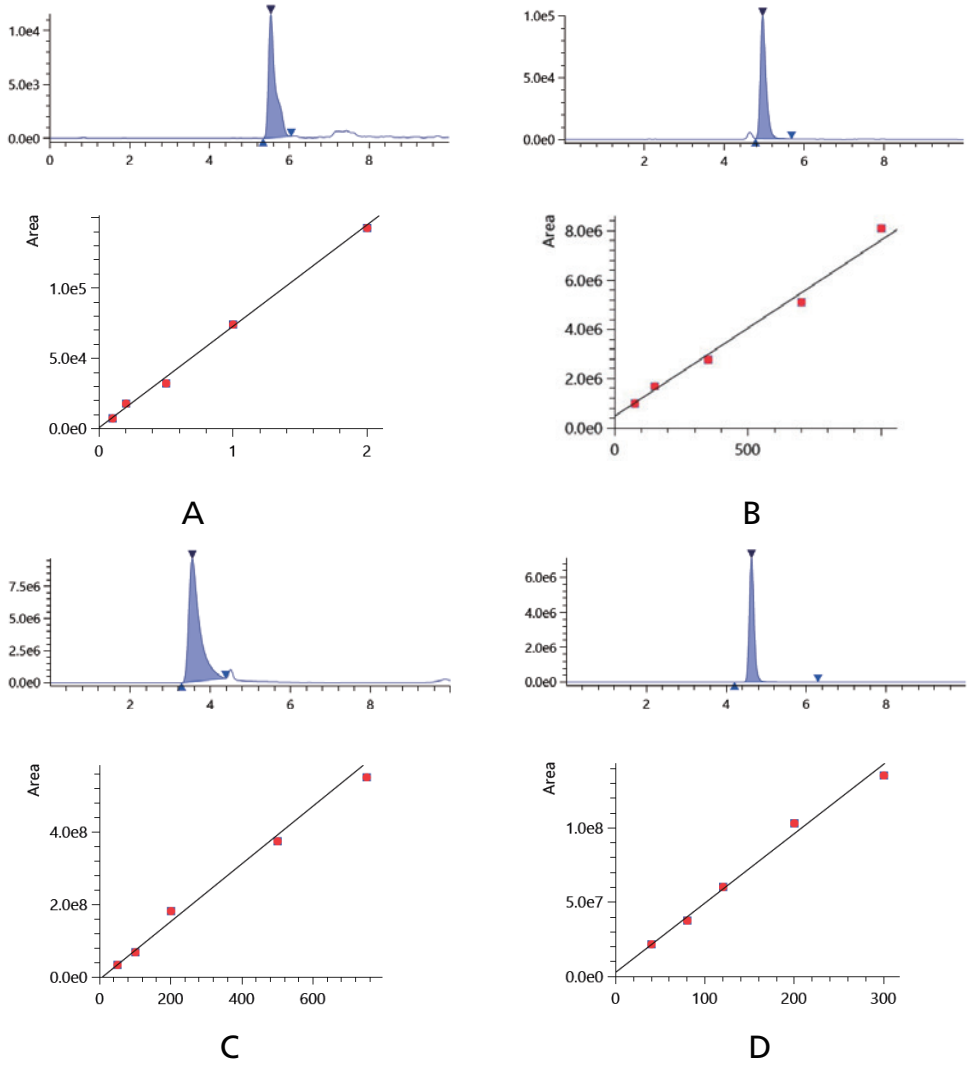

Figure 3: A for vitamin B9, B for vitamin B3, C for vitamin B1 and D for vitamin B6

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Conclusions

- Method was found to be simple, sensitive and rapid with easy sample preparation covering wide detection range for vitamins.
- Results matches with the label claim values (Table:4).

Reference

- ISO 21470/2020 guideline
- Ayano Kakitani, Tomonori Inoue, Keiko Matsumoto, Jun Watanabe, Yasushi Nagatomi & Naoki Mochizuki (2014) Simultaneous determination of water-soluble vitamins in beverages and dietary supplements by LC-MS/MS, FoodAdditives & Contaminants: Part A, 31:12, 1939-1948

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