SHIMADZU

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Rapid Analysis of Water-Soluble Vitamins in Peanut Butter using LCMS-2050

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1. Introduction

Peanut butter is a widely consumed food product that contains various essential nutrients, including water-soluble vitamins (Fig. 1), which are vital for maintaining human health. Fortified peanut butter is specifically formulated to aid in the nutritional rehabilitation of malnourished children, particularly in low-resource regions. Accurate quantification of these essential nutrients is crucial for proper labeling and quality control. However, traditional analytical techniques for vitamin analysis often involve extensive sample preparation and multiple detection methods, making the process time-consuming and labor-intensive. This study introduces a rapid and sensitive approach for the analysis of water-soluble vitamins in fortified peanut butter, utilizing a simplified extraction method[1,2].

In this study, Shimadzu's LCMS-2050, a single quadrupole mass spectrometer coupled with LC-2050C systems (Fig. 2) is demonstrated as an effective solution for the efficient and reliable analysis of water-soluble vitamins in fortified peanut butter.

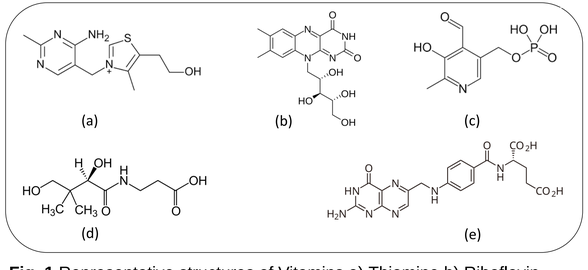

Fig. 1 Representative structures of Vitamins a) Thiamine b) Riboflavin, c) Pyridoxine d) Pantothenic acid e) Folic acid.

2. Methods and Materials

The reference standards of Thiamine (B1), Riboflavin (B2), Niacin (B3), Pyridoxine (B5), Pantothenic acid (B6), and Folic acid (B9) are sourced from a local supplier. The reagents such as sodium hydroxide, acetic acid, ammonium acetate, methanol are also procured from a local supplier. The fortified and unfortified peanut butter are sourced from local market.

Approximately 25 mg of individual standard is taken into 25 ml separate standard flask, makeup to the mark with distilled water, except folic acid and riboflavin. Since these two compounds have limited solubility in water, 50 µL of 1 M NaOH is added to each. Additionally, 10 µL of acetic acid is added to the riboflavin standard to aid in dissolution.

The calibration curve for the multivitamin standards is prepared based on the labeled fortification levels of vitamins in the peanut butter, taking into account the dilution factor used in the extraction procedure. For this analysis, five calibration levels ranging from 50% to 300% of the label claim are prepared in water. The calibration curve generated by using external standard calibration method and weighted regression of 1/C. Calibration standards and samples are injected into the LCMS-2050 system. The analytical parameters used for the analysis are summarized in Table 1.

The Shimadzu's LCMS-2050 is a compact, high-performance single quadrupole mass spectrometer designed for seamless integration with LC systems. It features a Heated Dual Ion Source (DUIS), combining ESI and APCI for broad compound coverage. This hybrid ionization enables simultaneous analysis of both high and low polarity compounds in one run. It's user friendly design enhances sensitivity while streamlining lab workflows.

Fig. 2 Shimadzu LCMS-2050 and LC-2050C.

Table 1: LC-MS method parameters.

Column	Shim-pack GIST C18, 4.6 mm x 250 mm; 5 µm
Mobile phase	A: 20 mM Ammonium acetate in water
Mobile phase	B: Methanol
Flow rate	1 mL/min
Gradient	10% B (0.01→1.50 min) → 50% B (10 →14 min) → 10% B (14.10 →15 min)
Injection volume	2 µL
Column temperature	40 °C
Ionization mode	DUIS (Positive/Negative)
Gas flow	Nebulizing gas:- 3 L/min; Heating gas:- 7 L/min; Drying:-5 L/min
MS temperature	Desolvation temperature :- 200 °C; DL Temperature:- 250 °C

Approximately 1 g of peanut butter is weighed into 50 mL centrifuge tubes, and 10 mL of water is added to each. Subsequently, 200 µL of 1 M NaOH and 50 µL of acetic acid are added. The mixtures are vortexed for 5 minutes and then centrifuged at 6000 rpm for 10 minutes. The supernatants are carefully collected and filtered through 0.45 µm membrane filters. The filtered samples are then diluted in a 1:1 ratio with water and injected into the LCMS for analysis. All samples, including test and spiked samples, are prepared in duplicates. Unfortified peanut butter sample is used for spiking. The spiking concentration is determined based on the label claim of the fortified peanut butter sample.

3. Results

The calibration curve showed excellent linearity, with a correlation coefficient (R^2) ≥ 0.99 and accuracy within the range of 80–120%. A recovery study was conducted by spiking known concentrations of vitamins, in duplicate, into unfortified peanut butter samples. The recoveries ranged from 70% to 120%, with relative standard deviations (RSD) found to be less than 20%. The detailed results of this study are shown in Table 2.

Table 2: Quantification results in fortified and spiked peanut butter sample.

Vitamins	Fortified Peanut R1 (mg/kg)	Fortified Peanut R2 (mg/kg)	% RSD	Spike Conc. (mg/kg)	Recovery (%)
Thiamine	19.53	19.84	1.11	20	120
Riboflavin	17.69	18.06	1.44	16	72
Niacin	8.49	8.51	0.18	50	89
Pantothenic acid	41.16	41.80	1.09	30	110
Pyridoxine	13.73	14.30	2.85	6	101
Folic acid	2.66	2.62	1.16	10	81

The observed limit of detections (LODs) and limit of quantifications (LOQs) are well below than requirement, confirming the high sensitivity and suitability of the method for nutritional analysis.

All data acquisition is conducted using Shimadzu's LabSolutions software, and quantification is carried out by using LabSolutions Insight. It is a tool to streamline data review and prioritize chromatograms that require critical attention. It automatically applies peak detection criteria to the data, flags any deviations, and enables more efficient data analysis. The calibration curves and the chromatograms are shown in Fig. 3.

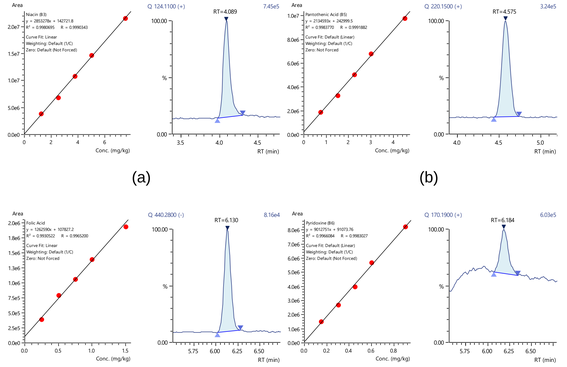

Fig. 3 Representative chromatograms of (a) Niacin (1.25 mg/kg) (b) Pantothenic acid (0.75 mg/kg) (c) Folic acid (0.25 mg/kg) (d) Pyridoxine (0.15mg/kg).

4. Conclusion

- A simple, sensitive, and rapid LCMS method is developed for the quantification of water-soluble vitamins using Shimadzu's LCMS-2050 coupled with LC-2050C systems.
- The simple extraction procedure is developed which ensures better recovery of water-soluble vitamins from fortified peanut butter sample. As well as the % recovery of spiked sample shows the efficiency of the extraction procedure.

Reference

1) 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, Food Additives & Contaminants: Part A, 31:12, 1939-1948.

2) Nitin Shukla, Durvesh Sawant, Sujit Patil, Pratap Rasam (2023) Quantitative analysis of vitamin B complex in Dietary supplement powder by LC-MS/MS.