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Analysis of Vitamins
~ Microbiological assay and HPLC ~

Introduction

Vitamins are nutrients essential for the normal functioning of the body and are organic compounds that must be taken in from the diet because they cannot be synthesized in the body or even when synthesized, their quantities are insufficient to meet the physiological demand. Currently, there are thirteen vitamins, which are broadly divided into two groups; four fat-soluble vitamins and nine water-soluble vitamins. In recent years, there has been increasing interest in health-consciousness and the functionality of foods. The contents of nutritional and functional ingredients, including vitamins, has become more important than ever. Therefore, accurate analytical data of these ingredients should be controlled and properly reflected on labeling. The Analytical methods for vitamins in the Food Labeling Standards [1)] in Japan are mainly based on microbiological assays and high-performance liquid chromatography (hereinafter referred to as HPLC). We analyzes several B-vitamins (vitamin B6, vitamin B12, folic acid, pantothenic acid, niacin, and biotin) using microbiological assay and HPLC. In this article, we introduce the characteristics of both analytical methods.

Analysis method

(1) Microbiological assay

Principle of the method

Microbiological assay is a method to quantify microorganisms that requires the vitamin of purpose as an essential nutrient. It quantifies vitamins by measuring the degree of growth when they are cultured in a medium without the vitamin.

The summary of the test method is as follows;.

1) Samples are weighed and then, pressurized extraction and enzymatic treatment are conducted to separate proteins and sugars which are converted into vitamins. These can be used by organisms for growth, and sample solutions are obtained.

2) Add the standard solution or sample solution and the test organism solution to the culture medium, and incubate in test tubes or microplates.

3) After incubation, measure the turbidity (600 nm) of the culture solution. The more vitamins are contained in the standard solution or sample solution, the greater the turbidity will be because the test organism grows and the culture solution becomes turbid (Figure 1).

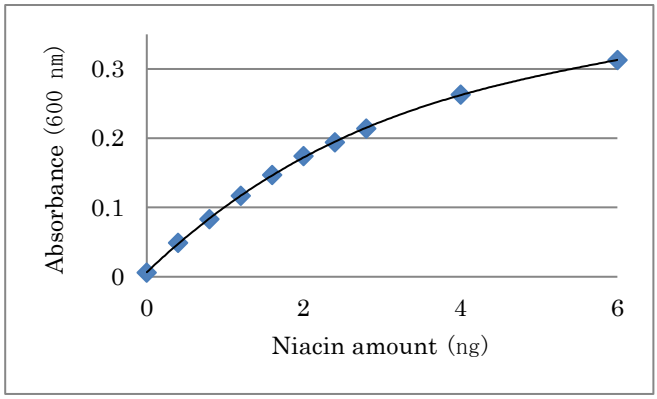

Figure 1 Example of a calibration curve for niacin.

Characteristics of the method

Microbiological assay is a highly sensitive method that can measure the targeted vitamin B to a low concentration range without being affected by impurities in the sample. It is also possible to simultaneously measure naturally originating substances and ingredients with the same activity. For example, vitamin B6 can measure pyridoxine, pyridoxal, pyridoxamine, and their phosphate forms at once and quantify the total amount of vitamin B6. On the other hand, the measurement range of the calibration curve is narrow, and the growth of the test organism may affect the results. It is also time consuming, since culture of the test organism is required. The handling of test organism requires a certain level of know-how, and is therefore a more difficult analytical method than HPLC. Other disadvantages are difficulties in separating and measuring each active ingredient.

(2) HPLC

Principle of the method

The target vitamins are extracted from the samples, separated from other impurities by HPLC columns, then detected and quantified by an ultraviolet-visible spectrophotometer or a fluorescence detector. This method is applied to samples with high levels of added vitamins so it does not require complex pretreatment.

Characteristics of the method

HPLC method is a reliable method which can obtain high precision data in a short time. It is also possible to measure the target substance individually. For example, it can measure only pyridoxine as vitamin B6. On the other hand, the detection sensitivity is less than that of microbiological assays, and it is not possible to measure total of natural vitamins such as analogs and various conjugates, simultaneously. Additionally, because of its low sensitivity and high precision character, impurities in the samples cause difficulties to obtain the results.

Comparisons between microbiological assays and HPLC

The Food Labeling Standards state examples of HPLC procedures for six vitamins (vitamin B6, vitamin B12, folic acid, pantothenic acid, niacin, and biotin) for which microbiological assay is the mainstay, and samples with high contents, such as health foods and vitamin preparations, can be analyzed by HPLC. Vitamin B6, folic acid, pantothenic acid, and niacin in these samples can generally be analyzed by HPLC, but vitamin B12 and biotin are generally contained in low quantities in foods, which makes the kinds of applicable sample limited. Table-1 compares the microbiological assay with HPLC

Table-1 Compares between microbiological assays and HPLC

	Microbiological assay	HPLC
Measurement sensitivity	Measurable from low to high concentration range	Measurable in the high concentration range
Measurement range of the calibration curve	Narrow	Wide
Analysis time	Require time	Can be analyzed in a short time
Analytical precision	Be slightly inferior	Precise
Test procedures	Require pretreatment	No complex pretreatment required
Influence of impurities	Low possibility to be affected by impurities in the sample	Impurities in the sample may preclude analysis.
Substances measured	Measured as total amount including conjugated vitamins and analogs	Individual measurement of target substances
Applied sample	General foods (fresh foods, processed foods, and health foods such as supplements), feeds	Health foods supplemented with vitamins for ingestion and reinforcement purposes (supplements, etc.) and vitamin preparations (premixes, etc.)

[1] Quantitation limit

Microbiological assays are very sensitive for detection. Our quantitation limit of HPLC method is 0.5 mg/100 g, but microbiological assays can measure from 0.03 μg/100 g to 0.05 mg/100 g. It is 10 to 10,000 times of HPLC (Table-2).

Table-2 Quantitation limits

Composition measured	Microbiological assay	HPLC
Vitamin B6	0.003 mg/100g	0.5 mg/100g (500 μg/100g)
Vitamin B12	0.03 μg/100g	
Folic acid	1 μg /100g	
Pantothenic acid,	0.05 mg/100g	
Niacin	0.03 mg/100g	
Biotin	0.3 μg/100g	

[2] Correlation between the two assays

Table-3 shows the values of vitamin B6 in three dietary supplements when analyzed by both microbiological assay and HPLC. Results show samples with high concentrations of vitamins have similar values using both methods.

Table-3 Comparable data of vitamin B6 values by microbiological assay and HPLC

Sample	Microbiological assay (mg/100g)	HPLC ※1 (mg/100g)
Tablet A	64.9	68.9
Tablet B	1,140	1,070
Tablet C	6,850	6,810

※1 Values converted from pyridoxine hydrochloride to vitamin B6. Conversion factor ;0.8227

[3] Analytical precision

Analytical precision of microbiological assay and HPLC is shown as the relative standard deviations (%) of the measured values (Table-4). Supplements added with high concentrations of each vitamin were analyzed 10-20 times, and their relative standard deviations (standard deviation/mean × 100) were calculated. The precision of HPLC analysis is better for all vitamins.

Table-4 Analytical precision [Relative standard deviation (%) of measurements].

Measurement component (concentration level)		Microbiological assay	HPLC
Vitamin B6	(1,000 mg/100 g)	10.1	2.69
Folic acid	(50 mg/100 g)	7.68	2.12
Pantothenic acid	(2,000 mg/100 g)	5.62	3.01
Niacin	(4,000 mg/100 g)	3.11	1.71

Closing

The microbiological assay has excellent detection sensitivity and enables the measurement of target vitamins in low concentration range, and enables the determination of the total amount of target vitamins, including naturally originating substances and analogs. However, the measured values have low reproducibility because they are affected by the growth of organisms, and the analytical precision is inferior to that of HPLC. The lacking reproducibility of the measured values may be due to changes in the conditions of the test organism, culture media, or seasonal variation. On the other hand, HPLC can be analyzed with high precision in a short time. It is a very useful analytical method when we want to analyze the amounts of vitamins in supplements and premixes which are supplemented with vitamins for fortification purpose as well as the change in the amount during their storage.

Vitamin B12 and biotin, which are not listed in Table-4, can also be analyzed depending on the amount added and the matrix of the sample.

In Japan, the Food Labeling Standard shows microbiological assay prior to HPLC for vitamin B6, vitamin B12, folic acid, pantothenic acid, and biotin. The official methods in the Food Labeling Standards is used for sampling inspections by the authorities. It is not mandatory to use official methods to obtain the data for labelling. You can use suitable method according to the sample matrices. Microbiological assay is applicable to various matrices of common food samples and able to detect low level of vitamins. For samples with added vitamins and when formulation is obvious, HPLC is applicable.

We are looking forward you contact regarding vitamin analysis.

References

1) Consumer Affairs Agency: Food Labeling Standards (Digestion Table No. 139 of March 30, 2015) Attachment Analytical Method of Nutrient Ingredients, etc.