

# Determination of watersoluble B – Vitamins with VitaFast® Tests in Cereals

VitaFast® – microbiological microtiterplate test for quality control procedures of folic acid, vitamin B12 (cyanocobalamin), vitamin B7 (biotin), vitamin B3 (niacin), vitamin B5 (pantothenic acid), vitamin B1 (thiamine), vitamin B2 (riboflavine), vitamin B6 (pyridoxine) and inositol

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## Introduction

An increasing number of food products is enriched with vitamins. The vitamin content is monitored by manufacturers and control authorities to check compliance with labelling regulation. Cereal based vitamin fortified products are difficult to analyse. In order to determine the total vitamin content (natural and added vitamins) an enzymatic treatment is necessary to release the vitamin from the matrix. For the different watersoluble B-vitamins different enzymes and sample preparations are necessary. This is why the manufacturer developed and validated sample preparation protocols for the vitamins in cereal products.

The golden standard in vitamin analysis was and is still the microbiological method. But the traditional microbiology is very tedious as microorganisms have to be cultured and stored, assay-mediums have to be controlled, etc. Chromatographic methods such as HPLC are also often the preferred method, but because of the low concentrations of folic acid, vitamin B12 and biotin, the measurement often presents problems with HPLC.

The ifp, Institute of Product Quality, Berlin, has developed a series of microbiological vitamin analysis products with a ready-to-use microtiter plate format. The trade name is VitaFast® and the test kits are distributed by R-Biopharm, Darmstadt.

## Method

The vitamin concentration in cereal materials was determined by using innovative microbiological assays in test kit format (VitaFast®). R-Biopharm presents a system of watersoluble B-vitamin determination which is rapid and based on AOAC, EN and DIN reference methods.

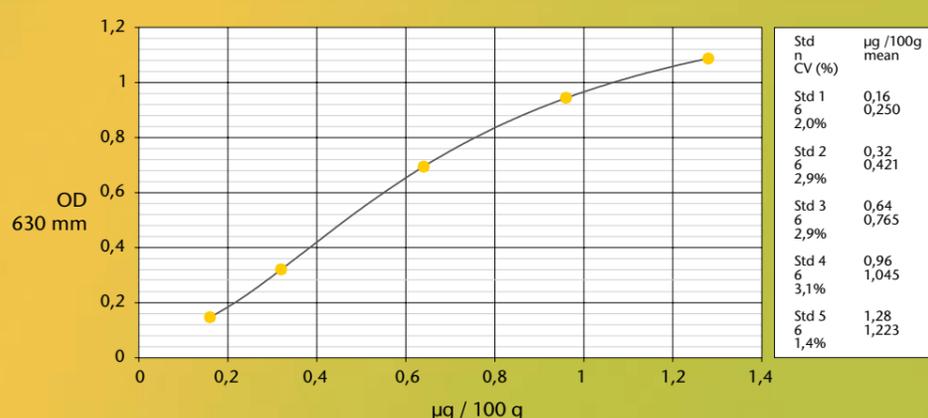
### Cereal sample preparation for Folic Acid (total content of natural and added):

- weigh exactly 1 g (ml) homogenized sample and 20 mg pancreatin into a 50 ml sterile centrifuge vial
- add 30 ml phosphate buffer (0.05 mol / l; 0.1 % ascorbate; pH 7.2), shake well and fill up to 40 ml with phosphate buffer
- incubate 2 h at 37 °C (98.6 °F) in the dark (shake at times); thereafter heat 30 min at 95 °C (203 °F) in a water bath; chill down quickly to below 30 °C (86 °F)
- transfer 1 ml of the sample extraction in a 1.5 ml sterile reaction vial and centrifuge 5 min (greater than 8,000 x g)

After the sample extraction of the vitamins, each 150 µl of the assay-medium and the diluted extract or standard are pipetted into the wells of a microtiter plate which is coated with specific microorganisms. The growth of the microorganisms is dependent on the supply of vitamin. Following the addition of standard or as a compound of the sample, the bacteria grow until the vitamin is consumed. The incubation is carried out in the dark at 37 °C (98.6 °F) for 44 - 48 h. The intensity of metabolism or growth in relation to the extracted vitamin is measured as turbidity and compared to a standard curve. The measurement is done using an ELISA reader at 610 - 630 nm (alternatively at 540 - 550 nm).



The VitaFast® test kit contains a microtiter plate (96 wells) coated with microorganisms, an additional holder, 3 adhesive foils, 3 bottles assay-medium, 3 standard bottles, 3 buffer bottles and 3 bottles sterilized water. The test procedure further requires sterile single disposable materials and a microtiter plate photometer. The VitaFast® test kits are ideal for routine analysis since the reagents are ready-to-use and the kit is very user-friendly. Therefore, a lot of food producers are now able to carry out vitamin analysis in-house.



The standard curve from the quality assurance certificate for VitaFast® Folic Acid measured at 630 nm. The coefficient of variation (CV) of the standards is smaller than 10 %. All test kit components are quality controlled, the purity of the assay-medium is also checked by the ISO certified manufacturer ifp.

## Validation and Quality Control

### Cereal Reference Materials

	Vitamin	Target concentration	Amount recovered with VitaFast®	CV's (%)	Percentage of target recovered (%)
FAPAS® 2148 Vitamins in Breakfast Cereal	Vitamin B2 (Riboflavin) (mg/100g)	1.99 (1.58-2.40)	2.17	5.3	109
	Vitamin B6 (Pyridoxin) (mg/100g)	2.05 (1.63-2.47)	2.08	1.5	101
	Niacin (mg/100g)	20.8 (17.8-23.8)	23.1	2.9	111
	Folic Acid (µg/100g)	438 (326-550)	499	7.9	114
AACC VMA 399	Vitamin B2 (Riboflavin) (mg/100g)	5.97 (4.89-7.60)	5.79	3.8	97
	Vitamin B12 (µg/100g)	21.2 (12.2-25.0)	20.8	6.1	98
	Pantothenic Acid (mg/100g)	37.4 (31.0-41.9)	37.8	5.8	101
	Folic Acid (µg/100g)	1395 (1160-1620)	1363	5.0	98
BCR CRM 121 (wholemeal flour)	Folic Acid (µg/100g)	50 (43-57)	48.5	7.7	97

### Flour Samples

	Vitamin	Target concentration	Amount recovered with VitaFast®	CV's (%)	Percentage of target recovered (%)
Multivitamin Crispies	Biotin (µg/100g)	4000	4039	5.4	101
	Vitamin B2 (Riboflavin) (mg/100 g)	0.75	0.64	5.8	85
	Vitamin B12 (µg/100g)	1.25	1.03	4.4	82
	Niacin (mg/100g)	6.9	6.9	3.3	100

### Folic Acid in Grain

	Target concentration* (µg / 100 g)	Amount recovered with VitaFast® (µg / 100g)	CV's (%)
Oat	35	39.1	7.6
Maize	25	22.1	4.6
Brown rice	16	22.1	3.7
Wheat flower	10	12.8	2.5

\* according to literature: Souci Fachmann Kraut; natural variation is expected

CV = Coefficient of variation (n=4)

## Discussion

Among other food a range of cereal samples like AACC VMA 399, BCR CRM 121 and FAPAS® materials were successfully tested with the microbiological VitaFast® tests. In cooperation with ifp, the VitaFast® parameters, folic acid, vitamin B12, niacin, pantothenic acid, vitamin B1, B2, pyridoxine and inositol were validated for the cereal samples mentioned above. The VitaFast® microtiter plate system has excellent handling and performance characteristics. Unlike other immunological assay systems, no washing steps are required. The tests excel through high accuracy and precision. The coefficient of variation (CV) is below 10%. All test steps and test reagents (microorganisms, assay-medium and standard) are optimally adjusted in harmony with each other. Compared to traditional microbiological vitamin assays, the time required usually for VitaFast® tests assay performance is roughly 60 – 70% less, and the materials consumption is around 30 times lower. In conclusion these microbiological tests can be used to determine successfully the added, natural and total vitamin content in food. The sample preparation varies accordingly.