R-Biopharm

Vitamin Determination in Infant Food and Enteral Clinical Nutrition

VitaFast® - microbiological test kits for quality control procedures of vitamin B12 (cyanocobalamin), folic acid, biotin and vitamin B5 (pantothenic acid)

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Introduction

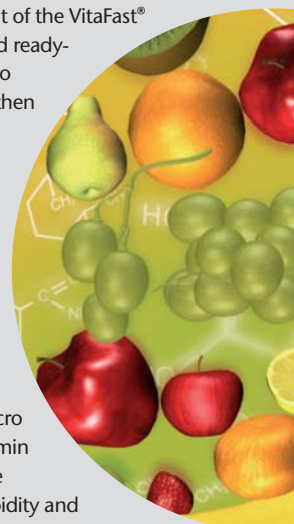
Infant food and food for special medical purpose (FSMP) are highly complex food matrices. They are made by blending fats, proteins and carbohydrates; vitamins as premixes are frequently added to increase the nutritional value. The raw materials used are proteins (e.g. animal milk or soybeans), fats and carbohydrates, diluents (e.g. skim milk or purified water), vitamins and emulsifiers/stabilizers. The natural vitamin content of the raw materials is subject to seasonal variation and has also to be taken into account since the "total vitamin content" (native + added) must be labelled as ingredients.

During the manufacturing process of mixing, pasteurisation and homogenisation significant vitamin losses can occur. This is why the manufacturer has to evaluate new formulations and the effect of changes in ingredients or processing conditions by means of a testing program designed to confirm uniformity of batches. The gold standard in vitamin analysis was and still is the microbiological method, as it allows the determination of both native and added vitamins. A further advantage is that vitamin B12, folic acid and biotin are also measured at low concentrations, which otherwise presents problems for HPLC analytics.

In cooperation with the Central Laboratories Friedrichsdorf GmbH, Germany, four VitaFast® parameters, biotin, pantothenic acid, folic acid and vitamin B12, were validated for infant milk formula (IMF), milk cereals and enteral clinical nutrition (ECN).

Method

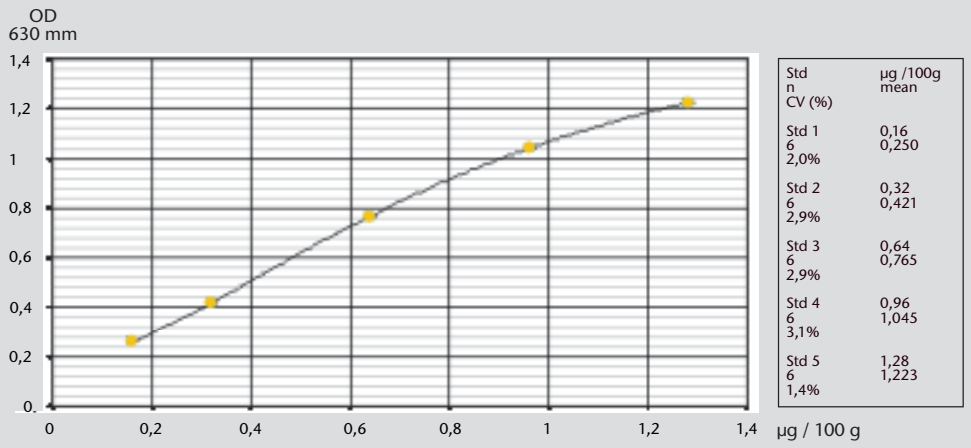
The VitaFast® tests are based on the traditional microbiological method. The advancement of the VitaFast® product line has resulted in all of the test components being available in standardized and ready-to-use forms. For example, the microtiter plate wells are already coated with specific micro organisms, and the medium and standard only have to be reconstituted with water and then be pipetted into the wells. Compared with traditional microbiological methods, there is no costly cultivation and storage of bacteria in a strain collection, or the time-consuming verification of the purity of the medium and the precise standard concentration.



Prior to analysis the vitamins are extracted from the food matrix. For the determination of added vitamins, a hot water extraction is usually sufficient. For measuring the total vitamin content, including the native vitamins, the vitamins are extracted by specific enzymatic treatment.

The assay-medium and the diluted sample extract or standard are pipetted into the wells of a microtiter plate which is coated with specific micro organisms. The growth of the micro organisms is dependent on the concentration of vitamin, 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 carried out using a microplate 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 (filters, tips, vials) and a microtiter plate photometer. The user-friendly VitaFast® product line has led to an increasing number of labs returning to performing their vitamin analytics themselves, because, compared with "traditional microbiology", VitaFast® is cheaper and faster.



The standard curve from the quality assurance certificate for VitaFast® Folic Acid is measured at 630 nm. The quality controlled VitaFast® test systems are a reliable measuring technique with low variation coefficients (< 10 %), which largely eliminate the need for repetition of tests. All test kit components are quality controlled, the purity of the assay medium is also checked by the ISO certified manufacturer ifp. The whole product line has been validated with internationally available reference materials from NIST, CRM and AACC and also in inter-laboratory studies. All these quality measures ensure a high analytical performance for the end user.

FAPAS® Proficiency Test 2143

Vitamin B12 in powdered baby food

Target value in µg / 100 g	Tolerance range in µg / 100 g	VitaFast® Vitamin B12 in µg / 100 g
1.73	0.97 - 2.5 (1.73 +/- 0.77)	Lab 29: 1.76 Lab 30: 1.69 Lab 39: 1.68

In January 2007, VitaFast® Vitamin B12 (Cyanocobalamin) test kits were successfully employed in the FAPAS® Proficiency Test 2143 "Vitamins in powdered baby food". 3 participants used VitaFast® test kits as the method of choice and all VitaFast® users obtained excellent results lying very close to the target value.

Vitamin content during processing

Folic acid concentration (µg / 100 g) of different infant formulas

	Raw material (VitaFast®)	Premix (label)	Expected conc. (calculated)	Measured conc. (VitaFast®)
Solid cereal / milk	12	97	109	104
Solid cereal / milk	10	107	117	113
Liquid milk	0.2	44	44	43
Liquid milk	0.2	31	31	33

Pantothenic acid concentration (mg / 100 g) of different infant formulas

	Raw material (VitaFast®)	Premix (label)	Expected conc. (calculated)	Measured conc. (VitaFast®)
Solid cereal / milk	1.4	1.9	3.3	3.1
Solid cereal / milk	1.4	1.9	3.3	3.3
Liquid milk	0.3	0.9	1.2	1.1
Liquid milk	0	0.9	0.9	0.9

The different production steps in the manufacturing process of different infant formulas were evaluated. The vitamin content of the raw material and the end product was determined with VitaFast® test kits. The expected concentration of the final end product is the concentration of the raw material plus the premix concentration (taken from the certificate of the premix manufacturer). The results obtained with VitaFast® for folic acid and pantothenic acid are very consistent and accurate.

Discussion

The VitaFast® product line showed reproducible and consistent results for the different production steps in the manufacturing process. The extraction procedures in general included hydrolysis and digestion with an appropriate enzyme.

Problems were observed when analysing hypoallergenic food (containing only fragments of cow's protein): No correlation was observed between the expected and the measured vitamin concentrations. For complex food matrices such as infant food and other specific foods further refinement of the extraction is necessary with respect to nature of the food matrix, the form in which the vitamin occurs naturally or is added (different bound forms of vitamins are often found in meat, plant and dairy products), the nature and relative amounts of potentially interfering substances and the stability of the vitamin towards heat and extremes of pH.

The challenge is that the extraction should also reflect the bioavailability of the vitamin by the body: vitamins existing as chemically bound complexes in the food matrix exhibit lower efficiencies of digestion and absorption compared with free (unbound) vitamin ingested, for example, in tablet form.

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