R-Biopharm

Vitamin Determination in Infant Food and Enteral Clinical Nutrition

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

Introduction

Infant food and food for special medical purposes (FSPM) 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, vitamins (e.g. skin milk or purified water), vitamins and minerals/trace elements. The native vitamin content of the raw materials is subject to natural variations and has also to be taken into account since the “total vitamin content” (native + added) must be labeled as ingredients.

During the manufacturing process of mixing, pasteurizing and homogenization 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 conformity of batches. The gold standard in vitamin analysis was and still ist the microbiological method. The vitamin content, including the native vitamins, the vitamins are extracted by specific microorganisms and are also measured at low concentrations, which otherwise present problems for HPLC analysis.

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 formula and enteral formula (EFM).

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 microorganisms, 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 stock collection, and the time-consuming verification of the purity of the microorganisms is eliminated.

Prior to analyze the samples 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 incubated with specific microorganisms. The growth of the microorganisms is dependent on the concentration of vitamins. The bacteria grow with the vitamin present. 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 native vitamin is measured as turbidity and compared to a standard curve. The measurement is carried out using a microplate reader at 630 ± 20 nm (alternatively at 410 – 530 nm).

The VitaFast® test kit contains a microtiter plate (96 wells) coated with microorganisms, an additional buffer, and a microtiter plate photometer. The user-friendly VitaFast® test kit allows an increasing number of labs returning to performing their vitamin analysis themselves, because compared with traditional microbiology, VitaFast® is cheaper and faster.

Discussion

The VitaFast® product line has shown reproducible and consistent results for the different production steps in the manufacturing process. Hydrolysis and digestion with an appropriate enzyme have been included at extractions procedures. Future development will focus on sample preparations of specialized food such as hypoallergenic formulae (containing only milk protein fragments).

Refinement of the extraction is necessary in complex food matrices such as infant food and products for specialized nutrition. There are a number of difficulties to overcome like matrix effects, different derivates of vitamins included in all extraction procedures.

The standard curve from the quality assurance certificate for VitaFast® Folic Acid is measured at 450 nm. The quality-controlled test systems are a multiple measuring technique with low variance coefficients (< 10 %), which largely eliminates the need for repetition of tests. All test kit components are quality controlled, the purity of the assay medium is checked by the ISO certificated 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.