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Original Article

Development of two reference materials for all trans-retinol, retinyl palmitate, α- and γ-tocopherol in milk powder and infant formula

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ABSTRACT

Vitamins are important food constituents that can be present in almost every foodstuff. Food quality and safety depends on food surveillance by reliable quantitative analysis enabled by appropriate quality control. Certified matrix reference materials are versatile tools to support quality assurance and control. However, in the case of vitamins, which are important in various foods, there is a lack of matrix reference materials. Two certified reference materials for the determination of all–*trans*-retinol, retinyl palmitate, and α- and γ-tocopherol in milk powder and infant formula have been developed by the National Institute of Standards, Egypt. This article presents the preparation, characterization, homogeneity, and stability testing as well as statistical treatment of data and certified value assignment. The assignment of the certified values and associated uncertainties in the prepared natural-matrix reference materials were based on the widely used approach of combining data from independent and reliable analytical methods.

1. Introduction

Infant formula as a breast-milk substitute must be manufactured to satisfy, by itself, the nutritional requirement of infants during the first months of life up to the introduction of appropriate complementary food. The relationship between nutrient levels and human health requires accurate determination of nutrient levels by laboratories making such measurements. Food control laboratories are required to use validated methods wherever possible. For this reason, analytical methods must be subjected to internal and/or external validation studies. Food and feed reference materials and especially certified reference materials play a key role in internal validation studies. They are a useful tool in the verification of the accuracy of analytical measurements and are employed in analytical quality assurance, quality control

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schemes, laboratory accreditation, as well as in the establishment of traceability in the framework of internationally agreed standards [1–3]. They can also be used for the process of measurement uncertainty estimation or the calibration of analytical instruments. At present, the quality of feed and foodstuffs is estimated not only using ecological indices (concentration of toxic elements, mycotoxins, etc.), but also by the concentration of valuable biologically active substances (amino acids, vitamins, provitamins, etc.). The concentration of biologically active substances, vitamins in particular, in foodstuffs is strictly regulated, because both the deficiency and excess of vitamins can cause harmful effects [4]. Vitamins have been divided into two groups based on their solubilities in fat solvents or in water. Thus, fat-soluble vitamins include A, D, E, and K, while vitamins of the B-complex and C are classified as water-soluble. In general, vitamin A refers to all–*trans*-retinol, which is the most active form of this vitamin, while vitamin E is a collective term for tocopherols (α-, β-, γ-, and δ) and tocotrienols [5,6]. In the present work, the process of preparation and characterization of matrix reference materials from milk powder and infant formula is described. These reference materials were certified for mass fraction of vitamins A and E using independent analytical methods [7] for sample preparation and chromatographic separation. Establishing the traceability of measurement results to SI units in addition to the homogeneity and stability testing of the prepared reference materials were also highlighted.

2. Materials and methods

2.1. Chemicals and reagents

High-performance liquid chromatography (HPLC) grade solvents, methanol, acetonitrile, n-hexane, acetone, petroleum ether, dichloromethane, cyclohexane, isopropanol, diethyl ether, and ethyl acetate were purchased from Fluka and Riedel (Munich, Germany) and were used in all procedures without further purification. Tert-butylhydroquinone was used as an antioxidant and was obtained from Sigma–Aldrich (Munich, Germany). K_2HPO_4 (extra pure), and trifluoroacetic acid were purchased from Merck (Darmstadt, Germany). Karl Fischer titration reagents were from Scharlau (Sentmenat, Spain) High purity water was obtained through a Milli-Q water purification system (Millipore, Bedford, MA, USA) and was used in all procedures. Vitamins A and E (all–*trans*-retinol, retinyl palmitate, α-tocopherol and γ-tocopherol) were of analytical reagent grade. All the vitamins were supplied by Sigma–Aldrich.

2.2. Chromatographic system used for determination of vitamins A and E

The chromatographic analysis was carried out using HPLC Agilent 1100 integrated system equipped with a G1313A automated injector, a G1311A pump and G1315B multiwavelength diode-array detector (Agilent, Santa Clara, CA, USA). The chromatographic separation of the compounds was achieved with a reversed phase columns, ZORBAX SB-C18 (3.0 mm × 250 mm, 5 μm) from Agilent and (ODS-hypersil 2.1 mm × 100 mm 3 μm) from Thermo Fisher (Waltham, MA, USA) as well as normal phase ZORBAX-RX Sil 4.6 mm × 250 mm 5 μm and Polaris 5 Si 4.6 mm × 100 mm columns from Agilent, operating at constant room temperature (20°C). The chromatographic data were analyzed using Agilent Chemstation Rev. B.02.01-SR1 (260). The compounds under study were identified by their retention times and their UV spectral characteristics.

2.3. UV-visible spectrophotometer

The purity of vitamins was determined by UV-visible spectrophotometry using a Specord 250 Plus (Analytik-Jena, Jena, Germany) equipped with a 15-sample tray.

2.4. Karl Fischer titrator

Moisture content was determined using a volumetric Karl Fischer titrator (852 Titrando; Metrohm, Tampa, FL, USA) in which about 0.2 g samples were accurately weighed and transferred to a Karl Fisher beaker containing methanol as extraction solvent. The method was applied for four bottles and each bottle was measured three times.

2.5. Materials processing and packaging

Milk powder and infant formula samples were obtained from local markets (Cairo, Egypt). Two kilograms of each material were homogenized and packed in 40-g brown glass bottles to prevent light exposure and humidity uptake, the bottles were stored at 4°C in the dark.

2.6. Sample preparation

2.6.1. Saponification

Five grams of powdered sample were reconstituted with 10 mL of distilled water, and then immersed in warm water (40°C), and mixed for 5 minutes until complete homogenization was achieved. Vortex was used to complete the homogenization of the sample. The sample was transferred to a 250 mL Erlenmeyer flask, and 0.5 mL of a 20% (m/v) hydroquinone solution and 50 mL of KOH ethanol solution were added. The preparation was subjected to continuous shaking for 2 hours at room temperature [8,9].

2.6.2. Extraction

The sample was transferred to a separating funnel and 25 mL of hexane were added and the mixture was shaken for 10 minutes. The upper/organic layer was then transferred to another separating funnel, and the aqueous layer was re-extracted with a further 25 mL of hexane. The two organic extracts were combined and washed twice by shaking for 1 minutes with a mixture of 10 mL ethanol and 25 mL of water. The organic layer was collected and evaporated to dryness in a turbo vap evaporator at 40°C. For the second method, the same steps were applied using ethyl acetate/diethyl ether/petroleum ether mixture with ratio (20:30:50) respectively.