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Infant formula and adult nutritionals — Determination of vitamin E and vitamin A by normal phase high performance liquid chromatography

Formules infantiles et produits nutritionnels pour adultes — Détermination de la teneur en vitamine E et de la teneur en vitamine A par chromatographie liquide à haute performance en phase normale

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 34, *Food products* in collaboration with AOAC INTERNATIONAL. It is being published by ISO and separately by AOAC INTERNATIONAL. The method described in this International Standard is equivalent to the AOAC Official Method 2012-10: *Infant formula and adult nutritionals — Determination of vitamin E and vitamin A by normal phase high performance liquid chromatography*.

Infant formula and adult nutritionals — Determination of vitamin E and vitamin A by normal phase high performance liquid chromatography

WARNING — The use of this International Standard can involve hazardous materials, operations and equipment. This International Standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this International Standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

1 Scope

This International Standard specifies a method for the simultaneous quantitative determination of vitamin E (α-tocopherol and α-tocopheryl acetate) and vitamin A (13-*cis* and all-*trans* isomers of retinyl palmitate and retinyl acetate) present in all forms of infant and adult formulas (powders, ready-to-feed liquids and liquid concentrates).

Retinol is not used for fortification purposes and therefore is not addressed in this method. The innate amount in products is insignificant.

Stereoisomers of vitamin E, α-tocopherol and α-tocopheryl acetate, are not differentiated in this method.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1
adult nutritional
nutritionally complete, specially formulated food, consumed in liquid form, which may constitute the sole source of nourishment, made from any combination of milk, soy, rice, whey, hydrolysed protein, starch and amino acids, with and without intact protein

2.2
infant formula
breast-milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding

[SOURCE: Codex Standard 72-1981]

3 Principle

This procedure utilizes the proteolytic enzyme, papain, to hydrolyze the hydrophilic protein coating of fat micelles in milk or soy-based infant formulations in an aqueous solution. The hydrophobic contents of the micelles are then extracted quantitatively into iso-octane in a single extraction. The extract is analysed by normal phase HPLC using an analytical column with gradient elution. Quantification of α-tocopherol and α-tocopheryl acetate is done using fluorescence detection with excitation and emission wavelengths at 280 nm and 310 nm. Retinyl palmitate (*cis* and *trans*) and retinyl acetate (*cis* and *trans*) are quantified using UV detection at 325 nm.

4 Reagents and materials

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

4.1 Methyl-*t*-butyl ether, also known as *tert*-butylmethylether, HPLC grade.

4.2 *n*-Hexane, HPLC grade.

4.3 Ethanol, HPLC grade.

4.4 Methanol, HPLC grade.

4.5 Iso-octane (2,2,4-trimethylpentane), HPLC grade.

4.6 Papain (from *Carica papaya*), ≥ 3 U/mg, Sigma 76220[1)] or equivalent.

4.7 Hydroquinone, Sigma H9003[1)] or equivalent.

4.8 Glacial acetic acid, analytical reagent grade.

4.9 Anhydrous sodium acetate.

4.10 Dilute hydrochloric acid solution.

Dilute 100 ml of a hydrochloric acid solution with a mass fraction of 36 % to 200 ml with water.

4.11 Papain solution, mass concentration ρ = 20 g/l.

Dissolve 100 mg hydroquinone and 4 g anhydrous sodium acetate in approximately 80 ml of water in a 100 ml one-mark volumetric flask (5.11). Adjust the pH to 5,0 with dilute hydrochloric acid solution (4.10). Add 2 g of papain and make up to volume. Prepare fresh prior to use.

4.12 Acidified methanol solution.

Add 20 ml of glacial acetic acid to 1 l of methanol and mix. Prepare fresh on the day of use.

4.13 HPLC mobile phase A.

n-Hexane, filtered and degassed for 15 min in an ultrasonic bath.

4.14 HPLC mobile phase B.

Mix 750 ml of *n*-hexane with 250 ml of methyl-*t*-butyl ether. Add 3 ml of methanol, filter and degas for 15 min in an ultrasonic bath.

4.15 Standard substances

4.15.1 Retinyl palmitate reference standard, primary reference standard. The standard shall contain antioxidant. CAS 78-81-2.

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1) This is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

4.15.2 Retinyl acetate reference standard, primary reference standard. CAS 127-47-9.

4.15.3 α-tocopheryl acetate reference standard, primary reference standard. CAS 7695-91-2

4.15.4 α-tocopherol reference standard, primary reference standard. CAS 10191-41-0.

4.16 Standard solutions

4.16.1 Retinyl palmitate stock standard solution.

Weigh to the nearest 0,01 mg, approximately 70 mg of retinyl palmitate (4.15.1) into a 50 ml volumetric flask (5.11). Dissolve in and dilute to volume with iso-octane (4.5).

4.16.2 Retinyl acetate stock standard solution.

Weigh, to the nearest 0,01 mg, approximately 35 mg of retinyl acetate (4.15.2) into a 50 ml volumetric flask (5.11). Dissolve in and dilute to volume with ethanol (4.3).

4.16.3 α-tocopheryl acetate stock standard solution.

Weigh, to the nearest 0,01 mg, approximately 180 mg of α-tocopheryl acetate (4.15.3) into a 50 ml volumetric flask (5.11). Dissolve in and dilute to volume with iso-octane.

4.16.4 α-tocopherol stock standard solution.

Weigh, to the nearest 0,01 mg, approximately 100 mg of α-tocopherol (4.15.4) into a 50 ml volumetric flask (5.11). Dissolve in and dilute to volume with iso-octane.

NOTE The above stock standard solutions are stable in a refrigerator at 4 °C to 8 °C for up to 7 days.

4.16.5 Combined working standard solution 1.

Transfer by pipette 4 ml of retinyl palmitate stock standard solution (4.16.1), 4 ml of retinyl acetate stock standard solution (4.16.2), 7 ml of α-tocopheryl acetate stock standard solution (4.16.3) and 20 ml of α-tocopherol stock standard solution (4.16.4), into a 50 ml volumetric flask (5.11) and dilute to volume with iso-octane. Prepare this solution freshly prior to use.

4.16.6 Combined working standard solution 2.

Transfer by pipette 8 ml of combined working standard solution 1 (4.16.5) into a 100 ml volumetric flask (5.11) and dilute to volume with iso-octane. Prepare this solution freshly prior to use.

4.16.7 Calibration standard solutions

Into separate 50 ml volumetric flasks (5.11), transfer by pipette 0,5 ml, 2 ml, 4 ml, 8 ml, 16 ml and 32 ml of combined working standard solution 2 (4.16.6), and dilute to volume with iso-octane. These solutions are used to construct a multipoint calibration curve. Prepare these solutions daily prior to use.

NOTE For routine testing and depending on the concentration range of the analytes in the test samples, a 3 or 4 point standard curve can be used, provided the ranges are within the lowest and highest points of the 6 point curve listed above.