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Infant formula and adult nutritionals — Determination of myo-inositol by liquid chromatography and pulsed amperometry

Formules infantiles et produits nutritionnels pour adultes — Détermination de la teneur en myo-inositol par chromatographie liquide et ampérométrie pulsée

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Foreword

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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 2011.18: *Myo-inositol (free and bound as phosphatidyl inositol) in infant and pediatric formula and adult nutritional*.

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Infant formula and adult nutritionals — Determination of myo-inositol by liquid chromatography and pulsed amperometry

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 determination of myo-inositol (free or free plus bound as phosphatidylinositol) in infant formula and adult nutritionals using liquid chromatography and pulsed amperometry with column switching.

2 Terms and definitions

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

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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

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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

Free myo-inositol and phosphatidyl bound myo-inositol are extracted using two different sample preparation procedures. Free myo-inositol is extracted from samples with dilute hydrochloric acid and water. Phosphatidylinositol is extracted from samples with chloroform and separated from other fats with silica solid phase extraction cartridges. Myo-inositol is then released from the glycerol backbone with concentrated acetic and hydrochloric acid at 120°C. The ion chromatographic method uses a combination of two different ion exchange columns with column switching and pulsed amperometric detection (PAD). The concentration of myo-inositol is calculated by comparison with external standards of known concentration.

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.

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4.1 Chemicals and solvents

4.1.1 Acetic acid, glacial, ACS.

4.1.2 Chloroform, high-purity, HPLC grade.

4.1.3 Diethyl ether, anhydrous, HPLC grade.

4.1.4 Drierite, (desiccant), anhydrous calcium sulfate, 8 mesh.

4.1.5 Helium, zero grade or equivalent.

4.1.6 Hexane, HPLC grade.

4.1.7 Hydrochloric acid, concentrated (36 % to 38 %), ACS.

4.1.8 Metaphosphoric acid, ACS.

4.1.9 Methanol, HPLC grade.

4.1.10 Myo-inositol, primary reference standard, official lot, store desiccated. See standard label for purity.

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4.1.11 Sodium chloride, ACS.

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4.1.12 Sodium hydroxide, 50 % (m/m), low carbonate form.

4.2 Preparation of reagents and standard solutions

4.2.1 General. All solutions can be scaled up or down for convenience provided good laboratory practices are observed. Solutions can be stored at refrigerated or at ambient temperature in tight, inert containers unless otherwise specified.

4.2.2 Myo-inositol stock standard solution (approximately 2 000 mg/l). Accurately weigh approximately 0,100 g myo-inositol and quantitatively transfer to a 50 ml volumetric flask. Dilute to volume with water. Mix well. Store refrigerated. Expiration: 3 months.

4.2.3 Myo-inositol intermediate standard solution (approximately 200 mg/l). Dilute 10,0 ml stock standard (4.2.2) to 100 ml with water and mix well. Discard after use.

4.2.4 Preparation of calibration standard solutions

4.2.4.1 Myo-inositol calibration standard solutions high, (approximately 4 mg/l, 2 mg/l, 1 mg/l, 0,5 mg/l).

Into separate volumetric flasks, dilute 2,0 ml, 1,0 ml and 0,5 ml myo-inositol intermediate standard (4.2.3) to 100 ml with water. Dilute 0,5 ml myo-inositol intermediate standard (4.2.3) to 200 ml with water. Expiration: 2 weeks

4.2.4.2 Myo-inositol calibration standard solutions low, (approximately 0,2 mg/l and 0,05 mg/l).

Into separate volumetric flasks, dilute 4 ml and 1 ml of the 0,5 mg/l myo-inositol calibration standard to 10 ml with water. Expiration: 2 weeks.

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4.2.5 Hydrochloric acid, 0,5 %. Add 1,25 ml concentrated hydrochloric acid to approximately 200 ml water in a 250 ml volumetric flask. Dilute to volume with water and mix well. Expiration: 6 months.

4.2.6 Sodium chloride, 1 mol/l. Dissolve 5,8 g sodium chloride and dilute to 100 ml with water. Expiration: 1 month.

4.2.7 Sodium hydroxide, 0,12 % or 30 mmol (Pump 1). Quickly weigh (4,8 ± 0,1) g of 50 % sodium hydroxide into a 2 000 ml volumetric flask containing approximately 1 900 ml water. It is important that the sodium hydroxide does not absorb carbon dioxide from the air. Swirl to mix well. Dilute to volume with water and mix well. Expiration: 1 month.

4.2.8 Sodium hydroxide, 4,0 % or 1 mol/l (Pump 2). Quickly weigh (160 ± 3) g of 50 % sodium hydroxide into a 2 000 ml volumetric flask containing approximately 1 900 ml water. It is important that the sodium hydroxide does not absorb carbon dioxide from the air. Swirl to mix well. Dilute to volume with water and mix well. Expiration: 1 month.

4.2.9 Metaphosphoric acid, 6 %. Weigh 6,0 g metaphosphoric acid into a 100 ml volumetric flask. Dissolve and dilute to volume with water. Mix well. Store refrigerated. Expiration: 1 week.

4.2.10 Phosphatidylinositol extraction solutions. Prepare fresh on day of use.

4.2.10.1 Chloroform:methanol (2:1). Mix 60 ml chloroform and 30 ml methanol.

4.2.10.2 Hexane:diethyl ether (80:20). Mix 80 ml hexane and 20 ml diethyl ether.

4.2.10.3 Hexane:diethyl ether (50:50). Mix 50 ml hexane and 50 ml diethyl ether.

4.2.10.4 Methanol:chloroform:water (75:15:10). Mix 75 ml methanol, 15 ml chloroform and 10 ml water.

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5 Apparatus

Usual laboratory glassware and equipment and, in particular, the following.

5.1 Analytical balance, minimum weighing capacity of at least 0,000 1 g.

5.2 Centrifuge.

5.3 Desiccator.

5.4 Nitrogen evaporator, with water bath or equivalent.

5.5 Oven, capable of maintaining 120 °C.

5.6 pH-meter, with pH 4 and 7 buffers.

5.7 Stir plate, multiposition with stir bars.

5.8 Vacuum manifold.

5.9 Vortex mixer.

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