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

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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*.

INTERNATIONAL STANDARD ISO 20637:2015(E)

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.

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

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.

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.

4.1.11 Sodium chloride, ACS.

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.

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.

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.

5.10 System HPLC, with corrosion-resistant components including an autosampler, two isocratic pumps, 6-port switching valve, pulsed amperometry detector with a gold electrode and PEEK or polytetrafluoroethylene (PTFE) 0,18 mm to 0,25 mm (0,007 inch to 0,01 inch) internal diameter tubing. Autosampler capable of injecting 20 µl.

5.11 Columns, Dionex CarboPac[1)] MA1 (4 mm × 250 mm) P/N, 44066, MA1 (4 mm × 50 mm) P/N 44067, and PA1 (4 × 50 mm) P/N 43096, or equivalent.

5.12 Beakers, assorted sizes.

5.13 Centrifuge tubes, 50 ml with polytetrafluoroethylene (PTFE)-coated caps.

5.14 Syringe filters, polyamide, 0,45 µm and 0,2 µm.

5.15 Filter paper, Whatman 2V[1)] or equivalent.

5.16 Conical flasks, 50 ml or 125 ml or equivalent.

5.17 Volumetric flasks, assorted sizes.

5.18 Funnels, suitable for use with filter paper.

5.19 Pipets, volumetric, assorted sizes.

5.20 Solid-phase extraction (SPE) cartridge, silica, 1 g[2)].

5.21 Syringes, 1 ml disposable and 25 ml gas-tight glass with 100 mm (4 in) stainless steel needles.

6 Procedure

6.1 Free myo-inositol

6.1.1 Sample preparation

6.1.1.1 General

Prepared samples that are constantly stored at 1 °C to 8 °C in closed containers are stable for up to 5 days. After 5 days, samples shall be prepared again. Mix liquid samples well to ensure homogeneity. If the powder sample homogeneity is unknown, assume that it is non-homogenous and proceed with the preparation of dry blended/non-homogenous powder samples as given in 6.1.1.3.

6.1.1.2 Liquid samples

For ready-to-feed liquid samples, accurately weigh (0,5 ± 0,05) g to (5 ± 0,5) g of product into a 100 ml volumetric flask and record the mass to the nearest 0,000 1 g.

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6.1.1.3 Dry blended powder samples

For dry blended/non-homogenous powder samples, reconstitute per the product label instructions. Accurately weigh 0,5 g to 5 g reconstituted product into a 100 ml volumetric flask. Record the mass to the nearest 0,000 1 g.

6.1.1.4 Wet blended powder samples

For wet blended/homogenous powder samples, accurately weigh 0,25 g to 1,5 g powder into a 100 ml volumetric flask and record the mass to the nearest 0,000 1 g. Add approximately 10 ml to 15 ml water to the volumetric flask and swirl or stir to completely dissolve the powder.

6.1.2 Extraction

Add enough 0,5 % hydrochloric acid (4.2.5) to each sample to adjust the sample pH to 4,5 ± 0,2 and swirl to mix.

Allow the samples to react with 0,5 % hydrochloric acid for a minimum of 2 min and then dilute to volume with water. Mix well. Filter samples through filter paper (5.15) into 125 ml conical flasks or appropriate glassware.

NOTE Although some samples will filter cloudy, the filtrates can still be used.

Filter an aliquot of sample filtrate through a 0,45 µm syringe filter (5.14) into an autosampler vial.

6.2 Myo-inositol bound as phosphatidylinositol

6.2.1 Sample preparation

6.2.1.1 General

Prepared samples that are constantly stored at 1 °C to 8 °C in closed containers are stable for up to 5 days. After 5 days, samples shall be prepared again. Thoroughly mix or stir products prior to sampling. Mix liquid samples well to ensure homogeneity. If the powder sample homogeneity is unknown, assume that it is non-homogenous and proceed with the preparation of dry blended/non-homogenous powder samples given in 6.2.1.3.

6.2.1.2 Liquid samples

For ready-to-feed liquid samples, accurately weigh (4 ± 0,4) g of product into a 50 ml centrifuge tube and record the mass to the nearest 0,000 1 g.

6.2.1.3 Dry blended powder samples

For dry blended/non-homogenous powder samples, reconstitute per the product label instructions. Accurately weigh (4 ± 0,4) g reconstituted sample into a 50 ml centrifuge tube. Record the mass to the nearest 0,000 1 g.

6.2.1.4 Wet blended powder samples

For wet blended homogenous powder samples, accurately weigh (1 ± 0,1) g powder into a 50 ml centrifuge tube and record the mass to the nearest 0,000 1 g. Add 4 ml water centrifuge tube and mix well.

6.2.2 Extraction

In a fume hood, add 10 ml methanol to each sample and stir for at least 20 min or vortex for at least 1 min and allow samples to set for at least 20 min. Add 20 ml chloroform and stir for at least 5 min or vortex for at least 1 min and allow samples to set for at least 5 min. If large clumps form when chloroform is

added, cap tube and shake well for at least 1 min to mix sample. Add 5 ml 6 % metaphosphoric acid (4.2.9) and 1 ml 1 mol/l NaCl (4.2.6) and mix well. Centrifuge until layers separate. Using a 25 ml glass gas-tight syringe with a stainless steel needle (5.21), transfer the bottom chloroform layer to a clean 50 ml centrifuge tube and evaporate the chloroform with nitrogen in a 60 °C water bath.

6.2.3 Cleanup

In a fume hood, condition a 1 g silica SPE cartridge (5.20) with 6 ml hexane. Dissolve residue in the bottom of the centrifuge tube in 1 ml chloroform:methanol (2:1). Quantitatively transfer dissolved residue to the conditioned silica SPE cartridge. Rinse the 50 ml centrifuge tube with 3 ml hexane:diethyl ether (80:20) and then transfer to the SPE cartridge. Discard the eluent. Rinse the 50 ml centrifuge tube with 3 ml hexane:diethyl ether (50:50) and then transfer to the SPE cartridge. Collect eluent in a clean 50 ml centrifuge tube. Rinse 50 ml centrifuge tube with 4 ml methanol and then transfer to the SPE cartridge. Collect eluent in the same 50 ml centrifuge tube. Rinse 50 ml centrifuge tube with 4 ml methanol:chloroform:water (75:15:10) and transfer to the SPE cartridge. Collect eluent in the same 50 ml centrifuge tube. Evaporate eluents collected from SPE cartridge with nitrogen in a 60 °C water bath.

6.2.4 Hydrolysis

In a fume hood, add 40 µl glacial acetic acid (4.1.1) and 2 ml concentrated hydrochloric acid (4.1.7) to the residue in the centrifuge tube from the sample cleanup step. Tightly cap tube. Heat in a 120 °C oven for 2 h. Cool. Add approximately 10 ml of water and swirl to mix. Add 1,25 ml 50 % (m/m) sodium hydroxide (4.1.12). Transfer sample to a 50 ml volumetric flask and dilute to volume with water. Filter an aliquot of sample filtrate through a 0,45 µm syringe filter into an autosampler vial.

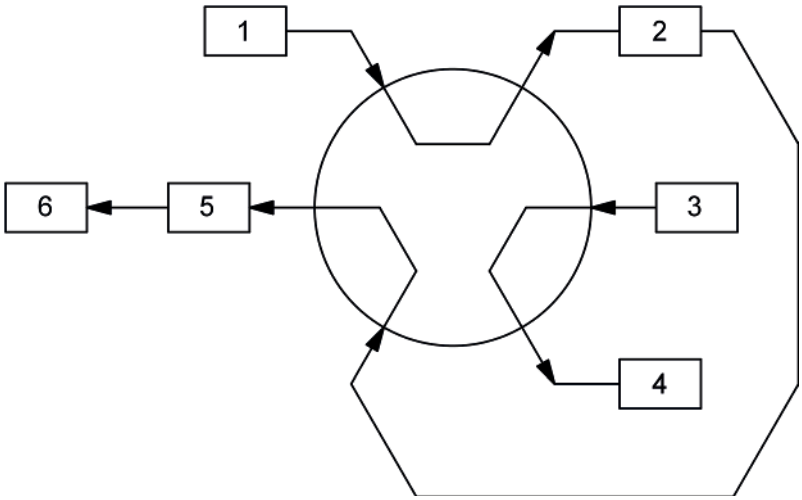
6.3 HPLC analysis

6.3.1 Instrument operating conditions

Pump 1 pressure limit	13 790 kPa (2 000 psi)
Pump 1 mobile phase	0,12 % (30 mmol/l) NaOH
Pump 1 flow rate	0,40 ml/min
Pump 2 pressure limit	13 790 kPa (2 000 psi)
Pump 2 mobile phase	4 % (1 mol/l) NaOH
Pump 2 flow rate	0,40 ml/min
Injection volume	20 µl
Myo-inositol retention time	11 min to 13 min
Run time	25 min

Switching valve configuration time:

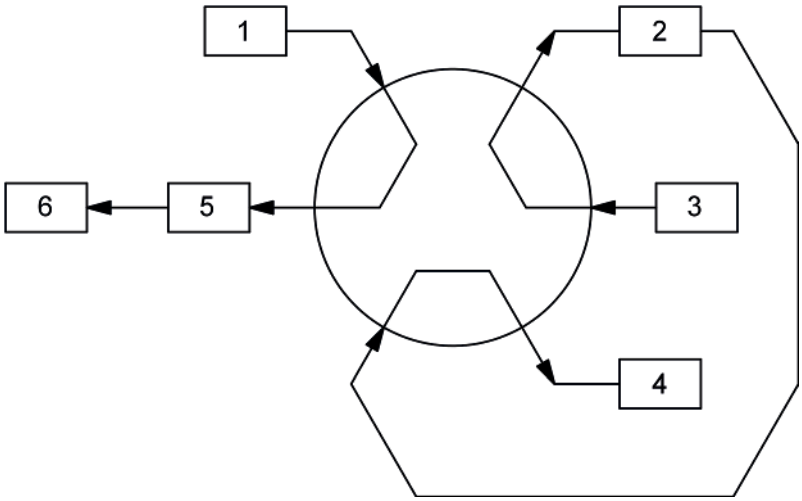
0,00 min	Configuration 1	See Figure 1
1,50 min	Configuration 2	See Figure 2
13,50 min	Configuration 1	See Figure 1



Key

1	Pump 1	4	waste
2	PA1 guard column	5	MA1 guard and analytical columns
3	Pump 2	6	electrochemical detector

Figure 1 — Switching valve configuration 1



Key

1	Pump 1	4	waste
2	PA1 guard column	5	MA1 guard and analytical columns
3	Pump 2	6	electrochemical detector

Figure 2 — Switching valve configuration 2

6.3.2 PAD settings with gold electrode

Analog range: 1 µC

Detector program: Dionex ICS 3000 or ICS 5000[3)]

0,0 s	+0,10 V
0,20 s	+0,10 V
0,40 s	+0,10 V
0,41 s	−2,00 V
0,42 s	−2,00 V
0,43 s	+0,60 V
0,44 s	−0,10 V
0,50 s	−0,10 V

Integration period: 0,20 s to 0,40 s

Examples of typical standard chromatograms are shown in Annex A.

6.3.3 Instrument startup

Prepare mobile phases. If necessary, helium sparge mobile phases and/or pressurize mobile phase reservoirs. If necessary, clean and polish the gold working electrode. Turn on the detector and pump mobile phase over the columns at a flow rate of 0,40 ml/min for at least 30 min to equilibrate the system. Verify that the detector is stable before beginning an analysis. Inject 20 µl of the most concentrated standard at least 5 times and note the peak areas or heights. If the system is equilibrated, the relative standard deviation (RSD) of the peak areas or heights of the last three standard injections should be ≤ 2,0 %.

6.3.4 Standard and sample analysis

Once the system has equilibrated, inject one standard at each concentration (4.2.4 and 4.2.5). After a set of standards has been injected, samples and a control sample can be injected before another set of standards should be injected.

6.3.5 System shutdown

After all samples and standards have been analysed, inject 20 µl of water to clean out the autosampler needle and tubing. Store the analytical columns in mobile phase [0,12 % (30 mmol/l) sodium hydroxide]. Turn off the electrochemical cell. Flush the pump heads with water to remove sodium hydroxide.

7 Calculations

7.1 General

Before calculating myo-inositol concentrations in samples, compare the myo-inositol standard peaks with the myo-inositol sample peaks and confirm that there are not any interfering compounds and that the myo-inositol sample peak areas or heights are within the range of the myo-inositol standard peak areas or heights. The concentration of myo-inositol cannot be calculated if there are interferences or if

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the separation is poor. The myo-inositol retention time should be 11 min to 13 min depending on the individual analytical column.

7.2 Concentration of calibration standards

Calculate the concentration of calibration standards using Formula (1):

$$C_W = m \times \frac{1}{0,05} \times \frac{1}{10} \times \frac{A_1}{V_1} \times \frac{A_2}{V_2} \times p = m \times 2 \times \frac{A_1}{V_1} \times \frac{A_2}{V_2} \times p \quad (1)$$

where

C_W is the concentration of the calibration standard solution in milligrams per litre;

m is the mass, in milligrams, of myo-inositol standard weighed;

0,05 is the dilution volume of the stock standard in litres (see 4.2.2);

1/10 is the intermediate standard dilution (10 ml to 100 ml);

A_1 is the aliquot of intermediate standard used, in millilitres (see 4.2.4.1);

V_1 is the dilution volume of the calibration standard high in millilitres (see 4.2.4.1);

A_2 is the aliquot of calibration standard high used, in millilitres, if applicable (see 4.2.4.2);

V_2 is the dilution volume of the calibration standard low in millilitres, if applicable (see 4.2.4.2);

p is the purity in mg/mg from the primary standard label or determined experimentally.

7.3 Preparation of standard curve

For each calibration standard concentration, average the peak areas or heights from each two consecutive sets of standards. Prepare a standard curve by performing linear least squares (regression) on the concentrations versus the averaged peak areas or heights.

7.4 Calculation of free or free plus bound myo-inositol in samples

7.4.1 Calculation of free myo-inositol

The concentration of free myo-inositol in a prepared sample is extrapolated from the standard curve prepared in 7.3. From the diluted, prepared sample concentration, the product concentration can be calculated using Formula (2):

$$C_f = \frac{C_d \times 100}{m_s} \quad (2)$$

where

C_f is the concentration of free myo-inositol in the product sample in milligrams per kilogram;

C_d is the concentration of myo-inositol in the prepared sample in milligrams per litre;

100 is the dilution volume in millilitres;

m_s is the sample mass in grams.

7.4.2 Calculation of bound myo-inositol

The concentration of bound myo-inositol in a prepared sample is extrapolated from the standard curve prepared in 7.3. From the diluted, prepared sample concentration, the product concentration can be calculated using Formula (3):

$$C_b = \frac{C_d \times 50}{m_s} \quad (3)$$

where

C_b is the concentration of bound myo-inositol in the product sample in milligrams per kilogram;

C_d is the concentration of myo-inositol in the prepared sample in milligrams per litre;

50 is the dilution volume in millilitres;

m_s is the sample mass in grams.

7.4.3 Calculation of free plus bound myo-inositol

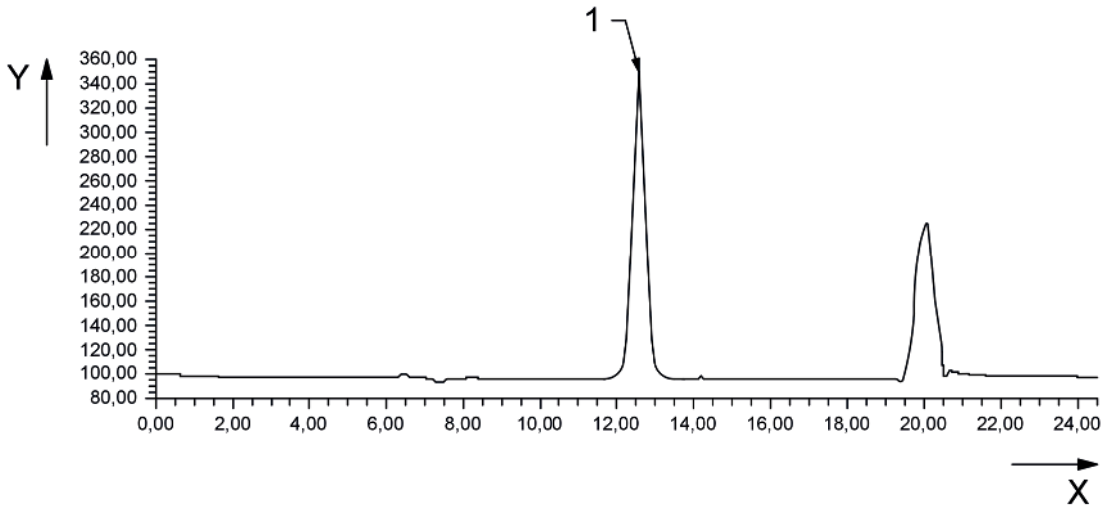
The concentration of free plus bound myo-inositol in a prepared sample is calculated using Formula (4):

$$C_T = C_f + C_b \quad (4)$$

where C_T is the concentration of free plus bound myo-inositol in product sample in milligrams per kilogram.

Annex A
(informative)

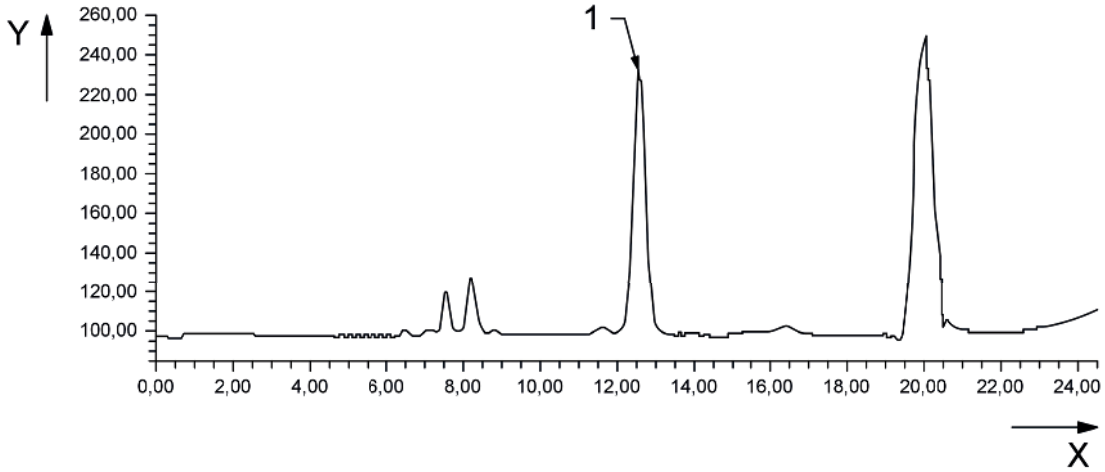
Examples of chromatograms



Key

X time, in min
Y arbitrary units
1 myo-inositol

Figure A.1 — Example of a typical standard chromatogram



Key

X time, in min
Y arbitrary units
1 myo-inositol

Figure A.2 — Example of a SRM 1849a chromatogram

Annex B
(informative)

Precision data

The data given in Tables B.1, B.2 and B.3 were obtained in an interlaboratory study and published in 2015,[3] in accordance with ISO 5725-2[4] and the AOAC-IUPAC Harmonized Protocol for collaborative study procedures, to assess precision characteristics of a method of analysis.[5] The method was validated for the quantitation of free myo-inositol and myo-inositol from phosphatidylinositol in infant and adult nutritionals. Repeatability was determined from duplicate analyses performed on multiple days. Accuracy was determined from spike recovery experiments (free myo-inositol and myo-inositol from phosphatidylinositol). Instrument limits of detection and quantitation were determined statistically from injections of low-level standards and by spiking samples with low levels of free myo-inositol. The study was performed based on requirements given in Reference [6].

More information on the validation of the method can be found at http://standards.iso.org/iso/20637

Table B.1 — Unbound (free) myo-inositol

Sample type	Total No. laboratories excluding outliers	Number of outlier laboratories	Total No. replicates accepted	Mean (mg/100 g RTF)	s_r	s_R	$C_{V,r}$	$C_{V,R}$	HorRat[a]
NIST SRM 1849a	10	0	22	412[b]	11,3	11,4	2,75	2,77	0,43
Infant formula powder soy-based	10	0	22	4,22	0,127	0,305	3,03	7,26	0,80
Infant formula powder milk-based	10	0	20	4,26	0,168	0,232	3,95	5,43	0,60
Infant formula RTF milk-based	9	0	20	7,17	0,095	0,207	1,33	2,89	0,34
Infant formula powder partial hydrolysed milk-based	10	0	22	3,65	0,035	0,412	0,97	11,4	1,22
Infant formula powder partial hydrolysed soy-based	10	0	22	3,11	0,089 9	0,389	2,92	12,61	1,32
Child formula powder	10	0	22	5,10	0,185	0,246	3,61	4,81	0,54
Infant elemental powder	10	0	22	5,10	0,227	0,318	4,45	6,24	0,71
Infant formula RTF milk-based, unfortified	9	0	20	3,17	0,058 2	0,091 0	1,84	2,87	0,30

[a] HorRat value, according to Reference [7].

[b] Results in mg/kg powder.

RTF is ready-to-feed.

Table B.2 — Myo-inositol bound as phosphatidylinositol

Sample type	Total No. laboratories excluding outliers	Number of outlier laborato-ries	Total No. replicates accepted	Mean (mg/100 g RTF)	s_r	s_R	$C_{V,r}$	$C_{V,R}$	HorRat[a]
NIST SRM 1849a	9	0	20	9,51[b]	1,82	2,62	18,7	26,8	2,36
Infant formula powder soy-based	9	0	20	2,10	0,150	0,501	6,94	23,2	2,30
Infant formula powder milk-based	9	0	18	0,667	0,026 1	0,172	3,92	25,9	2,15
Infant formula RTF milk-based	8	0	18	0,348	0,030 1	0,090 9	8,36	25,2	1,91
Infant formula powder partial hydrolysed milk-based	9	0	20	0,214	0,010 3	0,057 6	4,72	26,4	1,86
Infant formula powder partial hydrolysed soy-based	9	0	20	1,64	0,093 6	0,358	5,53	21,1	2,02
Child formula powder	9	0	20	0,328	0,023 4	0,087 8	6,89	25,8	1,94
Infant elemental powder	9	0	20	0,00	0,00	0,00	0,00	0,00	0,00
Infant formula RTF milk-based, unfortified	8	0	18	0,305	0,024 4	0,085 0	7,71	26,9	2,00

[a] HorRat value, according to Reference [7].

[b] Results in mg/kg powder.

RTF is ready-to-feed.

Table B.3 — Unbound (free) myo-inositol plus myo-inositol bound as phosphatidylinositol

Sample type	Total No. laboratories excluding outliers	Number of outlier laboratories	Total No. replicates accepted	Mean (mg/100 g RTF)	s_r	s_R	$C_{V,r}$	$C_{V,R}$	HorRat[a]
NIST SRM 1849a	9	0	20	422[b]	11,9	11,9	2,83	2,83	0,44
Infant formula powder soy-based	9	0	20	6,27	0,147	0,446	2,32	7,05	0,82
Infant formula powder milk-based	9	0	18	4,92	0,184	0,314	3,74	6,38	0,72
Infant formula RTF milk-based	8	0	18	7,50	0,106	0,218	1,41	2,90	0,35
Infant formula powder partial hydrolysed milk-based	9	0	20	3,84	0,035	0,426	0,91	11,2	1,21
Infant formula powder partial hydrolysed soy-based	9	0	20	4,71	0,152	0,357	3,22	7,55	0,84
Child formula powder	9	0	20	5,42	0,203	0,307	3,73	5,63	0,64
Infant elemental powder	9	0	20	5,08	0,237	0,324	4,67	6,40	0,72
Infant formula RTF milk-based, unfortified	8	0	18	3,46	0,065 9	0,128	1,90	3,70	0,39

[a] HorRat value, according to Reference [7].

[b] Results in mg/kg powder.

RTF is ready-to-feed.

Bibliography

[1] J. AOAC Int. 2012, **95** p. 937

[2] J. AOAC Int. 2012, **95** p. 295

[3] 2011.18, *Determination of Myo-Inositol in Infant, Pediatric, and Adult Formulas by IC-PAD and Column Switching: Collaborative Study*

[4] ISO 5725-2:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

[5] AOAC INTERNATIONAL. AOAC Official Methods Program, Associate Referee's Manual on Development, Study, Review, and Approval Process. Part IV AOAC Guidelines for Collaborative Studies, 1995, pp. 23–51

[6] AOAC SMPR 2011.07, Standard Method Performance Requirements for Myo-inositol in infant formula and Adult/Pediatric Nutritional formula

[7] THOMPSON M. Recent Trends in Inter-Laboratory Precision at ppb and sub-ppb Concentrations in Relation to Fitness for Purpose Criteria in Proficiency Testing. Analyst (Lond.). 2000, **125** pp. 385–386

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