

CODEX ALIMENTARIUS COMMISSION



Food and Agriculture
Organization of the
United Nations



World Health
Organization

Viale delle Terme di Caracalla, 00153 Rome, Italy - Tel: (+39) 06 57051 - E-mail: codex@fao.org - www.codexalimentarius.org

Agenda Item 1&13

NFSDU/41 CRD 7
Original language only

JOINT FAO/WHO FOOD STANDARDS PROGRAMME CODEX COMMITTEE ON NUTRITION AND FOODS FOR SPECIAL DIETARY USES

Forty-first Session

Dusseldorf, Germany
24 – 29 November 2019

METHODS OF ANALYSIS IN THE STANDARD FOR INFANT FORMULA AND FORMULAS FOR SPECIAL MEDICAL PURPOSES INTENDED FOR INFANTS (CODEX STAN 72-1981)

Prepared by the United States of America

EXECUTIVE SUMMARY

This document outlines a proposal to replace and complement methods of analysis for the determination of nutrients in infant formula, which are listed in CXS 234-1999 and referenced in CODEX STAN 72-1981 and CAC/GL 10-1979. It is proposed that these new methods be considered during the 41st Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) in November 2019 and that they either: (1) replace current Type II/III methods which may be outdated, and/or methods that were not validated on all types of infant formula; or (2) serve as Type II methods where such methods do not currently exist.

These new methods have been developed through the Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) project, which is managed by AOAC INTERNATIONAL (AOAC). They have also either been developed by the International Organization for Standardization (ISO) and International Dairy Federation (IDF) as ISO or ISO/IDF Standards, or are in the process of being adopted as such. It is proposed that these new methods be adopted as Codex Type II methods to enable them to be utilized as needed for the purposes of dispute resolution internationally.

INTRODUCTION

The *Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants* (CODEX STAN 72-1981) was revised in 2007. At the 30th CCNFSDU Session in 2008, the electronic Working Group (eWG) on methods of analysis for infant formula recommended the Committee to periodically review the infant formula methods listed in the *Recommended Methods of Analysis and Sampling* (CXS 234-1999) to keep them updated (ALINORM 09/03/26). In 2009, the Codex Committee on Methods of Analysis and Sampling (CCMAS) endorsed the status of several methods of analysis for nutrients in CODEX STAN 72-1981 based on the best available methods in matrices at the time (ALINORM 09/32/23 paras. 45-71). These methods were adopted by the Codex Alimentarius Commission in 2009, including various Type I, I, III and/or IV methods, and are included in CXS 234-1999.

BACKGROUND

Some methods referenced in CODEX STAN 72-1981 and CXS 234-1999 are outdated and/or not validated for infant formula. Further, for some required nutrients and many optional ingredients, Codex Type I or Type II methods are lacking.

Since 2015, new methods of analysis for nutrients in infant formula, which were validated by AOAC International, ISO and IDF through the SPIFAN project, have been adopted and published in the *Journal of AOAC INTERNATIONAL*, adopted and published by ISO and IDF as ISO or ISO/IDF Standards, and have been submitted to Codex for review. Methods are introduced by CCNFSDU, referred to CCMAS for technical review, typing and endorsement, and then submitted to the Codex Alimentarius Commission (CAC) for adoption. To date, methods for vitamin A, vitamin C, vitamin B₁₂, vitamin D, vitamin E, vitamin K, biotin, pantothenic acid, folate, myo-inositol, fatty acid profile, total nucleotides, iodine, chloride, calcium, magnesium, phosphorus, potassium, sodium, copper, iron, manganese, zinc, chromium, selenium, and molybdenum have been brought through this process and all have been adopted by CAC as Type II methods for the purpose of dispute resolution.

PROPOSAL AND RATIONALE

The Committee is requested to consider recommending that the following methods be referred to CCMAS for technical review and typing. The Committee is also requested to recommend that CCMAS review the existing Type II and Type III methods for these nutrients listed in CXS 234-1999, to determine if the methods meet the specifications in CODEX STAN 72. Based on this review, CCMAS may either retain the existing methods, make necessary changes to the method Types, or revoke any methods which do not meet the specifications. Rationale supporting each method is provided below. Table 1 presents the summary of the proposed changes.

With regard to potential questions regarding certain technologies, it should be noted that CCFSDU has previously considered questions from CCMAS related to expensive instrumentation and whether some countries may not have the capacity to run certain methods. It should be noted a Type II method is not required to be used except in the case of resolving a dispute that cannot otherwise be settled, and in all other cases any approved Codex method may be used. The United States supports the desire of countries to use all approved Codex methods for routine nutrient analysis. However, there is a strong need to have one Type II method for each nutrient or group of nutrients in infant formula that will be used as the referee method in the case of a dispute that cannot otherwise be settled. As such, CCFSDU and CCMAS have endorsed several methods that use the ICP-MS and other sophisticated instrumentation as Type II.^{1,2}

THIAMINE (VITAMIN B1), RIBOFLAVIN (VITAMIN B2), NIACIN (VITAMIN B3), VITAMIN B6 (AOAC 2015.14 / ISO DIS 21470)

AOAC 2015.14 is an Official Final Action method which has undergone successful multi-laboratory validation (MLV) using 15 infant, child and adult nutritional formulas covering the fortification range. 10 laboratories in eight countries participated in the MLV of the LC-MS/MS method, which simultaneously determines the concentrations of thiamine, riboflavin, niacin and vitamin B6 in infant formula. The method has been approved as AOAC Official Final Action and is in publication. It is also expected that ISO will publish this method as a final ISO Standard no later than April 2020. AOAC 2015.14 / ISO DIS 21470 is proposed as Type II for the determination of thiamine (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3) and vitamin B6 in infant formula.

CHOLINE, CARNITINE (AOAC 2015.10 / ISO DIS 21468)

There is currently no Type II method in CXS 234-1999 for the determination of carnitine in infant formula.

AOAC 2015.10 has undergone successful MLV using 10 infant, child and adult nutritional formulas covering the fortification range. Nine laboratories in seven countries participated in the MLV of the LC-MS/MS method, which simultaneously determines the concentrations of choline and carnitine in infant formula. AOAC 2015.10 has been approved as Official Final Action method and is in publication. It is also expected that ISO will publish this method as a final ISO Standard no later than April 2020. AOAC 2015.10 / ISO DIS 21468 is proposed as Type II for the determination of choline and carnitine in infant formula.

FRUCTANS (FRUCTOOLIGOSACCHARIDES AND/OR INULIN) (AOAC 2016.14 / ISO DIS 22579 | IDF 241)

There is currently no Type II method in CXS 234-1999 for the determination of fructooligosaccharides (FOS) in infant formula. In addition, fructans or FOS are not included in CXS 72-1981 or CAC/GL 10-1979. However, infant formulas containing FOS are being traded globally so there is the possibility of regulatory disputes regarding the level of FOS in a particular product. In addition, CXS 234-1999 contains several methods for the determination of fiber in certain products, but none of these methods have been validated on infant formula. To address potential regulatory disputes and because fiber is already referenced in CXS 234-1999, it is appropriate to propose a Type II method for the determination of fructans in infant formula.

AOAC 2016.14 / ISO DIS 22579 | IDF 241 is an Official Final Action method which has undergone successful MLV using seven infant, child and adult nutritional formulas covering the fortification range. 12 laboratories in nine countries participated in the MLV of the enzymatic digestion with HPAEC-PAD method, which simultaneously determines the concentrations of fructans in infant formula. AOAC 2016.14 has been approved as AOAC Official Final Action and is in publication. It is also expected that ISO/IDF will publish this method as a final ISO and IDF Standard no later than April 2020. AOAC 2016.14 / ISO DIS 22579 | IDF 241 is proposed as Type II for the determination of fructans in infant formula.

BETA CAROTENE, LYCOPENE (AOAC 2016.13 / ISO DIS 23443)

There are currently no Type II methods in CXS 234-1999 for the determination of beta carotene or lycopene in infant formula. In addition, while beta carotene is referenced in CAC/GL 10-1979, lycopene is not included in CXS 72-1981 or CAC/GL 10-1979. However, infant formulas containing both beta carotene and lycopene are being traded globally so there is the possibility of regulatory disputes regarding the level of lycopene in a

¹ AOAC 2011.19 | ISO 20649 | IDF 235

² AOAC 2012.15 | ISO 20647 | IDF 234

particular product. To address potential regulatory disputes, it is appropriate to propose a Type II method for the determination of beta carotene in infant formula.

AOAC 2016.13 / ISO DIS 23443 is an Official Final Action method which has undergone successful MLV using seven infant, child and adult nutritional formulas covering the fortification range. 10 laboratories in seven countries participated in the MLV of the UHPLC method, which simultaneously determines the concentrations of beta carotene and lycopene. AOAC 2016.13 has been approved as AOAC Official Final Action and is in publication. It is also expected that ISO will publish this method as a final ISO Standard in April 2020. AOAC 2016.13 / ISO DIS 23443 is proposed as Type II for the determination of beta carotene and lycopene in infant formula.

BIOTIN (AOAC 2016.02 / ISO 23305)

A method for the determination of biotin in infant formula, AOAC 2016.02, was introduced at the 39th CCNFSDU Session. At that time, work to develop a complementary ISO Standard had not yet begun, so CCNFSDU agreed to refer AOAC 2016.02 to CCMAS for technical review and typing.³ At the time of the 39th CCMAS Session, ISO DIS 23305 was in development; however, CCMAS only endorsed AOAC 2016.02 as Type II.⁴ The Codex Alimentarius Commission adopted AOAC 2016.02 as Type II during its 41st Session.⁵

ISO/DIS 23305 has been published⁶, and the Final ISO Standard is expected to be published prior to CCMAS.

RECOMMENDATION

That the Committee:

- Refer AOAC 2015.14 / ISO DIS 21470 to CCMAS, for review and endorsement with the recommendation of a Type II method.
- Refer AOAC 2015.10 / ISO DIS 21468 to CCMAS, for review and endorsement with the recommendation of a Type II method.
- Refer AOAC 2016.14 / ISO DIS 22579 | IDF 241 to CCMAS, for review and endorsement with the recommendation of a Type II method.
- Refer AOAC 2016.13 / ISO 23443 to CCMAS, for review and endorsement with the recommendation of a Type II method.
- Refer ISO DIS 23305 to CCMAS, for review and endorsement with the recommendation of a Type II method.

Request that CCMAS review the existing Type II and Type III methods listed in CXS 234-1999, to determine if the methods meet the specifications in CXS 72-1981. Based on that review, either retain the existing methods, making any necessary changes to the Type, or revoke any methods which do not meet the specifications.

³ REP18/NFSDU, para. 152 and Appendix VII

⁴ REP 18/MAS, Appendix II

⁵ REP 18/CAC, Appendix III

⁶ <https://www.iso.org/standard/75198.html>

TABLE 4. AOAC Official Methods validated in Infant Formula

Commodity	Provision	Method	Principle	Proposed Type
Infant Formula	Thiamine	AOAC 2015.14 / ISO DIS 21470	Enzymatic digestion and LC-MS/MS	II
		EN 14122	HPLC with pre- or post-column derivatization to thiochrom	‡ III
		AOAC 986.27	Fluorimetry	III
	Riboflavin	AOAC 2015.14 / ISO DIS 21470	Enzymatic digestion and LC-MS/MS	II
		EN 14152	HPLC	‡ III
		AOAC 985.31	Fluorimetry	III
	Niacin	AOAC 2015.14 / ISO DIS 21470	Enzymatic digestion and LC-MS/MS	II
		EN 15652	HPLC	‡ III
		AOAC 985.34	Microbioassay and turbidimetry	III
	Vitamin B6	AOAC 2015.14 / ISO DIS 21470	Enzymatic digestion and LC-MS/MS	II
		AOAC 2004.07 / EN 14164	HPLC	‡ III
		AOAC 985.32	Microbioassay	III
		EN 14166	Microbioassay	III
	Choline	AOAC 2015.10 / ISO DIS 21468	LC-MS/MS	II
		AOAC 999.14	Enzymatic Colorimetric Method with limitations on applicability due to choline and ascorbate concentration	‡ III
	Carnitine	AOAC 2015.10 / ISO DIS 21468	LC-MS/MS	II
Fructans	AOAC 2016.14 / ISO DIS 22579 IDF 241	Enzymatic digestion with HPAEC-PAD	II	
Beta Carotene	AOAC 2016.13 / ISO DIS 23443	UHPLC	II	
Lycopene	AOAC 2016.13 / ISO DIS 23443	UHPLC	II	
Biotin	AOAC 2016.02 / ISO 23305	HPLC-UV	II	
	EN 15607	HPLC-fluorescence	III	