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

(Prepared by United States of America)

An in-session physical working group (PWG) was held on the 26th of December 2018 during the CCNFSDU meeting. The focus of the working group was to provide consensus on the recommendations for Methods of Analysis in the Standard for Infant Formula and Formulas for Special Medical Purposes intended for Infants (CXS 72-1981), specifically for vitamin K, folic acid, and 9 minerals (Ca, Mg, P, K, Na, Cu, Fe, Mn, Zn). The working group reviewed the proposed new methods in CRD3 for Type II status. A summary of the discussions and consensus points of the physical working group are outlined below.

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 the *Recommended Methods of Analysis and Sampling* (CXS 234-1999) and referenced in the *Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants* (CXS 72-1981). It is proposed that these new methods be considered during the 40th Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) in November 2018 and that they either: (1) replace current Type II/III methods which may be outdated, and/or methods that were not validated on all types of infant formula; or (2) serve as Type II methods where such methods do not currently exist.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* (CXS 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 CXS 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 CXS 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 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, biotin, pantothenic acid, myo-inositol, fatty acid profile, total nucleotides, iodine, chloride, 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 was requested to consider recommending to CCMAS that the current methods for Minerals and Trace Elements (i.e. calcium, magnesium, phosphorus, potassium, sodium, copper, iron, manganese, zinc), vitamin K, and folic acid in CXS 234-1999 are outdated and/or not validated for infant formula, that they be replaced by the below methods, and that they be removed or reclassified within CXS 234-1999.

Additional rationale supporting each method is provided below. Table 4 presents the summary of the proposed changes.

Methods for MINERALS AND TRACE ELEMENTS (Ca, Mg, P, K, Na, Cu, Fe, Mn, Zn) (AOAC 2015.06 / ISO 21424 | IDF 243)

The PWG discussed that the AOAC 2015.06 is an Official Final Action method which has undergone successful multi-laboratory validation (MLV) using eighteen infant, child and adult nutritional formulas covering the fortification range. Ten laboratories in seven countries participated in the MLV of the inductively couple plasma- mass spectrometry (ICP-MS) method, which determines the concentrations of nine nutritional elements. Method performance data from the MLV is described by Pacquette and Thompson.[1] It is also expected that ISO/IDF will publish this method as a final ISO|IDF Standard in November 2018. AOAC 2015.06 / ISO 21424 | IDF 243 was proposed as Type II for the determination of all nine elements in infant formula. This method allows for detection and quantitation of minerals at lower levels.

The PWG had the following discussion:

- There was concern expressed that the emission spectroscopy methodology would be no longer considered as an acceptable method of analysis; however, as it would move to Type III with acceptance of the above method as Type II, it would still be considered acceptable for use.
- The AOAC representative cited that both methods, including emission spectroscopy, could still be used for determination of ultra-trace minerals.
- A question was asked about the process of referral to CCMAS.

There was no disagreement among members of the working group.

Methods for VITAMIN K (AOAC 2015.09 / ISO 21446)

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

The PWG discussed the proposed new method (AOAC 2015.09 / ISO 21446) as an AOAC Official Final Action method[2] which determines trans and **total Vitamin K1** in the product matrix using high performance liquid chromatography with post column reduction fluorescence detection. The method underwent an eight laboratory, six country MLV, using nineteen infant formula matrices. Method validation performance data is described by Schimpf *et al.*[3] Based on the method performance, AOAC 2015.09 / ISO 21446 is proposed as a Type II method for the determination of Vitamin K in infant formula.

There was no disagreement among members of the working group.

Methods for FOLIC ACID (AOAC 2011.06)

The current Codex Type II method for the determination of folic acid in infant formula is AOAC 992.05 / EN 14131, Total Folate/Folic Acid (Pteroylglutamic Acid) in Infant Formula. The AOAC method is not capable of estimating bound natural folates and is therefore invalid for infant formulas. EN 14131 has not been validated for infant formula matrices. Additionally, both methods are based on microbiological technique which is a lengthy and laborious and can easily be compromised by variable vitamer response. The microbiological assays are known to be non-specific and are very susceptible to matrix interferences as well have poor precision.

AOAC 2011.06 is an AOAC Official Final Action method which has undergone an eleven laboratory MLV using eleven infant and adult nutritional formulas covering a wide folic acid fortification and total folate range as well as infant formula matrix types. AOAC 2011.06, using a trienzyme extraction, solid phase extraction and liquid chromatography-tandem mass spectrometry to determine total folate/folic acid in infant and adult nutritional formulas. **Folic acid (common name Pteroyl-L-glutamic acid), 5-CH3-THF (common name calcium-L-**

methyl folate), and 5-CHO-THF are quantified, and total folate is estimated and expressed as folic acid. The method is also capable of estimation of total folic acid and natural folates separately enabling calculation of results as Dietary folate equivalents (DFUs) if required. Based on the method performance reported by Bhandaru et al.[4] AOAC 2011.06 is proposed as a Type II method for the determination of folic acid in infant and adult nutritional formulas.

There was no disagreement among members of the working group.

PWG RECOMMENDATIONS that the Committee:

- Refer methods for the **9 Minerals and Trace Elements** in Table 1 (AOAC 2015.06 / ISO 21424 | IDF 243) 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.
- Confirm that the method for **Vitamin K** (AOAC 2015.09 / ISO21446) determines the analytes consistent with the form in CXS 72-1981[5] and Infant Formula provisions in the *Advisory Lists of Nutrient Compounds for Use in Foods for Special Dietary Uses Intended for Infants and Young Children* (CXG 10-1979)[6]. If the appropriate forms are being determined by the method, refer the method, as a Type II, for CCMAS review and endorsement.
- Confirm that the method for **folate** (AOAC 2011.06) determines the analytes consistent with the form(s) in CXS 72-1981[7] and infant formula provisions in CXG 10-1970[8]. If the appropriate analytes and forms are being determined, refer the method, as a Type II, for CCMAS review and endorsement

Comment on the current use/applicability of the existing methods and if they should be retained as Type III.

1 Pacquette, L. H.; Thompson, J. J. *J AOAC Int*. **2018**, 101 (2), 536.

2 Bidlack M, Butler Thompson LD, Jacobs WA, Schimpf KJ. J AOAC Int. 2015 Sep-Oct;98(5):1382-9

3 Schimpf, K. J.; Butler Thompson, L. D.; Pan, S-J. *J AOAC Int*. **2018**, 101.

4 Bhandary, S. D; Gao, M.; Szpylka, J. *J AOAC Int.* **2018**, 101.

5 CXS 72-1981 lists Vitamin K.

6 CXG 10-1979 lists the nutrient source as "13. Vitamin K1; 13.1 Phytomenadione (2-Methyl-3-phytyl-1,4-naphthoquinone/ Phylloquinone/ Phytonadione) for infant formula.

7 CXS 72-1981 lists folic acid.

8 CXG 10-1979 lists the nutrient source as N-Pteroyl-L-glutamic acid for infant formula and calcium-L-methyl folate for infant formula Section B.

TABLE 1. AOAC Official Methods validated in Infant Formula

Commodity	Provision	Method	Principle	Proposed Type
	Calcium	**AOAC 2015.06 / ISO 21424 | IDF 243**	**ICP mass spectrometry**	**II**
		ISO 8070 | IDF 119	Flame atomic absorption spectrophotometry	~~II~~ **III**
		AOAC 985.35	Flame atomic absorption spectrometry	III
	Copper	**AOAC 2015.06 / ISO 21424 | IDF 243**	**ICP mass spectrometry**	**II**
		AOAC 985.35	Flame atomic absorption spectrophotometry	~~II~~ **III**
		~~AOAC 984.27~~	~~ICP emission spectroscopy~~	~~III~~
	Iron	**AOAC 2015.06 / ISO 21424 | IDF 243**	**ICP mass spectrometry**	**II**
		AOAC 985.35	Flame atomic absorption spectrometry	III
		~~AOAC 984.27~~	~~ICP emission spectroscopy~~	~~III~~
	Magnesium	**AOAC 2015.06 / ISO 21424 | IDF 243**	**ICP mass spectrometry**	**II**
		ISO 8070 | IDF 119	Flame atomic absorption spectrophotometry	~~II~~ **III**
		AOAC 985.35	Flame atomic absorption spectrometry	III
	Manganese	**AOAC 2015.06 / ISO 21424 | IDF 243**	**ICP mass spectrometry**	**II**
		AOAC 985.35	Flame atomic absorption spectrometry	~~II~~ **III**
		~~AOAC 984.27~~	~~ICP emission spectroscopy~~	~~III~~
	Phosphorus	**AOAC 2015.06 / ISO 21424 | IDF 243**	**ICP mass spectrometry**	**II**
		~~AOAC 984.27~~	~~ICP emission spectroscopy~~	~~III~~
		AOAC 986.24	Spectrophotometry (molybdovandate)	~~II~~ **III**
	Potassium	**AOAC 2015.06 / ISO 21424 | IDF 243**	**ICP mass spectrometry**	**II**
		ISO 8070 | IDF 119	Flame atomic absorption spectrophotometry	~~II~~ **III**
		~~AOAC 984.27~~	~~ICP emission spectroscopy~~	~~III~~
	Sodium	**AOAC 2015.06 / ISO 21424 | IDF 243**	**ICP mass spectrometry**	**II**
		ISO 8070 | IDF 119	Flame atomic absorption spectrophotometry	~~II~~ III
		~~AOAC 984.27~~	~~ICP emission spectroscopy~~	~~III~~
	Zinc	**AOAC 2015.06 / ISO 21424 | IDF 243**	**ICP mass spectrometry**	**II**
		AOAC 985.35	Flame atomic absorption spectrometry	~~II~~ **III**
		~~AOAC 984.27~~	~~ICP emission spectroscopy~~	~~III~~
	Vitamin K	**AOAC 2015.09 / ISO 21446**	**HPLC**	**II**

	Folic acid	**AOAC 2011.06**	**LC-MS/MS**	**II**
		AOAC 992.05 / EN 14131	Microbioassay	~~II~~ **III**
		J AOAC Int. 2000:83; 1141-1148	Optical Biosensor Immunoassay	IV
		J Chromatogr. A., 928, 77-90, 2001	HPLC, incorporating immunoaffinity clean-up and conversion to 5-methyltetrahydrofolate	IV