CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda Item 3 **MAS/39 CRD/3**

ORIGINAL LANGUAGE ONLY

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS SAMPLING

Thirty-ninth Session
Budapest, Hungary, 7 – 11 May 2018

ENDORSEMENT OF METHODS OF ANALYSIS PROVISIONS AND SAMPING PLANS IN CODEX STANDARDS

(Information submitted by AOAC, ISO and IDF)

Executive Summary

This document presents recommendations and supporting information for each recommendation from AOAC INTERNATIONAL (AOAC), the International Standardization Organization (ISO) and the International Dairy Federation (IDF) regarding infant formula methods of analysis topics to be discussed during the 39th Session of the Codex Committee on Methods of Analysis and Sampling (CCMAS39).

Recommendations to CCMAS39

AOAC/ISO/IDF recommends CCMAS39 to take the following actions:

1. Endorse AOAC 2016.02 | ISO DIS 23305 as Type II and reclassify EN 15607 as Type III.
2. Endorse AOAC 2016.05 | ISO 20636 as Type II, reclassify EN 12821 as Type III and remove AOAC 992.26 and AOAC 995.05 from CODEX STAN 234-1999.
3. Endorse AOAC 2016.03 | ISO 21422 | IDF 242 as Type II.

Agenda Item #3: Endorsement of Methods of Analysis and Sampling Plans for Provisions in Codex Standards

Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU39)

Methods of analysis for provisions in the Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants (CODEX STAN 72-1981)

Biotin

CCNFSDU39 agreed to submit AOAC 2016.02, Determination of Biotin by Liquid Chromatography Coupled with Immunoaffinity Column Cleanup Extraction, to CCMAS39 for technical review, typing, endorsement, and inclusion in the Recommended Methods of Analysis and Sampling (CODEX STAN 234-1999) in Part A, section "Foods for Special Dietary Uses," with the description "Infant Formula." This method reflects the most recent scientific method of analysis for biotin in infant formula and was fully validated in these products. AOAC Multi-Laboratory Testing (MLT) and reproducibility validation information for AOAC 2016.02 has been published.[1] Development of an International Standardization Organization (ISO) Draft International Standard (DIS 23305) for this method has been initiated and will be published for review by May 2018. CCNFSDU39 also asked CCMAS39 to re-type the related existing methods for biotin in CODEX STAN 234-1999.

The current Type II method for the determination of biotin in infant formula is EN 15607. For this method, D-biotin is extracted from a sample after an enzymatic treatment and quantified by HPLC with post column binding reaction with the fluorescent marker avidin-FTIC (Fluorescein 5-isothiocyanate). The enzymatic digestion is time consuming as it involves overnight incubation as part of the sample preparation. The avidin-FTIC is relatively expensive and the stability is questionable and it has to be monitored at frequent intervals with calibration standards to ensure the validity of the post-column reaction.

[1] *J. AOAC Int*. (2018) **101**, in press DOI: https://doi.org/10.5740/jaoacint.17-0242

AOAC 2016.02 has been published as Official Final Action and has extensive Single Laboratory (SLV) and MLT study data. The MLT study utilized 14 carefully selected infant, child and adult nutritional formulas covering a broad matrix range. 12 laboratories covering 10 countries produced data with excellent precision and accuracy.

The SLV and the MLT data provide systematic scientific evidence for a simple, selective, accurate and precise method for the purposes of dispute resolution for the determination of total biotin in all forms of infant, adult, and/or pediatric formula.

The analytical platform is inexpensive and the method can be used in almost any labs worldwide with basic facilities. The immunoaffinity column (IAC) clean-up extraction is the key step to successful analysis. Although R-Biopharm IAC was used for the MLT, alternative IACs were compared during the SLV with comparable results.

The performance parameters of the method are summarized below.

Parameters	**AOAC 2016.02 | ISO DIS 23305**	**EN 15607**
Analyte	Total biotin	Total D-biotin
Repeatability (RSD_r)	5%	11.6%
Reproducibility (RSD_R)	10%	29.8%
Recovery (>1 µg/100 g)	95 to 105%	Not Available
NIST SRM 1849a (certified value 199 ± 13 µg/100g)	197 ± 10 µg/100 g	Not Available
Limit of Quantitation	0.017 µg/100 kcal	Not Available
Infant formula matrices used in MLT validation	14	Not Available

The validated limit of quantitation of AOAC 2016.02 | ISO DIS 23305 is 0.01 µg/100 ml RTF which is equivalent to 0.017 µg/ 100 kcal based on the minimum energy value of 60 kcal /100 ml RTF as per CODEX STAN 72-1981. The performance of the procedure ensures that the method is able to selectively, accurately and precisely determine biotin well below 1.5 µg/100 kcal, the minimum level for biotin in CODEX STAN 72-1981.

AOAC/ISO/IDF recommends CCMAS39 to endorse AOAC 2016.02 as Type II and reclassify EN 15607 as Type III, as indicated in the below table.

Table 1. Recommended Methods of Analysis and Sampling (CODEX STAN 234-1999)

Commodity	**Provision**	**Method**	**Principle**	**Type**
Infant Formula	Biotin	EN 15607	High Performance Liquid Chromatography	~~**II**~~ **III**
		AOAC 2016.02 | ISO DIS 23305	**Liquid Chromatography**	**II**

Vitamin D

CCNFSDU39 agreed to submit AOAC 2016.05 | ISO DIS 20636, Analysis of Vitamin D_2 and Vitamin D_3 in Fortified Milk Powders and Infant and Nutritional Formulas by Liquid Chromatography – Tandem Mass Spectrometry, to CCMAS39 for technical review, typing, endorsement, and inclusion in the Recommended Methods of Analysis and Sampling (CODEX STAN 234-1999) in Part A, section "Foods for Special Dietary Uses," with the description "Infant Formula." This method reflects the most recent scientific method of analysis for vitamin D in infant formula and was fully validated in these products. AOAC MLT and reproducibility validation information for AOAC 2016.05 has been published.[2,3] This method will also be published as an ISO Standard in 2018. CCNFSDU39 also asked CCMAS39 to re-type the related existing methods for vitamin D in CXS 234-1999.

[2] *J. AOAC Int*. (2018) **101**, 256-263 DOI: https://doi.org/10.5740/jaoacint.17-0149

[3] For calculation purposes, 1 µg/hg = 1 µg/100g.

The current Type II method for the determination of vitamin D in infant formula is EN 12821. For this method, following sample saponification, vitamin D is extracted into organic solvent, with additional matrix separation by semi-preparative normal phase HPLC, with subsequent reversed-phase analytical HPLC. Quantitation is by use of vitamin D_2 as internal standard for vitamin D_3 analysis (vice versa for vitamin D_2 analysis). The saponification, extraction, evaporation and semi-preparative HPLC steps all contribute to a significant analysis time for this method. This method has limited sample throughput due to the serial nature of the semi-preparative step, and in a single analysis can only test a single vitamin D form (vitamin D_2 or vitamin D_3). Additionally, inter-laboratory validation for EN 12821 was performed on only two infant formula matrices.

The proposed new Type II method (AOAC 2016.05 | ISO DIS 20636) is an AOAC Official Final Action method and allows rapid and high throughput generation of precise and accurate analytical results for the simultaneous determination of both vitamin D_2 and vitamin D_3 in pediatric and adult nutritional formulas. Samples are saponified at high temperature, with lipid-soluble components extracted into isooctane and vitamin D derivatized with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) to form a high-molecular mass, easily ionizable adduct, which is analyzed by LC-MS/MS. Stable isotope labeled internal standards are used for quantitation to correct for losses in extraction and variation in derivatization and ionization efficiencies. Due to the highly selective nature of tandem mass spectrometry, coupled with the highly accurate analyte recovery afforded by use of isotope labelled internal standards, LC-MS/MS has become the “gold standard” analytical technique for the analysis of small molecules in complex matrices.

The performance of AOAC 2016.05 | ISO DIS 20636 was evaluated extensively by both Single Laboratory Validation (SLV) and Multi-Laboratory Testing (MLT). A sample set of over 20 matrices (SPIFAN Kit) used during both SLV and MLT method validation stages was developed specifically for this purpose and encompasses a broad range of infant and follow-on formula covering a wide nutrient fortification range. 10 laboratories from six countries participated in the MLT study and produced data with excellent precision and accuracy for all samples in the SPIFAN Kit.

The SLV and the MLT data provide systematic scientific evidence for a simple, selective, accurate and precise method for the purpose of dispute resolution for the determination of vitamin D_2 and vitamin D_3 in all forms of infant, adult, and pediatric formulas.

The performance parameters of the methods are summarized below:

Parameters	**AOAC 2016.05 | ISO DIS 20636**	**EN 12821**
Analyte	Simultaneous vitamin D_2 and D_3	Either vitamin D_2 or D_3
Repeatability (RSD_r)	1.9–5.8%	2.4–5.9%
Reproducibility (RSD_R)	6.4–12.7%	7.1–12.1%
Recovery (100 µg/kg)	Vitamin D_2: 97.0–99.2% vitamin D_3: 96.0–101.0%	93.9—102%
NIST SRM 1849a (Certified value 111 ± 17 µg/kg)	Vitamin D_3: 101 $\pm$ 11 µg/kg	Not available
Limit of Quantitation	0.029 µg/100 kcal	Not available
Infant formula matrices used in MLT validation	> 20	2

The validated limit of detection of AOAC 2016.05 | ISO DIS 20636 is 0.005 µg/100 ml RTF and the limit of quantitation is 0.0175 µg/100 ml RTF,[4] which is equivalent to 0.029 µg/100 kcal based on the maximum energy values of approximately 60 kcal/100 ml RTF. The performance of the procedure ensures that the method should be able to selectively, accurately and precisely determine vitamin D at well below 1 µg/100 kcal, the minimum level for vitamin D in CODEX STAN 72-1981.

AOAC/ISO/IDF recommends CCMAS39 to endorse AOAC 2016.05 | ISO 20636 as Type II and reclassify EN 12821 as Type III. In addition, it is recommended that AOAC 992.26 and AOAC 995.05 be removed from CODEX STAN 234-1999. Neither method uses an internal standard, which is widely regarded as necessary for highly manipulative analytical methods.

[4] Gill, B.D.; Zhu, X.; Indyk, H.E. (2015) A rapid method for the determination of vitamin D3 in milk and infant formula by liquid chromatography-mass spectrometry. Journal of AOAC International 98, 431–435.

Table 2. Recommended Methods of Analysis and Sampling (CXS 234-1999)

Commodity	Provision	Method	Principle	Type
		~~AOAC 992.26~~	~~High Performance Liquid Chromatography~~	~~III~~
		EN 12821	High Performance Liquid Chromatography	~~II~~ **III**
		~~AOAC 995.05~~	~~High Performance Liquid Chromatography~~	~~III~~
Infant Formula	Vitamin D	**AOAC 2016.05 | ISO DIS 20636**	**Liquid Chromatography – Mass Spectrometry**	**II**

Chloride

CCNFSDU39 agreed to submit AOAC 2016.03 | ISO DIS 21422 | IDF 242, Determination of Chloride in Milk, Milk Powder, Whey Powder, Infant Formula, and Adult Nutritionals by Potentiometric Titration Method, to CCMAS39 for technical review, typing, endorsement, and inclusion in the Recommended Methods of Analysis and Sampling (CODEX STAN 234-1999) in Part A, section "Foods for Special Dietary Uses," with the description "Infant Formula." This method reflects the most recent scientific method of analysis for chloride in infant formula and was fully validated in these products. AOAC Multi-Laboratory Testing and reproducibility validation information for AOAC 2016.03 has been published.[5] This method will be published as a joint ISO/IDF Standard in 2018. CCNFSDU39 also asked CCMAS39 to re-type the related existing methods for chloride in CODEX STAN 234-1999.

Codex adopted AOAC 986.26 as a Type III method for the detection of chloride in infant formula in 2009. Some validation data (collaborative study) was published in JAOAC 69, 777(1986). These data need updating with a wider range of infant formula and adult nutritional products.

AOAC 971.27 is based on the same principle as AOAC 986.26 and was designated as Type II (reference method) for "Foods for Special Dietary uses" by CCMAS. However, the field of application of this AOAC method is "general." This method is not appropriate for infant formula and adult nutritional products, since these products are not included in the scope of application.

The proposed new Type II method (AOAC 2016.03 | ISO DIS 21422 | IDF 242) is an AOAC Official Final Action method with extensive SLV and MLT data using 19 carefully selected infant and adult nutritional formulas covering the matrix range. 16 laboratories covering nine countries produced data with excellent precision and accuracy.

The SLV and the MLT data provide systematic scientific evidence for a simple, accurate and precise method for the purposes of dispute resolution for the determination of chloride in all forms of infant, adult, and/or pediatric formula.

The analytical platform is inexpensive and the method can be used in almost any labs worldwide with basic facilities. The autotitrator is not required so this broadens the analysis implementation options. The performance parameters of the method are summarized below.

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Parameters	**AOAC 2016.03 | ISO DIS 21422 | IDF 242**	**AOAC 986.26**
Analyte	Chloride	Chloride
Repeatability (RSD_r)	<2%	Not Available
Reproducibility (RSD_R)	<4%	Not Available

[5] *J. AOAC Int*. (2016) **99**, 1113-1117 DOI: http://dx.doi.org/10.5740/jaoacint.16-0123

Recovery	99 to 103%	Not Available
NIST SRM 1849a (Reference value 701 + 17.0 mg/100 g)	701 ± 17.1 mg/100 g	Not Available
Limit of Quantitation	2.3 mg/100 kcal	Not Available

The validated limit of quantitation of AOAC 2016.03 | ISO DIS 21422 | IDF 242 is 1.4 mg/100 g RTF, which is equivalent to 2.3 mg/100 kcal based on the maximum energy values of approximately 60 kcal/100 g RTF. The performance of the procedure ensures that the method is able to selectively, accurately and precisely determine chloride at well below 50 mg/100 kcal, the minimum level for chloride in CODEX STAN 72-1981.

AOAC/ISO/IDF recommends CCMAS39 to endorse AOAC 2016.03 | ISO DIS 21422 | IDF 242 as Type II. With regard to other existing Codex methods, AOAC 986.26 is Type III for Infant Formula. There is no need for reclassification or removal of this method.

Table 3. Recommended Method of Analysis and Sampling (CXS 234-1999)

Commodity	**Provision**	**Method**	**Principle**	**Type**
Infant Formula	Chloride	AOAC 986.26	Potentiometry	III
		AOAC 2016.03 | ISO DIS 21422 | IDF 242	**Potentiometric Titration**	**II**

Recommendations to CCMAS39

AOAC/ISO/IDF recommends CCMAS39 to take the following actions:

1. Endorse AOAC 2016.02 | ISO DIS 23305 as Type II and reclassify EN 15607 as Type III.
2. Endorse AOAC 2016.05 | ISO DIS 20636 as Type II, reclassify EN 12821 as Type III and remove AOAC 992.26 and AOAC 995.05 from CODEX STAN 234-1999.
3. Endorse AOAC 2016.03 | ISO DIS 21422 | IDF 242 as Type II.