

**European Union Comments**  
**CODEX COMMITTEE ON NUTRITION AND FOODS FOR SPECIAL**  
**DIETARY USES**

**Thirty-seventh Session**

**Bad Soden am Taunus, Germany, 23 – 27 November 2015**

**Agenda item 11 a):**

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

**CX/NFSDU 15/37/10**

*Mixed competence*

*Member States vote*

The European Union and its Member States would like to submit the following comments:

The proposed AOAC/ISO/(IDF) methods have been recently validated by an AOAC led consortium of infant formula manufacturers. They represent the current state-of-the-art and as some of the related methods listed in STAN 234 are indeed outdated it is appropriate that CCNFSDU requests CCMAS to review them and decide whether they can be endorsed. For some of the provisions contained in STAN 72 methods of analysis are indeed missing in STAN 234. For example, no method has been endorsed and included in STAN 234 for myo-inositol and nucleotides, although the latter are only mentioned under optional ingredients and no minimum/maximum values are given in the standard. For the other provisions mentioned STAN 234 contains endorsed methods and a number of them, in particular the European Norms, are still valid and in use (see overview below).

<b>Provision</b>	<b>Method included in STAN 234</b>	<b>Principle</b>	<b>Method scope</b>
Vitamin B12	AOAC 986.23	Microbiological assay (no validation data available)	Milk/infant formula
Myo-inositol	No method	--	--
Chromium	EN 14082 EN 14083 AOAC 2006.03	AAS dry ashing AAS pressure ashing ICP-OES	Milk Milk Fertilisers
Selenium	AOAC 996.16/17 EN 14627 AOAC984.27	Spectroscopy/Fluorimetry Hydride AAS ICP-OES	Feed/premixes  Se not in method scope
Molybdenum	EN 14083	AAS pressure ashing	
5'-Mono-nucleotides	No method	--	--

Vitamin A	EN 12083-1	HPLC-UV	Milk
Vitamin E	AOAC 992.03 EN 12822	HPLC-UV HPLC-UV	Infant formula Milk
Fatty acids	AOAC 996.06 <sup>1)</sup> AOCS Ce 1h-05 <sup>2)</sup>	GLC-FID GLC-FID	
Iodine	AOAC 992.24	Ion-selective electrode	Infant formula
Pantothenic acid	AOAC 992.07	Microbiological assay	Infant formula

<sup>1)</sup> AOAC 996.06 has been designed to determine total fat and has not been validated for the estimation of individual fatty acids, including trans fatty acids.

<sup>2)</sup> AOCS Ce 1h-05 has been specifically designed and validated for the determination of cis-, trans-, saturated, monounsaturated and polyunsaturated fatty acids in vegetable or non-ruminant oils and fats.

<b>Provision</b>	<b>Proposed amendment</b>	<b>Principle</b>	<b>Remark</b>
Vitamin B12	AOAC 2011.10 ISO/DIS 20634	HPLC-UV	Extensive validation data
Myo-inositol	No method	HPLC-amperometric detection	Extensive validation data
Chromium	AOAC 2011.19 ISO/DIS 20649 IDF 235	ICP-MS	Validation data provided not very informative
Selenium	AOAC 2011.19 ISO/DIS 20649 IDF 235	ICP-MS	Validation data provided not very informative
Molybdenum	AOAC 2011.19 ISO/DIS 20649 IDF 235	ICP-MS	Validation data provided not very informative
5'-Mono-nucleotides	AOAC 2011.20 ISO/DIS 20638	HPLC-UV	AOAC 2011.20 does not contain validation data; however, they are available (J. AOAC International 98, 2015, 971-979); method not suitable for non-fortified infant-formula
Vitamin A	AOAC 2012.10 ISO/DIS 20633	HPLC-UV	AOAC 2012.10 does not contain validation data, while ISO/DIS 20633 does. Furthermore, the standard does not allow to determine all potential forms of vitamin A and E.
Vitamin E	AOAC 2012.10 ISO/DIS 20633	HPLC-UV	AOAC 2012.10 does not contain validation data, while ISO/DIS 20633 does. Furthermore, the standard does not allow to determine

			all potential forms of vitamin A and E.
Fatty acids	AOAC 2012.13 ISO/DIS 16958 IDF 231	GLC-FID	AOAC 2012.13 does not contain validation data.
Iodine	AOAC 2012.15 ISO/DIS 20647 IDF 234	ICP-MS	Extensive validation data
Pantothenic acid	AOAC 2012.16 ISO/DIS 20639	HPLC-MS	AOAC 2012.16 does not contain validation data, while ISO/DIS 20639 does.

### Conclusions and recommendations

As all concerned provisions relate to rationale analytes where the stoichiometry of the compounds concerned is known, the criteria approach is applicable. This would allow keeping the currently used methods in the system, provided they fulfil the required criteria, and let analysts choose among those methods that best meet their needs.

No validation data are available for the determination of vitamin B12 by AOAC 986.23 and it could be replaced by the newly developed AOAC 2011.10 as the Type II method if CCNFSDU does not opt for the criteria approach.

For the determination of chromium, selenium and molybdenum appropriate European Standards exist (EN 14082, EN 14083, EN 14627) which should be kept in the Codex system, preferentially by using the criteria approach.

The proposed AOAC/ISO method for vitamin A does not quantify all forms of the vitamin, only retinyl palmitate and retinyl acetate, while the provisions of STAN 72 relate to all-trans-retinol. Since no saponification is foreseen in the AOAC/ISO method, naturally present retinyl esters of milk fat escape determination. Regarding vitamin E STAN 72 relates to d- $\alpha$ -tocopherol, while the measurands in the AOAC/ISO standard is dl-  $\alpha$ -tocopherol and dl-  $\alpha$ -tocopheryl acetate, which have a different biological activity compared to d- $\alpha$ -tocopherol. Moreover, other forms of vitamin E such as  $\beta$ -,  $\gamma$ -,  $\delta$ -tocopherol are not covered by the proposed AOAC/ISO standard. Therefore, CCNFSDU may be invited to specify to greater detail the provision for vitamin E, in particular which vitamers are considered to represent vitamin E. For this reason it is not recommended to request submission of AOAC 2012.10/ISO/DIS 20633 for review to CCMAS. In case, the committee decides otherwise, the criteria approach is recommended since appropriate European Standards (EN 12083-1, EN 12822) exist and are in use.

For the determination of fatty acids by AOAC 2012.13/ISO/DIS 16958/IDF 231 no validation data were found in AOAC 2012.13; however, they may be included in the equivalent ISO and IDF standards.