Method Guidance Notes on Nutritional Composition and Nutrition Labelling of Infant Formula, Follow-up Formula and Prepackaged Food for Infants and Young Children
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Nutritional Composition and Nutrition Labelling of Infant Formula,
Follow-up Formula and Prepackaged Food for Infants and Young Children

Centre for Food Safety
Food and Environmental Hygiene Department

INTRODUCTION

The Government is committed to protecting the health of infants and young children. The Food and Drug (Composition and Labelling) (Amendment) (No.2) Regulation 2014 (“the Amendment Regulation 2014”) amends the Food and Drugs (Composition and Labelling) Regulations (Cap. 132W) to provide for regulatory control over infant formula, follow-up formula and prepackaged food for infants and young children. The Amendment Regulation 2014 covers nutritional composition requirement of infant formula and nutrition labelling requirement for infant formula, follow-up formula (collectively, “formula products”) and prepackaged food for infants and young children.

2. In order to assist the trade to comply with the Food and Drugs (Composition and Labelling) Regulations (Cap. 132W) as amended by the Amendment Regulation 2014 (“the Amended Regulations”), the Centre for Food Safety (“CFS”) of the Food and Environmental Hygiene Department (“FEHD”) has prepared this Method Guidance Notes to provide technical information on the requirement and to answer some of the most frequently asked questions, which in turn offer guidance to the trade and laboratory service providers in making nutrition labels. The members of trade should also make reference to other related guidelines published by the CFS, such as “Technical Guidance Notes on Nutritional Composition and Nutrition Labelling of Infant Formula, Follow-up Formula and Prepackaged Food for Infants and Young Children”.

DISCLAIMER

3. The Method Guidance Notes are not part of the legislation and are intended for general reference only. It should be read in conjunction with the relevant legislation including but not limited to the Amendment Regulation 2014. Information contained in the Method Guidance Notes may not be exhaustive or complete. Specific issues should be considered on a case by case basis and independent legal advice should be sought in case of doubt. The ultimate authority for interpretation of the legislation rests with the Courts.
BACKGROUND

Objective of Legislative Amendment

4. The Food and Drugs (Composition and Labelling) Regulations (Cap. 132W) have introduced a Nutrition Labelling Scheme ("the Scheme") for prepackaged food products since July 2010 to regulate nutrition labelling and nutrition claims. However, the Scheme does not cover formula products and prepackaged food intended for infants and young children under the age of 36 months as the Codex Alimentarius Commission (Codex)\(^1\) has established different standards for these foods.

5. Providing nutrition information on labels of formula products and prepackaged food intended for infants and young children is an important communication tool to assist the parents to obtain specific nutrition information on individual food products.

6. The introduction of the Amendment Regulation 2014 aims to (i) protect the health of infants and young children; (ii) assist the parents in making informed food choices; and (iii) encourage food manufacturers to apply sound nutrition principle in the formulation of formula products and prepackaged food intended for infants and young children.

DEFINITIONS

7. The Amendment Regulation introduces some newly added or amended definitions, including the following (unless otherwise stated, references to provisions are references to provisions in the Amended Regulations) –

- **folic acid** (葉酸), in relation to any infant formula or follow-up formula, means N-pteroyl-L-glutamic acid.
- "niacin" (煙酸) —
  (a) in relation to any infant formula, means nicotinamide together with nicotinic acid; and
  (b) in relation to any follow-up formula, means nicotinamide.
- "nutrient" (營養素) -
  (a) means any substance present in food which-
  (i) belongs to, or is a component of, one of the following categories—
      (A) protein;
      (B) carbohydrates;
      (C) fat;

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\(^1\) The Codex Alimentarius Commission (Codex) was established in 1963 by the Food and Agriculture Organization of the United Nations and World Health Organization as an international authority to set food-related standards and guidelines.
(D) dietary fibre;
(E) vitamins;
(F) minerals; and

(ii) satisfies any of the following conditions -
(A) the substance provides energy;
(B) the substance is needed for growth, development and normal function of the body;
(C) a deficit of the substance will cause characteristic bio-chemical or physiological changes to occur; and

(b) in relation to any infant formula includes myo-inositol, L-carnitine and taurine.

• “vitamin A” (維他命 A) in relation to any infant formula or follow-up formula means all-trans retinol calculated in terms of Retinol Equivalent (RE) or in International Unit (IU) (with 1 μg RE as being equivalent to 3.33 IU).

• “vitamin C” (維他命 C) —
(a) in relation to any infant formula, means ascorbic acid together with dehydroascorbic acid; and
(b) in relation to any follow-up formula, means ascorbic acid.

• “vitamin E” (維他命 E)—
(a) in relation to any infant formula, means d-alpha-tocopherol, calculated in terms of alpha-Tocopherol Equivalent (α-TE) or International Unit (IU) (with 1 IU as being equivalent to 0.67 mg α-TE); and
(b) in relation to any follow-up formula, means alpha-tocopherol compounds, calculated in terms of α-TE or IU—
(i) (for alpha-tocopherol compounds from any natural source) with 1 IU as being equivalent to 0.67 mg α-TE; or
(ii) (for alpha-tocopherol compounds from any synthetic source) with 1 IU as being equivalent to 0.45 mg α-TE.

• “vitamin K” (維他命 K) in relation to any infant formula and follow-up formula, means vitamin K1.

LABORATORY TESTING

Selecting an Analytical Laboratory

8. Manufacturers, importers, vendors, or any other relevant parties, are recommended to engage laboratory testing to verify their own nutrition label declarations. There are laboratories in Hong Kong and overseas that analyze food products for nutrition labelling purpose. CFS recommends the selection of local commercial laboratories that are accredited to ISO/IEC 17025 standard under the Hong Kong Laboratory Accreditation Scheme (“HOKLAS”) by the Hong Kong Accreditation Service
(“HKAS”) or overseas accredited commercial laboratories. These accredited commercial laboratories could be found in the Directory of Accredited Laboratories of HKAS or websites of overseas accreditation bodies, such as National Association of Testing Authorities, Australia, China National Accreditation Service for Conformity Assessment, Singapore Accreditation Council, United Kingdom Accreditation Service, American Association for Laboratory Accreditation, etc. CFS does not impose the use of only accredited laboratories but recommends them as a first choice.

9. Laboratories performing nutrient analyses should be able to demonstrate that they operate under a documented quality assurance programme that provides assurance that samples are adequately logged, stored, sampled, analyzed, and archived (if needed); that the integrity of the data collected is maintained; that analysts are appropriately trained; that equipment is calibrated; that analyses are conducted by appropriately validated methods and according to standard operating procedures; and that data are checked for errors and for reasonableness of results. Standard operating procedures for each method should include the use of standard reference materials, spiked samples, or other validation materials. Besides, laboratory should participate regularly in proficiency testing or interlaboratory comparison programmes and present their results to their client when requested.

Selecting the Analytical Methodology

10. CFS recommends using appropriate methods published in international standards such as Official Methods of Analysis of AOAC International, ISO International Standards or European Standards. If no international standard method is available or appropriate, other reliable and appropriate analytical procedures may be used. For the testing of dietary fibre, however, only AOAC official methods are acceptable.

11. CFS recommends international standard methods because these methods have undergone collaborative evaluations with respect to the following:

   Accuracy: the closeness of agreement between a test result or measurement result and a reference value;

   Precision: the closeness of agreement between independent test/measurement results obtained under stipulated conditions;

   Selectivity: the extent to which a method can determine particular analyte(s) in a mixture(s) or matrix(s) without interferences from other components of similar behaviour;

   Sensitivity: quotient of the change in the indication of a measuring system and the corresponding change in the value of the quantity being measured;
Linearity: the ability of a method of analysis, within a certain range, to provide an instrumental response or results proportional to the quantity of analyte to be determined in the laboratory sample.

12. It is well recognized that modifications of international standard methods may be needed because international standard methods are not currently available for all nutrients of interest in all food matrices. With appropriate modifications, some international standard methods that appear to be of limited applicability can be modified for use in other food matrices. When original methods are modified, the precision and accuracy of the new applications should be established. While precision can usually be demonstrated with replicate assays, determination of accuracy requires a material or a standard with a certified concentration of the analyte being measured. A number of standard reference materials available from the European Union’s Community Bureau of Reference, European Union’s Institute of Reference Materials and Measurements, United Kingdom’s Laboratory of Government Chemist, United States of America’s National Institute of Standard and Technology, etc. are certified for elemental composition and some organic nutrients and are representative of some foods.

13. Alternative methodology is recommended only in the absence of internationally recognized standard methods. If alternative methods are developed and/or used, they should be accompanied by documentation that describe in detail the analytical procedures and performance characteristics of the methods. The document “AOAC recommended guidelines for stakeholder panel on infant formula and adult nutritionals single-laboratory validation” issued by AOAC is a suitable reference for formula products.

14. The method selected should be chosen on the basis of practicability and preference should be given to methods which have applicability for routine use. Analytical methods for analyzing fatty acids, vitamins and other nutrients in formula products are set out in Annex I for reference. The CFS does not require laboratories to use these methods. As improvements in methodology become available, there may be changes to the adoption of these methods any time.

Definition of nutrients

15. The Amendment Regulation 2014 adopted Codex’s standards principally and definitions of certain nutrients in relation to formula products are different from the definitions specified in the Scheme or its guidelines. As such, definitions of certain nutrients have been amended by the Amendment Regulation 2014 accordingly. For consistency, the definitions of those nutrients mentioned in the previous Amendment Regulation (2008) and corresponding guidelines are maintained in certain aspects. Traders and laboratories should pay particular attention to the contents of folic acid, niacin, vitamin A, C, E and K in relation to formula products or prepackaged food intended for infants and young children that are claimed to be suitable for persons of any age from 36 months onwards.
16. For vitamin testing, laboratories should critically evaluate the Codex recommended methods before adopting it for testing since some of these methods do not have sufficient specificity to quantify stereoisomers individually. Subsequently, the quantitative results obtained from non-specific method might be overestimated.

17. Last but not least, details on the testing of certain nutrients are provided in the section on Frequently Asked Questions at Annex II.

Centre for Food Safety
Food and Environmental Hygiene Department
January 2015
Analytical methods for analyzing fatty acids, vitamins and other nutrients in formula products.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Codex recommended methods*</th>
<th>Other standard methods</th>
</tr>
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<tbody>
<tr>
<td>Linoleic, α-linolenic, lauric, myristic and erucic acid, trans, polyunsaturated and total fatty acids</td>
<td>AOAC 996.06 (GC); AOCS Ce 1h-05 (09) (GC)</td>
<td>AOAC 2012.13 (GC)</td>
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<tr>
<td>Vitamin A</td>
<td>AOAC 992.04 (HPLC); AOAC 992.06 (HPLC); AOAC 974.29 (Colourimetry); EN 12823-1 (HPLC)</td>
<td>AOAC 2011.07 (UPLC); AOAC 2011.15 (HPLC); AOAC 2012.09 (HPLC); AOAC 2012.10 (HPLC)</td>
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<td>Vitamin D</td>
<td>AOAC 992.26 (HPLC); AOAC 995.05 (HPLC); EN 12821 (HPLC); NMIKL 167 (HPLC)</td>
<td>AOAC 2011.11 (UPLC-MS/MS); AOAC 2011.12 (UPLC-MS/MS); AOAC 2011.13 (LC-MS/MS); AOAC 2012.11 (LC-MS/MS)</td>
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<td>Vitamin E</td>
<td>AOAC 992.03 (HPLC); EN 12822 (HPLC)</td>
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<td>Vitamin K</td>
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<td>AOAC 992.27 (HPLC);</td>
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<tr>
<td>Thiamine</td>
<td>AOAC 986.27 (Fluorimetry); EN 14122 (HPLC)</td>
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<td>Riboflavin</td>
<td>AOAC 985.31 (Fluorimetry); EN 14152 (HPLC)</td>
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<td>Niacin</td>
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<td>Vitamin B6</td>
<td>AOAC 985.32 (MBA); AOAC 2004.07 (HPLC); EN 14164 (HPLC); EN 14164 (MBA); EN 14663 (HPLC)</td>
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<tr>
<td>Vitamin B12</td>
<td>AOAC 986.23 (Turbidimetry)</td>
<td>AOAC 2011.01 (SPR); AOAC 2011.08 (HPLC); AOAC 2011.09 (HPLC); AOAC 2011.10 (HPLC); AOAC 2011.16 (SPR); AOAC 2014.02 (UPLC)</td>
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<td>Vitamin</td>
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<td>AOAC 2011.05 (OBA); AOAC 2011.06 (UPLC-MS/MS); AOAC 2013.13 (UPLC-MS/MS)</td>
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<tr>
<td>Vitamin C</td>
<td></td>
<td>AOAC 2012.21 (HPLC); AOAC 2012.22 (UPLC)</td>
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<tr>
<td>Biotin</td>
<td>EN 15607 (HPLC)</td>
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<tr>
<td>Iodine</td>
<td>AOAC 992.24 (Ion-selective potentiometry)</td>
<td>AOAC 992.22 (HPLC); AOAC 2012.14 (ICP-MS); AOAC 2012.15 (ICP-MS); EN 15111 (ICP-MS); ISO 14378 (HPLC)</td>
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<td>Choline</td>
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<td>Myo-Inositol</td>
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<tr>
<td>L-Carnitine</td>
<td></td>
<td>AOAC 2012.17 (LC-MS/MS)</td>
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Note: * CODEX STAN 234-1999 (the most updated version of the method should be used)

The CFS does not require laboratories to use these testing methods.


AOCS refers to American Oil Chemist’s Society
EN refers to European standard.
GC refers to gas chromatography.
HPLC refers to high performance liquid chromatography.
IC refers to ion chromatography.
ICP-MS refers to inductively coupled plasma – mass spectrometry.
ISO refers to International Organization for Standardization.
MBA refers to microbiological assay.
MS refers to mass spectrometry.
NMKL refers to Nordic Committee on Food Analysis
OBA refers optical biosensor assay.
SPR refers to surface plasmon resonance.
UPLC refers to ultra performance liquid chromatography.
Frequently Asked Questions

General

1. Do the tolerance limits cover the measurement uncertainty of test methods?

Measurement uncertainty is a quantitative indicator of the analytical variability of a test result and has not been taken into consideration when corresponding tolerance limits were set. Since measurement uncertainty and tolerance limit are independent of each other, the measurement uncertainty will be dealt with separately in the event of compliance checking.

Proficiency test

2. Which organizations provide proficiency test for macronutrients?

Food Analysis Performance Assessment Scheme (FAPAS) of the Food and Environment Research Agency, United Kingdom organizes a number of proficiency tests (PT) on macronutrients, including moisture, ash, total fat, nitrogen, etc. in infant formula and milk powder. Similarly, the Laboratory of Government Chemist, United Kingdom organizes PT for most macronutrients in ready-to-eat and cereal products.²

3. Which organizations provide proficiency test for micronutrients, including vitamins, in formula products?

“Bureau Interprofessionnel des Etudes Analytiques” (BIPEA – International Bureau for Analytical Studies) organizes a comprehensive PT programme (dietary products and nutritional labeling) on micronutrients in infant milk / baby milk. The main parameters includes fluoride, iodide, lauric acid, Ca, Cu, Fe, K, Mg, Mn, Na, Se, P, Zn, vitamins (A, B1, B2, B5, B6, B9, B12, C, D, E, H, K, PP), choline, carnitine, inositol, etc. Besides, FAPAS organizes PT on vitamin A, D3 and elements in milk powder.²

4. How to interpret proficiency test findings?

There is a number of different ways to present the PT findings. One of the common ways is z-score. If the z-score value is between -2 and +2, it is classified as ‘satisfactory’. When the z-score value is between -2 and -3 or +2 and +3, it is normally classified as ‘questionable’. For z-score value is less

² Please be reminded that PT providers are not limited to the organizations mentioned in this guidance notes. Laboratory can search for other PT scheme that offer relevant food matrix and test parameter. CFS recommends the selection of PT providers that are accredited to ISO/IEC 17043 standard as a first choice.
than -3 or greater than +3, the result is said to be ‘unsatisfactory’ and the laboratory needs to investigate the source of unsatisfactory result.

**Energy**

5. Do I need to determine available carbohydrates before calculate energy content of an infant formula?

For prepackaged food, energy is obtained by the summation of the energy contributed by available carbohydrates, protein, total fat, ethanol, and organic acids, multiplied by corresponding conversion factors. However, the Codex’s “Standard for infant formula and formulas for special medical purposed intended for infants, CODEX STAN 72-1981 (Amended 2011)” (“Codex Stan 72”) has set minimum and maximum level for ‘total carbohydrates’ in infant formula only and there is no specific requirement set for dietary fibre in this standard. Therefore, ‘total carbohydrates’ is used for energy calculation and ‘Energy’ in infant formula is calculated by the following formula:

(\text{weight in grams} \times [4 \times \text{total carbohydrates} + 4 \times \text{protein} + 9 \times \text{total fat}] \text{ kcal in 100 mL of infant formula prepared ready for consumption})

For follow-up formula and prepackaged food intended for infants and young children, ‘available carbohydrates’ is used for energy calculation. Please refer to the “Method Guidance Notes on Nutrition Labelling and Nutrition Claims” for the energy calculation formula.

**Protein**

6. Which nitrogen to protein conversion factor should be used for infant formula based on soy protein or goat’s milk protein?

According to Codex Stan 72, nitrogen to protein conversion factor should be based on 6.25 unless a scientific justification for the use of a different conversion factor for a particular product. Besides, most countries that regulate infant formula used 6.25 as nitrogen to protein conversion factor. Thus, CFS uses 6.25 as nitrogen to protein conversion factor too. Please note that Australia and New Zealand use 6.38 as conversion factor for milk proteins and their partial protein hydrolysates.

**Fat**

7. Regarding the additional requirements on lauric, myristic and erucic acid and trans fatty acids, can total fat instead of total fatty acids be used for calculation?
In the Amendment Regulation 2014, it is stated clearly that the requirements were based on total fatty acids. Using total fat for calculation would lead to underestimation since total fat also contains phospholipids, wax ester, sterols and other minor amount of non-fatty material.

8. What is polyunsaturated fat?

Polyunsaturated fat refers to fatty acids with cis-cis methylene double bonds and commonly the sum of 14 polyunsaturated fatty acids including $\text{C}_{18:2}(9\text{-cis}, \text{C}_{18:3}(6,9,12\text{-cis}), \text{C}_{18:3}(9,12,15\text{-cis}), \text{C}_{20:2}(11,14\text{-cis}), \text{C}_{20:3}(8,11,14\text{-cis}), \text{C}_{20:3}(11,14,17\text{-cis}), \text{C}_{20:4}(5,8,11,14\text{-cis}), \text{C}_{20:5}(5,8,11,14,17\text{-cis}), \text{C}_{22:2}(13,16\text{-cis}), \text{C}_{22:3}(13,16,19\text{-cis}), \text{C}_{22:4}(7,10,13,16\text{-cis}), \text{C}_{22:5}(4,7,10,13,16\text{-cis}), \text{C}_{22:5}(7,10,13,16,19\text{-cis})$ and $\text{C}_{22:6}(4,7,10,13,16,19\text{-cis})$.

9. Does beta-carotene count as vitamin A in infant and follow-up formula?

With reference to the Codex Stan 72 and 156, the Amendment Regulation 2014 recognizes only all trans-retinol as vitamin A. Therefore, the method used for quantification should be able to separate the all trans-retinol from other stereoisomers. Please note that beta-carotene counts as vitamin A for prepackaged food including those intended for infants and young children.

10. Is there any difference for definition of vitamin E in infant formula, follow-up formula and prepackaged food intended for infants and young children?

Only d-alpha-tocopherol is defined as vitamin E in infant formula while both d- and l- isomer of alpha-tocopherol are counted as vitamin E in follow-up formula. For prepackaged food intended for infants and young children, please refer to FAQ 27 of the “Method Guidance Notes on Nutrition Labelling and Nutrition Claims” for details.

11. How can I distinguish vitamin E obtained from natural source or synthetic source?

Vitamin E obtained from synthetic source is a racemic mixture. In order to distinguish them, chiral separation of alpha-tocopherol isomers is necessary.

12. How to calculate vitamin E in terms of IU if a follow-up formula contains vitamin E from both natural source and synthetic source?

In such case, both d- and l- form of alpha-tocopherol should be determined. Since synthetic sources of vitamin E consists of racemic mixtures, i.e. d- and l- form in 1:1 ratio, the content of synthetic form can be found. Subsequently, the content of natural form can be calculated.
13. Are there suitable methods for separating erythorbic acid, L-dehydroascorbic acid and ascorbic acid in formula products?

The D-isomer (erythorbic acid) of ascorbic acid, which is used as an antioxidant food additive, does not show vitamin C activity. Hence, it is a good suggestion to determine these compounds simultaneously. At present, standard method cannot be found to separate them simultaneously. However, Doner and Hicks\(^3\) of US Department of Agriculture and Kall and Andersen\(^4\) of Danish Veterinary and Food Administration published their developed methods to quantify these compounds in foods and are suitable references.

14. Is AOAC 985.34 suitable for testing niacin in follow-up formula?

AOAC 985.34 determines nicotinic acid and nicotinamide by microbiological assay and cannot provide the content of nicotinic acid and nicotinamide separately. Hence, the quantitative results of niacin may be overestimated.

15. N-pteroyl-L-glutamic acid is another name for folic acid. Does it need to be defined?

The ‘folic acid’ referred in the Amendment Regulation 2014 is free folic acid and does not include food bounded folate. Thus, digestion or hydrolysis step before extraction and/or quantification should be avoided. Otherwise, overestimation may occur.

**Nutritional requirements**

16. How to calculate vitamin E to polyunsaturated fatty acids ratio?

The denominator of the ratio depends on the composition of polyunsaturated fatty acids (PUFA). The general formula for calculating the minimum ratio is given below:

\[
\text{Minimum } \alpha\text{-TE/g PUFA (mg/g PUFA)} = \{0.5 \times [C_{18:2}(9,12\text{-cis}) + C_{20:2}(11,14\text{-cis}) + C_{22:2}(13,16\text{-cis})] + 0.75 \times [C_{18:3}(6,9,12\text{-cis}) + C_{18:3}(9,12,15\text{-cis}) + C_{20:3}(8,11,14\text{-cis}) + C_{20:3}(11,14,17\text{-cis}) + C_{22:3}(13,16,19\text{-cis})] + 1.0 \times [C_{20:4}(5,8,11,14\text{-cis}) + C_{22:4}(7,10,13,16\text{-cis})] + 1.25 \times [C_{20:5}(5,8,11,14,17\text{-cis}) + C_{22:5}(4,7,10,13,16\text{-cis}) + C_{22:5}(5,8,11,14,17\text{-cis})] + C_{22:6}(7,10,13,16\text{-cis})] + 1.5 \times [C_{22:6}(4,7,10,13,16,19\text{-cis})] / \{C_{18:2}(9,12\text{-cis}) + C_{20:2}(11,14\text{-cis}) + C_{22:2}(13,16\text{-cis}) + C_{18:3}(9,12,15\text{-cis}) + C_{18:3}(9,12,15\text{-cis}) + C_{20:3}(8,11,14\text{-cis}) + C_{20:3}(11,14,17\text{-cis}) + C_{22:3}(13,16,19\text{-cis}) + C_{22:5}(4,7,10,13,16\text{-cis}) + C_{22:5}(5,8,11,14,17\text{-cis}) + C_{22:6}(7,10,13,16,19\text{-cis}) + C_{22:6}(4,7,10,13,16,9\text{-cis})] \}
\]

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where \([C_{xy}(n1, n2..., \text{cis})]\) refers to the concentration of individual PUFAs with the same concentration unit.

Using the above equation, for an infant formula sample containing PUFA at the level of 7.92 g/100 g, of which the concentrations of \(C_{18:2}(9,12\text{-cis})\), \(C_{18:3}(9,12,15\text{-cis})\) and \(C_{22:6}(4,7,10,13,16,19\text{-cis})\) are 7.54 g/100 g, 0.30 g/100 g and 0.08 g/100 g respectively, the minimum content of vitamin E is calculated to be 0.52 mg per g PUFA in sample.

17. Is AOAC 954.05 or 905.03 suitable for testing fluoride in infant formula?

AOAC 954.05 and 905.03 are qualitative methods only and cannot be used for quantitative analysis of fluoride in infant formula. In order to determine fluoride in food quantitatively, AOAC 944.08 may be considered. Other fully validated methods for quantitative determination of fluoride in infant formula can also be used.