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The Practical Application of the Principles of the Criteria Approach for Methods Which use Sum of Components

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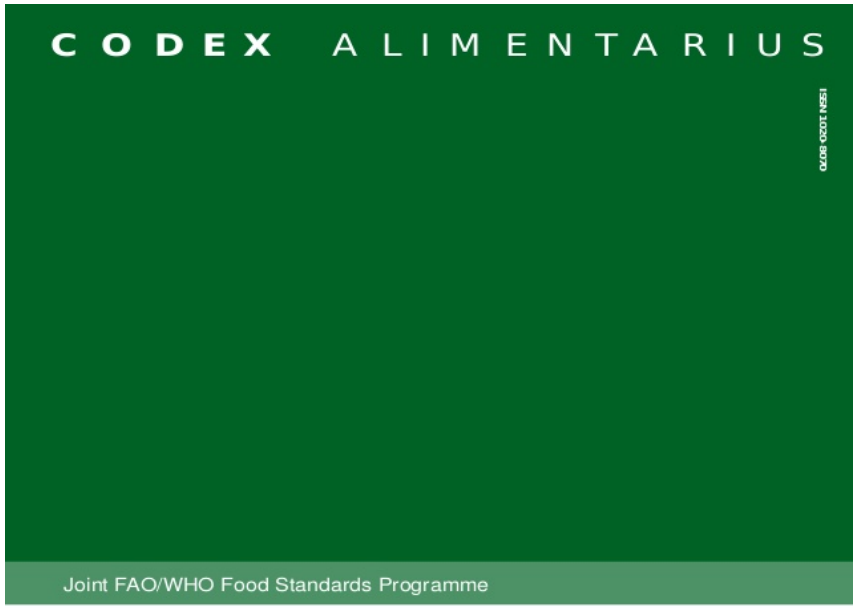

CODEX ALIMENTARIUS COMMISSION

PROCEDURAL MANUAL

Twenty-first edition

World Health Organization

Food and Agriculture Organization of the United Nations

ftp://ftp.fao.org/codex/Publications/ProcManuals/Manual_24e.pdf

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General Criteria for the Selection of Methods of Analysis

(a) Official methods of analysis elaborated by international organizations occupying themselves with a food or group of foods should be preferred.

(b) Preference should be given to methods of analysis the reliability of which have been established in respect of the following criteria, selected as appropriate:

(i) selectivity

(ii) accuracy

(iii) precision; repeatability intra-laboratory (within laboratory), reproducibility inter-laboratory (within laboratory and between laboratories)

(iv) limit of detection

(v) sensitivity

(vi) practicability and applicability under normal laboratory conditions

(vii) other criteria which may be selected as required.

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General Criteria for the Selection of Methods of Analysis

(c) The method selected should be chosen on the basis of practicability and preference should be given to methods which have applicability for routine use.

(d) All proposed methods of analysis must have direct pertinence to the Codex Standard to which they are directed.

(e) Methods of analysis which are applicable uniformly to various groups of commodities should be given preference over methods which apply only to individual commodities.

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Foods for Special Dietary Uses				
Follow-up formula	Vitamin A (retinol isomers)	AOAC 992.04	HPLC	II
Follow-up formula	Vitamin A (retinol) (above 500 IU/l milk after reconstitution)	AOAC 992.06	HPLC	III
Follow-up formula	Vitamin K	AOAC 999.15 EN 14148 (vitamin K_1) (Measures either aggregated cis + trans K_1 or can measure individual cis and trans forms depending on LC column.)	HPLC with C30 column to separate the cis- and the trans- K vitamins	II
Foods with low-sodium content (including salt substitutes)	Iodine	AOAC 925.56	Titrimetry	II
Foods with low-sodium content (including salt substitutes)	Silica (colloidal, calcium silicate)	AOAC 950.85N	Gravimetry	IV
Gluten-free foods	Gluten	Enzyme-Linked Immunoassay R5 Mendez (ELISA) Method *Eur J Gastroenterol Hepatol* 2003; 15: 465-474	Immunoassay	I
Infant formula	Biotin	EN 15607 (d-biotin) (Measures total D-biotin (free + D-biocytin)	HPLC	II
Infant formula	Calories (by calculation)	Method described in CAC/Vol IX-Ed.1, Part III[7]	Calculation	I
Infant formula	Calcium	ISO 8070 | IDF 119	Flame atomic absorption spectrophotometry	II

[7] Section 9. Calories by calculation – Section 9.2 Conversion Factors

(a) protein 4 kcal per g
(b) carbohydrate 4 kcal per g
(c) fat 9 kcal per g
(d) monosaccharides 3.75 kcal per g
(e) specific food ingredients See "Energy and Protein Requirements"(FAO Nutrition Meeting Report Series No. 52 or WHO Technical Report Series No. 522)
(f) other specific calorie conversion factors maybe used where the formulation of the food and the nutrient content are known and where such specific conversion factors are physiologically more meaningful than the factors listed above

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General Criteria for the Selection of Methods of Analysis using the Criteria Approach

In the case of Codex Type II and Type III methods, method criteria may be identified and values quantified for incorporation into the appropriate Codex commodity standard.

Two approaches for establishing criteria are described in the Procedural Manual.

- The first utilizes the specified limit (maximum or minimum limit) to establish numeric criteria for the characteristics mentioned and is summarized in Table 1.
- The second involves the conversion of a specific method to establish numeric criteria for the parameters listed in Table 1.

Although the method should be validated and appropriate for the analyte and commodity, there is not a specific requirement that the method be endorsed prior to being “converted” to criteria.

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Table 1 - The Criteria Approach

Applicability:	The method has to be applicable for the specified provision, specified commodity and the specified level(s) (maximum and/or minimum) (ML). The minimum applicable range of the method depends on the specified level (ML) to be assessed, and can either be expressed in terms of the reproducibility standard deviation (s_R) or in terms of LOD and LOQ.
Minimum applicable range:	For ML ≥ 0.1 mg/kg, [ML - 3 s_R , ML + 3 s_R] For ML < 0.1 mg/kg, [ML - 2 s_R , ML + 2 s_R] s_R [14] = standard deviation of reproducibility
Limit of Detection (LOD):	For ML ≥ 0.1 mg/kg, LOD ≤ ML · 1/10 For ML < 0.1 mg/kg, LOD ≤ ML · 1/5
Limit of Quantification (LOQ):	For ML ≥ 0.1 mg/kg, LOQ ≤ ML · 1/5 For ML < 0.1 mg/kg, LOQ ≤ ML · 2/5

Precision:	For ML ≥ 0.1 mg/kg, HorRat value ≤ 2 For ML < 0.1 mg/kg, the RSD_{TR} < 22%. RSD_R [15] = relative standard deviation of reproducibility. RSD_R ≤ 2. $PRSD_R$			
Recovery (R):	**Concentration**	**Ratio**	**Unit**	**Recovery (%)**
	100	1	100% (100g/100g)	98 – 102
	≥10	10^{-1}	≥ 10% (10g/100g)	98 – 102
	≥1	10^{-2}	≥ 1% (1g/100g)	97 – 103
	≥0.1	10^{-3}	≥ 0.1% (1mg/g)	95 – 105
	0.01	10^{-4}	100 mg/kg	90 – 107
	0.001	10^{-5}	10 mg/kg	80 – 110
	0.0001	10^{-6}	1 mg/kg	80 – 110
	0.00001	10^{-7}	100 µg/kg	80 – 110
	0.000001	10^{-8}	10 µg/kg	60 – 115
	0.0000001	10^{-9}	1 µg/kg	40 – 120
Trueness:	Other guidelines are available for expected recovery ranges in specific areas of analysis. In cases where recoveries have been shown to be a function of the matrix other specified requirements may be applied. For the evaluation of trueness preferably certified reference material should be used.			

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For concentration ratios ≥ 10^{-7} (≥ 0.1 mg/kg) the Horwitz' equation is applied:

$PRSD_R$ (%) = 100 · s_R/c =$2C^{-0.1505}$

where

$PRSD_R$ is the "predicted" relative standard deviation,

s_R is the predicted standard deviation

c is the concentration of interest, which here is the ML and

C is the concentration ratio, i.e. the concentration ratio of ML ($\mathbf{C}_{ML}$)

By rearranging the equation with respect of s_R, the following equation is obtained:

$$S_R = \frac{c \cdot 2C^{-0.1505}}{100} = \frac{ML \cdot 2 \cdot C_{ML}^{-0.1505}}{100}$$

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Example Criteria (Histamine)

Fish and Fishery Products				
Sturgeon Caviar	Salt content	Described in CODEX STAN 167-1989	Titrimetry (Mohr) Salt determined as chloride expressed as sodium chloride	I
Live and raw bivalve molluscs	Paralytic shellfish toxicity	AOAC 959.08	Mouse bioassay	IV
Live and raw bivalve molluscs	Paralytic shellfish toxicity	AOAC 2011.27	Receptor binding assay	IV

Method Performance Criteria for histamine in smoked fish, smoke-flavoured fish and smoke-dried fish

Provision	ML (mg/100 g)	Minimum applicable range (mg/100 g)	LOD (mg/100 g)	LOQ (mg/100 g)	RSD_R(%)	Recovery	Applicable methods that meet the criteria	Principle
Histamine	10 (average)	8 – 12	1	2	16.0	90 – 107	AOAC 977.13 | NMKL 99, NMKL 196,	Fluorometric HPLC
Histamine	20 (each unit)	16 – 24	2	4	14.4	90 – 107	AOAC 977.13 | NMKL 99, NMKL 196,	Fluorometric HPLC

Determination of Biotoxins in live and raw bivalve molluscs

The method selected should be chosen on the basis of practicability and preference should be given to methods which have applicability for routine use.

Criteria for determination of Toxin Analogues by chemical methods

Methods shall meet the numerical criteria listed in Table 1 and may either meet the minimum applicable range, or LOD and LOQ criteria listed.

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What About MLs that are a Sum of Components?

Cereals, Pulses and Legumes and Derived Products				
Degermed maize (corn) meal and maize (corn) grits	Protein	ICC Method No 105/1	Titrimetry, Kjeldahl digestion	I
Durum wheat semolina and durum wheat flour	Ash (semolina)	AOAC 923.03 ISO 2171	Gravimetry	I
Durum wheat semolina and durum wheat flour	Moisture	ISO 712 ICC 110/1	Gravimetry	I
Durum wheat semolina and durum wheat flour	Protein (N x 5.7)	ICC 105/1	Titrimetry, Kjeldahl digestion	I
Instant Noodles	Extraction of oil from instant noodles	described in the standard	Gravimetry	I
Instant Noodles	Acid Value	described in the standard	Titrimetry	I
Instant Noodles	Moisture	described in the standard	Gravimetry	I
Maize (corn)	Moisture	ISO 6540	Gravimetry	I
Peanuts (raw)	Aflatoxins, total	AOAC 991.31	Immunoaffinity column (Aflatest)	II
Peanuts (raw)	Aflatoxins, total	AOAC 993.17	Thin layer chromatography	III
Peanuts (intended for further processing)	Aflatoxins, total	AOAC 975.36	Romer minicolmn	III
Peanuts (Cereals, shell-fruits and derived products (including peanuts))	Sum of aflatoxins B_1, B_2, G_1 and G_2	EN 12955 ISO 16050:	HPLC with post column derivatization and immunoaffinity column clean up	III
Peanuts (intended for further processing)	Aflatoxins, total	AOAC 979.18	Holaday-Velasco minicolumn	III
Pearl millet flour	Ash	AOAC 923.03	Gravimetry	I
Pearl millet flour	Colour	*Modern Cereal Chemistry*, 6th Ed., D.W. Kent-Jones and A.J. Amos (Ed.), pp. 605-612, Food Trade Press Ltd, London, 1969.	Colorimetry using specific colour grader	IV
Pearl millet flour	Fat, crude	AOAC 945.38F; 920.39C	Gravimetry (ether extraction)	I
Pearl millet flour	Fibre, crude	ISO 5498: (B.5 Separation)	Gravimetry	I
Pearl millet flour	Moisture	ISO 712: ICC 110/1	Gravimetry	I

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Example Analyte Groups that Require a Sum of Components Analysis

- Colours (e.g. carotenoids, synthetic dyes)
- Desmethylsterols (e.g. cholesterol, campesterol, β-sitosterol)
- Tocopherols (e.g. α-tocopherol, β-tocopherol, γ-tocopherol, α-tocotrienol)
- Mycotoxins (e.g. aflatoxins B1 + B2 + G1 + G2)
- Polychlorinated Biphenyls (e.g. in natural mineral water)
- Polycyclic Aromatic Hydrocarbons (e.g. in natural mineral water)
- Organochlorine Pesticides (e.g. in natural mineral water)
- Scoville Units (e.g. total capsaicinoids)
- Shellfish Toxins (e.g. saxitoxin (STX) group, okadaic acid (OA) group)

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A Simple Example of How a Sum of Components Specification Could Theoretically be Converted to Method Performance Criteria

Example A:

Aflatoxin, consisting of 4 analytes, B_1, B_2, G_1 and G_2, in peanuts.

The ML = 15 µg/kg,

As there are 4 analytes, n = 4,

ML/n = 15/4 µg/kg = 3.75 µg/kg

Using the excel spreadsheet on www.nmkl.org under "how to get method criteria based on ML", the following are established:

Minimum Applicable Range for the individual components:	0.002* - 0.022** mg/kg = 2 - 22 µg/kg *corresponding to ML/*n* = 3.75 µg/kg **corresponding to ML = 15 µg/kg
Limit of Detection (LOD) for the individual components:	0.75 µg/kg
Limit of Quantification (LOQ) for the individual components:	1.5 µg/kg
Precision for the individual components:	$RSD_R \leq 44\%$
Recovery (R):	40-120%

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Fumonisins in Grain

Table 3. Performance criteria for Fumonisin B1+ B2.

Maize Grain

Analyte	**ML** (mg/Kg)	**LOD** (mg/Kg)	**LOQ** (mg/Kg)	**RSD_R**	**Recovery (%)**
FB1 + FB2	4.0	-	-	-	-
FB1		≤ 0.3*	≤ 0.6*	HorRat ≤ 2 (< 27%)	80 - 110
FB2		≤ 0.15*	≤ 0.3*	HorRat ≤ 2 (< 32%)	80 - 110

* - The LOD and LOQ were derived based upon typical B1:B2 ratio of 5:2 in naturally-contaminated samples

Maize Flour/Meal

Analyte	**ML** (mg/Kg)	**LOD** (mg/Kg)	**LOQ** (mg/Kg)	**RSD_R**	**Recovery (%)**
FB1 + FB2	2.0	-	-	-	-
FB1		≤ 0.15*	≤ 0.3*	HorRat ≤ 2 (< 30%)	80 – 110
FB2		≤ 0.06*	≤ 0.15*	HorRat ≤ 2 (< 34%)	80 – 110

* - The LOD and LOQ were derived based upon typical B1:B2 ratio of 5:2 in naturally-contaminated samples

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Issues

- If *n* is large (e.g. *n*>>5) then the LOD and LOQ might become unrealistically small.
- The simple approach does not take into account analyte weighting nor issues where geographical location might be important (e.g. shellfish toxins) although the fumonisins example does indicate a way forward.
- How do we deal with methods that involve the use of TEQs and TEFs?
- How do we deal with limits that contain provisions that are both single analyte and sum of components (e.g. sterols in oils and fats)?
- If methods have been formally validated using a sum of components basis it might be simplest to just convert the method into method performance criteria (e.g. shellfish biotoxins) rather than use the ML.

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So What Happens Next?

- ***There is no single approach to converts MLs that are a sum of components into method performance criteria. Decisions on how to undertake the conversion need to be made on a case by case basis.***
- A new eWG was proposed at the 37th session of CCMAS to further develop practical guidance on how to generate method performance criteria for limits that are based upon a sum of components. This work will be led by the United Kingdom.
- The sum of components issue is likely to gain importance as potentially more limits that involve a sum of components are adopted.
- The sum of components issue is not limited to Codex. It has application and relevance with other regional and national legislation.

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And Finally, Something Further to Consider

- How do we determine the measurement uncertainty of results that are a sum of components? Is there an issue here which needs to be addressed at some stage in the future?

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