

PRINCIPLES FOR THE ESTABLISHMENT OF CODEX METHODS OF ANALYSIS

Purpose of Codex Methods of Analysis

The methods are primarily intended as international methods for the verification of provisions in Codex standards. They should be used for reference, in calibration of methods in use or introduced for routine examination and control purposes.

Methods of Analysis

Definition of types of methods of analysis

(a) Defining Methods (Type I)

Definition: A method which determines a value that can only be arrived at in terms of the method per se and serves by definition as the only method for establishing the accepted value of the item measured.

Examples: Howard Mould Count, Reichert-Meissl value, loss on drying, salt in brine by density.

(b) Reference Methods (Type II)

Definition: A Type II method is the one designated Reference Method where Type I methods do not apply. It should be selected from Type III methods (as defined below). It should be recommended for use in cases of dispute and for calibration purposes.

Example: Potentiometric method for halides.

(c) Alternative Approved Methods (Type III)

Definition: A Type III Method is one which meets the criteria required by the Codex Committee on Methods of Analysis and Sampling for methods that may be used for control, inspection or regulatory purposes.

Example: Volhard Method or Mohr Method for chlorides

(d) Tentative Method (Type IV)

Definition: A Type IV Method is a method which has been used traditionally or else has been recently introduced but for which the criteria required for acceptance by the Codex Committee on Methods of Analysis and Sampling have not yet been determined.

Examples: chlorine by X-ray fluorescence, estimation of synthetic colours in foods.

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.
- (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.

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. Method criteria which are developed will include the criteria in section Methods of Analysis, paragraph (c) above together with other appropriate criteria, e.g. recovery factors.

General Criteria for the Selection of Single-Laboratory Validated Methods of Analysis

Inter-laboratory validated methods are not always available or applicable, especially in the case of multi-analyte/multi substrate methods and new analytes. The criteria to be used to select a method are included in the General Criteria for the Selection of Methods of Analysis. In addition the single-laboratory validated methods must fulfil the following criteria:

- (i) the method is validated according to an internationally recognized protocol (e.g. those referenced in the harmonized IUPAC Guidelines for Single-Laboratory Validation of Methods of Analysis)
- (ii) the use of the method is embedded in a quality system in compliance with the ISO/IEC 17025: 1999 Standard or Principles of Good Laboratory Practice;

The method should be complemented with information on accuracy demonstrated for instance with:

- regular participation in proficiency schemes, where available;
- calibration using certified reference materials, where applicable;
- recovery studies performed at the expected concentration of the analytes;
- verification of result with other validated method where available.

Working Instructions for the Implementation of the Criteria Approach in Codex

Any Codex Committee may continue to propose an appropriate method of analysis for determining the chemical entity and/or develop a set of criteria to which a method used for the determination must comply. In either case the specified maximum level, minimum level, any other normative level or the concentration range of interest has to be stated.

When a Codex Committee decides that a set of criteria should be developed, in some cases the Committee may find it easier to recommend a specific method and request the Codex Committee on Methods of Analysis and Sampling (CCMAS) to convert that method into appropriate criteria. The Criteria will then be considered by the CCMAS for endorsement and will, after the endorsement, form part of the standard. If a Codex Committee wishes to develop the criteria, it should follow instructions given for the development of specific criteria as outlined in Table 1.

Note: These criteria are applicable to fully validated methods except for methods such as PCR and ELISA, which require other set of criteria.

Table 1: Guidelines for establishing numeric values for the criteria:

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 $ML \geq 0.1$ 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^{12} = standard deviation of reproducibility
Limit of Detection (LOD):	For $ML \geq 0.1$ mg/kg, $LOD \leq ML \cdot 1/10$ For $ML < 0.1$ mg/kg, $LOD \leq ML \cdot 1/5$
Limit of Quantification (LOQ):	For $ML \geq 0.1$ mg/kg, $LOQ \leq ML \cdot 1/5$ For $ML < 0.1$ mg/kg, $LOQ \leq ML \cdot 2/5$

Precision:	For $ML \geq 0.1$ mg/kg, HorRat value ≤ 2 For $ML < 0.1$ mg/kg, the $RSD_{TR} < 22\%$. RSD_R^{13} = relative standard deviation of reproducibility. $RSD_R \leq 2$. $PRSD_R$			
Recovery (R):	Concentration	Ratio	Unit	Recovery (%)
	100	1	100% (100 g/100g)	98 . 102
	≥ 10	10^{-1}	$\geq 10\%$ (10 g/100g)	98 . 102
	≥ 1	10^{-2}	$\geq 1\%$ (1 g/100g)	97 . 103
	≥ 0.1	10^{-3}	$\geq 0.1\%$ (1 mg/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 μ g/kg	80 . 110
	0.000001	10^{-8}	10 μ g/kg	60 . 115
0.0000001	10^{-9}	1 μ g/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.			

The criteria in Table 1 must be approved for the determination in question.

However, the primary responsibility for supplying information about the specified Codex level(s), methods of analysis and criteria resides with the referring Committee. If the Committee fails to provide a method of analysis or criteria despite numerous requests, then the CCMAS may establish appropriate criteria as above.

¹² The s_R should be calculated from the Horwitz / Thompson equation. When the Horwitz/ Thompson equation is not applicable (for an analytical purpose or according to a regulation) or when converting methods into criteria then it should be based on the s_R from an appropriate method performance study.

¹³ The RSD_R should be calculated from the Horwitz/Thompson equation. When the Horwitz/Thompson equation is not applicable (for an analytical purpose or according to a regulation) or when converting methods into criteria then it should be based on the RSD_{s_R} from an appropriate method performance study.

GUIDELINES FOR ESTABLISHING NUMERIC VALUES FOR METHOD CRITERIA AND/OR ASSESSING METHODS FOR COMPLIANCE THEREOF

1. Recommendations for establishing numeric values for method criteria

Only the provision for the commodity along with its ML (maximum level, minimum level, normative level or concentration range) is needed when establishing numeric values for method criteria.

Note: These criteria are applicable to fully validated methods except for methods such as PCR and ELISA, which require other set of criteria.

1.1 The applicability

The method has to be applicable to the particular analyte(s)/provision(s) in the specified matrix/ commodity or food category. For horizontal methods the relevant food categories should have been tested. Furthermore, it should have been shown that the method is applicable for concentrations levels around the specified ML, i.e. the ML should be within the validated range.

- For $ML \geq 10^{-7}$, the minimum applicable range should be: $ML \pm 3s_R$
- For $ML < 10^{-7}$, the minimum applicable range should be: $ML \pm 2s_R$

The minimum applicable concentration range should correspond to an interval containing a large fraction of the expected variation (due to measurement uncertainty) in the results around the specified limit (ML). For collaboratively validated methods the expected variation would be the reproducibility standard deviation (s_R) multiplied with a coverage factor. A coverage factor of 2 corresponds to a confidence level of approx. 95%, and a coverage factor of 3 corresponds to a confidence level about 99%. As 99% is often used as an action level in control charts, a coverage factor of 3 is recommended for concentration ratios at or above 10^{-7} , (≥ 0.1 mg/kg). For concentrations lower than 0.1 mg/kg, a coverage factor of 2 is recommended, as a coverage factor of 3 would make it hard to find applicable methods for certain analytes/provisions due to the low level.

Calculation of the minimum applicable range for specified MLs:

The minimum applicable range can be estimated based on the Horwitz/Thompson equation for reproducibility standard deviation, s_R .

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

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

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 (C_{ML})

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

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

Example 1: ML = 0.1 mg/kg, C_{ML} = 10⁻⁷:

$$0.1 \pm 3 \cdot S_R = 0.1 \pm 3 \cdot \frac{0.1 \cdot 2 \cdot (0.0000001)^{-0.1505}}{100} = 0.1 \pm 0.07 \text{ mg/kg}$$

The minimum applicable range for a ML of 0.1 mg/kg is then 0.03 to 0.17 mg/kg

Example 2: For a ML of 1 mg/kg (i.e. 10⁻⁶):

$$1.0 \pm 3 \cdot S_R = 1.0 \pm 3 \cdot \frac{1.0 \cdot 2 \cdot (0.000001)^{-0.1505}}{100} = 1.0 \pm 0.48 \text{ mg/kg}$$

The minimum applicable range for ML of 1 mg/kg is then 0.5 to 1.5 mg/kg

1.1.2 For concentration ratios < 10⁻⁷, the Thompson theory is applied, i.e. PRSD_R = 22% and hence s_R = 0.22 · ML

Example 3: ML = 0.01 mg/kg (i.e. 10⁻⁸):

$$0.01 \pm 2 \cdot s_R = 0.01 \pm 2 \cdot (0.22 \cdot \text{ML}) = 0.01 \pm 0.44 \cdot 0.01 = 0.01 \pm 0.0044 \text{ mg/kg}$$

The minimum applicable range for a ML of 0.01 mg/kg is then 0.006 to 0.014 mg/kg.

In Table 1, a number of minimum applicable concentration ranges for specified MLs are given.

Table 1: Recommended criteria for minimum application range for specified MLs

ML (mg/kg)	0.01	0.02	0.05	0.1	1	10	100
Lower level:	0.006	0.011	0.028	0.03	0.52	6.6	76
Upper level: *	0.014	0.029	0.072	0.17	1.48	13.3	124

* Upper level will seldom be the limiting factor like the lower level.

1.2 Limit of Detection (LOD) and limit of Quantification (LOQ)

As an alternative to establishing minimum applicable range, the criteria could be numeric values for LOD and LOQ.

The numeric value for the limit of detection (LOD), should be:

- no more than 1/10 of the specified ML for levels at or above 0.1 mg/kg, and
- no more than 1/5 of the specified ML for levels below 0.1 mg/kg.

The numeric value for the limit of quantification (LOQ) should be:

- no more than 1/5 of the specified ML for levels at or above 0.1 mg/kg, and
- no more than 2/5 of the specified ML for levels below 0.1 mg/kg.

1.3 The method precision, derived from collaborative method performance studies

The precision should be expressed as the obtained relative reproducibility standard deviation (RSD_R) obtained from collaborative method performance studies, which is compared to the predicted relative reproducibility standard deviation ($PRSD_R$)

According to Horwitz, the ratio between the found and the predicted value should be ≤ 2 (known as the HorRat value), this is also applicable for Thompson equation of $PRSD_R = 22\%$:

$$\frac{RSD_R}{PRSD_R} \leq 2 \Leftrightarrow RSD_R \leq 2 \cdot PRSD_R$$

The numeric values for the precision given in table 2 are also based on the Horwitz/Thompson equation. For some analyses, using advanced techniques, a better precision can be obtained.

Table 2. Precision requirement at different concentrations based on the Horwitz/Thompson equation.

	Thompson	Horwitz equation ($2C^{-0.1505}$)							
Concentration ratio (C)	$< 10^{-7}$	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1
Concentration unit	< 0.1 mg/kg	0.1 mg/kg	1 mg/kg	10 mg/kg	0.1 g/kg	1 g/kg	10 g/kg	100 g/kg	1000 g/kg
$PRSD_R$ (%)	22	22	16	11	8	6	4	3	2
$RSD_R \leq 2 \cdot PRSD_R$ (%)	≤ 44	≤ 44	≤ 32	≤ 22	≤ 16	≤ 12	≤ 8	≤ 6	≤ 4

$PRSD_R$ = predicted value for relative standard deviation of reproducibility.
 RSD_R = found value for the relative standard deviation of reproducibility in a collaborative study.

1.4 Recovery

Evaluation and estimation of recovery is included in the method validation. Whether or not recovery is of relevance depends on the method procedure.

1.5 Trueness

For the evaluation of trueness preferably appropriate certified reference materials (CRMs) should be analysed and demonstrated to give the certified value (allowing for measurement uncertainty) is achieved.

1.6 Examples on how to establish criteria for a provision

In order to illustrate how to set criteria for a provision the following example is used:

According to Codex Standard 1993-1995, Rev 2-2006, General Standard for contaminants and toxins in food, the ML for lead in fruit juices is 0.05 mg/kg. According to the recommendations for obtaining numeric values for the characteristics based on the ML, the criteria would be those in table 3:

Table 3. Recommendation for numeric criteria values for lead in fruit juice

Applicability: Analyte:	Lead
Matrix/provision:	Juice
ML:	0.05 mg/kg
Lower level of min. application range:	$\leq 0.03 \text{ mg/kg}$ ($= \text{ML} - 2s_R = 0.05 \text{ mg/kg} - 0.44 \cdot 0.05 \text{ mg/kg}$). See Table 1
LOD:	$\leq 0.01 \text{ mg/kg}$ ($= \text{ML} \cdot 1/5 = 0.05 \text{ mg/kg} \cdot 1/5$)
LOQ:	$\leq 0.02 \text{ mg/kg}$ ($= \text{ML} \cdot 2/5 = 0.05 \text{ mg/kg} \cdot 2/5$)
Precision:	For concentration at 0.05 mg/kg, the $\text{RSD}_R \leq 44\%$, See Table 2
Recovery:	The method procedure does not include an extraction step and hence recovery is of no relevance.
Trueness:	Use of CRM.

2. Method criteria at different MLs (maximum level, minimum level, normative level or concentration range)

In table 4 examples on method criteria are given for certain MLs.

Table 4: Method criteria for MLs at increasing orders of magnitude.

ML unit	0.001 mg/kg	0.01 mg/kg	0.1 mg/kg	1 mg/kg	10 mg/kg	100 mg/kg	1 g/kg	10 g/kg
Concentration ratio of ML (C _{ML})	10 ⁻⁹	10 ⁻⁸	10 ⁻⁷	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	10 ⁻³	10 ⁻²
Minimum applicable Range	From 0.0006 to 0.0014 (mg/kg)	From 0.006 to 0.014 (mg/kg)	From 0.03 to 0.17 (mg/kg)	From 0.52 to 1.48 (mg/kg)	From 6.6 to 13.3 (mg/kg)	From 76 to 124 (mg/kg)	From 0.83 to 1.2 (g/kg)	From 8.8 to 11 (g/kg)
LOD (≤ mg/kg)	0.0002	0.002	0.01	0.1	1	10	100	1000
LOQ (≤ mg/kg)	0.0004	• 0.004	0.02	0.2	2	20	200	2000
RSD _R (≤ %)	44	44	44	32	22	16	12	8
Recovery (%) *	40 - 120	60 - 115	80 - 110	80-110	80 - 110	90 - 107	95 - 105	97 - 103

* 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.

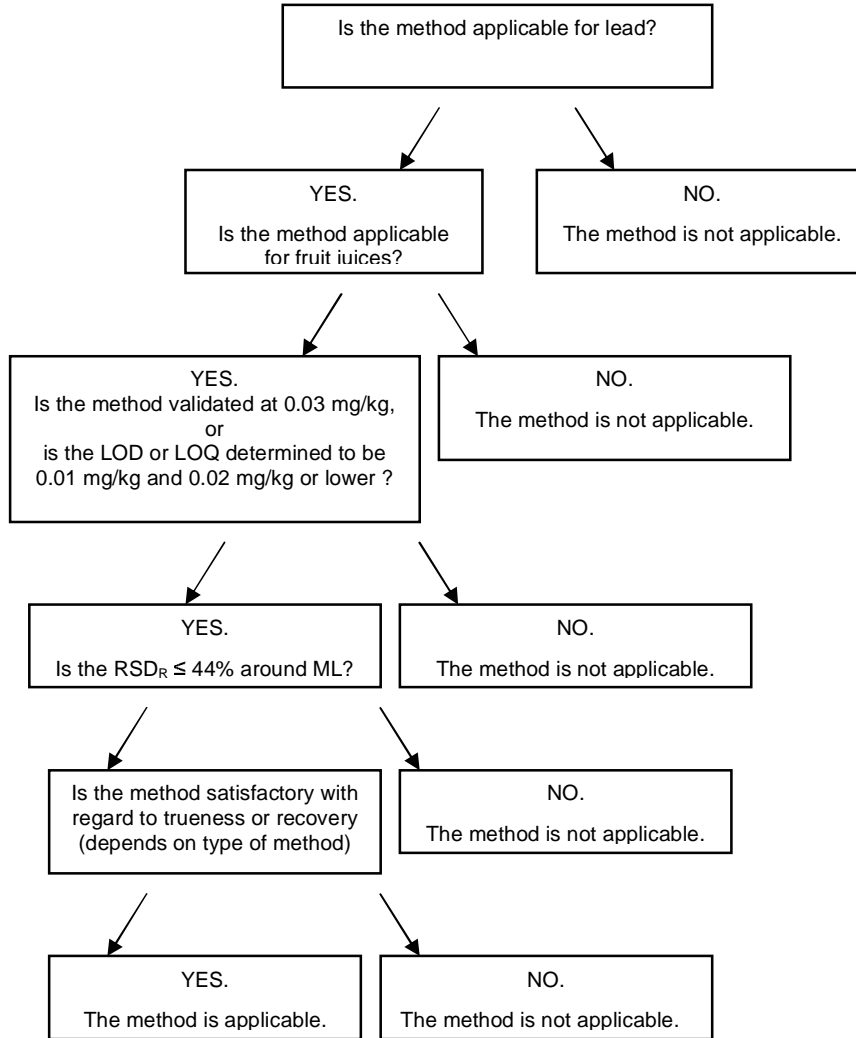
2.1 How to elucidate a method's compliance with the criteria.

To review a method for possible compliance with the established criteria, the method performance characteristics have to be assessed. The result of a method performance study is available in the method and/or published in an international journal.

2.1.1 Example on assessing methods for compliance

Continuing the example above on lead in fruit juice, having ML of 0.05 mg/kg, the methods considered should be able to quantify lead in fruit juice as low as 0.03 mg/kg, with a precision, PRSD_R of 22%, the RSD_R obtained from the method performance study should then not be higher than 44% (corresponding to a 95% confidence interval).

When assessing a method for compliance, the following steps should be considered:



In order to find appropriate methods for this purpose, information are collected on methods for determination of lead. (As this is an example in the Procedural Manual, the methodsqidentification is omitted):

Section II: Elaboration of Codex texts

Table 5: Collaboratively validated methods for analysis of lead

Method No	Applicability	Principle	Assessed level (mg/kg)	LOD (mg/kg)	RSD _R (%)	Applicable Yes/No and why
1	All foods	Flame AAS	2.2 - 29		4.9-36	NO Flame AAS will not be able to detect at 0.05 mg/kg
2	All Foods (Chicken, apple)	Anodic stripping voltammetry	0.03-2.8	0.03	17-106	NO The RSD _R is 106% (not <44%) at 0.03 mg/kg
3	Sugars	GF-AAS	0.03-0.50		12-30	YES Even if the applicability does not say Juice (or all foods) it should be considered applicable as fruit juice contains a lot of sugar. The precision is satisfactory.
4	Fats and Oils	GF-AAS	0.018-0.090		5.9-30	NO The method describes sample prep. for fats and oils only.
5	Natural mineral water	AAS	0.0197-0.977	< 0.01	2.8-4.2	NO The method describes sample prep. for water only.
6	All foods	GF-AAS after dry ashing	0.045-0.25	< 0.01	26-40	NO The lowest validated level is not low enough, however as the technique is GF-AAS, it should be applicable for 0.03 mg/kg.
7	All foods except oils, fats and extremely fatty products.	AAS after microwave oven digestion under pressure.	0.005-1.62	0.014	26-44	YES Validation level and RSD _R are ok
8	All foods	ICP-MS after pressure digestion	0.013-2.45	< 0.01	8-47	YES Validation level and RSD _R are ok for levels of 0.03 mg/kg and above.

AAS = Atomic Absorption Spectrometry

GF-AAS = Graphite Furnace Atomic Absorption Spectrometry

ICP-MS = Inductive Coupled Plasma - Mass Spectrometry

Conclusion: Methods No. 3, 7 and 8 are found applicable for the determination of lead in fruit juices for the given ML of 0.05 mg/kg. Assessing methods for compliance requires knowledge about the methods; sample preparation, procedures and instrumentation. Thus the methods cannot be judged by numeric values for the criteria alone.

Conversion of Specific Methods of Analysis to Method Criteria by the CCMAS

When a Codex Commodity Committee submits a Type II or Type III method to CCMAS for endorsement, it should also submit information on the specified Codex level(s) along with the provision to enable the CCMAS to convert it into suitable generalized analytical characteristics:

- trueness
- applicability (matrix, concentration range and preference given to 'general' methods)
- limit of detection
- limit of quantification
- precision; repeatability intra-laboratory (within laboratory), reproducibility inter-laboratory (within laboratory and between laboratories), but generated from collaborative trial data rather than measurement uncertainty considerations
- recovery
- selectivity
- sensitivity
- linearity

These terms are defined in the Analytical Terminology for Codex Use, as are other terms of importance.

The CCMAS will assess the actual analytical performance of the method which has been determined in its validation. This will take account of the appropriate precision characteristics obtained in method performance studies which may have been carried out on the method together with results from other development work carried out during the course of the method development. The set of criteria that are developed will form part of the report of the CCMAS and will be inserted in the appropriate Codex Standard.

In addition, the CCMAS will identify numeric values for the criteria for which it would wish such methods to comply.

Assessment of the Acceptability of the Precision Characteristics of a Method of Analysis

The calculated repeatability and reproducibility values can be compared with existing methods and a comparison made. If these are satisfactory then the method can be used as a validated method. If there is no method with which to compare the precision parameters then theoretical repeatability and reproducibility values can be calculated from the Horwitz equation. (M. Thompson, *Analyst*, 2000, 125, 385-386).