codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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Agenda Item 6 **CX/MAS 09/30/7**

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING
Thiertieth Session
Balatonalmadi, Hungary, 9 - 13 March 2009

GUIDELINES FOR ESTABLISHING METHODS CRITERIA FOR THE IDENTIFICATION OF RELEVANT ANALYTICAL METHODS

(Prepared by Sweden, NMKL + working group[1])

BACKGROUND

At the 29th session the Committee agreed to forward the proposed amendments to the *Working Instructions for the Implementation of the Criteria Approach in Codex* (Annex I), to the 31st Session of the Codex Alimentarius Commission (CAC) for adoption and inclusion in the Procedural Manual. The CAC adopted the amendments at the 31st Session, in June/July 2008.

Further, the 29th session of the Committee agreed that an electronic working group coordinated by Sweden, with the assistance of NMKL, should redraft Section II of the paper presented at the 28th meeting, in order to provide guidelines (examples) for establishing method criteria for inclusion in the Procedural Manual for consideration at the 30th session of CCMAS. (Annex II)

RECOMMENDATION

The working group proposes that the 30th session of CCMAS reviews and discuss **Annex II** *Guidelines for establishing numeric values for method criteria and/or assessing methods for compliance thereof*, for possible inclusion in the Procedural Manual as a Guide to the *Working Instructions for the Implementation of the Criteria Approach in Codex.*

(Please note that Annex I is adopted by CAC and hence not to be discussed at the 30th Session.)

[1] Argentina, Brazil, European Community, Finland, France, Japan, Germany, Netherlands, Norway, United Kingdom, United States, IDF and ISO.

AMENDMENTS TO THE PROCEDURAL MANUAL

WORKING INSTRUCTIONS FOR THE IMPLEMENTATION OF THE CRITERIA APPROACH IN CODEX

(This replaces the *Working Instructions for the Implementation of the Criteria Approach in Codex* in the *Principles for the Establishment of Codex Methods of Analysis*)

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.

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

<table>
<tr><td>Applicability:</td><td colspan="4">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.</td></tr>
<tr><td>Minimum applicable range:</td><td colspan="4">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^2 = standard deviation of reproducibility</td></tr>
<tr><td>Limit of Detection (LOD):</td><td colspan="4">For ML ≥ 0.1 mg/kg, LOD ≤ ML · 1/10
For ML < 0.1 mg/kg, LOD ≤ ML · 1/5</td></tr>
<tr><td>Limit of Quantification (LOQ):</td><td colspan="4">For ML ≥ 0.1 mg/kg, LOQ ≤ ML · 1/5
For ML < 0.1 mg/kg, LOQ ≤ ML · 2/5</td></tr>
<tr><td>Precision:</td><td colspan="4">For ML ≥ 0.1 mg/kg, HorRat value ≤ 2
For ML < 0.1 mg/kg, the RSD_{TR} < 22%
RSD_R = relative standard deviation of reproducibility</td></tr>
<tr><td>Recovery (R):</td><td colspan="4"><table>
<tr><td>Concentration</td><td>Ratio</td><td>Unit</td><td>Recovery (%)</td></tr>
<tr><td>100</td><td>1</td><td>100% (100 g/100g)</td><td>98 – 102</td></tr>
<tr><td>≥10</td><td>10^{-1}</td><td>≥ 10% (10 g/100g)</td><td>98 – 102</td></tr>
<tr><td>≥1</td><td>10^{-2}</td><td>≥ 1% (1 g/100g)</td><td>97 – 103</td></tr>
<tr><td>≥0.1</td><td>10^{-3}</td><td>≥ 0.1% (1 mg/g)</td><td>95 – 105</td></tr>
<tr><td>0.01</td><td>10^{-4}</td><td>100 mg/kg</td><td>90 – 107</td></tr>
<tr><td>0.001</td><td>10^{-5}</td><td>10 mg/kg</td><td>80 – 110</td></tr>
<tr><td>0.0001</td><td>10^{-6}</td><td>1 mg/kg</td><td>80 – 110</td></tr>
<tr><td>0.00001</td><td>10^{-7}</td><td>100 µg/kg</td><td>80 – 110</td></tr>
<tr><td>0.000001</td><td>10^{-8}</td><td>10 µg/kg</td><td>60 – 115</td></tr>
<tr><td>0.0000001</td><td>10^{-9}</td><td>1 µg/kg</td><td>40 – 120</td></tr>
</table></td></tr>
</table>

[2] 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 RDS_R from an appropriate method performance study.

	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.
Trueness:	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.

CONVERSION OF SPECIFIC METHODS OF ANALYSIS TO METHOD CRITERIA BY THE CCMAS

When a Codex 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 method performance study 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).

PROPOSAL FOR INCLUSION IN THE PROCEDURAL MANUAL

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.

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}, ($\geq$ 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 $\geq 10^{-7}$ ($\geq$ 0.1 mg/kg) the Horwitz' equation is applied:

$$RSD_T\,(\%) = s_{TR}/c \cdot 100 = 2C^{-0.1505}$$

where

RSD_T is the "theoretical" relative standard deviation,
s_{TR} is the "theoretical" 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 ($Ratio_{ML}$)

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

$$s_R = \frac{c \cdot 2C^{-0.1505}}{100} = \frac{ML \cdot 2Ratio_{ML}^{-0.1505}}{100}$$

Example 1: ML =0.1 mg/kg, $Ratio_{ML} = 10^{-7}$:

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

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^{-7}):

$$1.0 \pm 3 \cdot s_R = 1.0 \pm 3 \cdot \frac{1.0 \cdot 2 \cdot (0.0000001)^{-0.1505}}{100} = \mathbf{1.0 \pm 0.48\ mg/kg}$$

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. $RSD_T = 22\%$ and hence $s_R = 0.22 \cdot ML$

Example 3: ML = 0.01 mg/kg (i.e. 10^{-8}):

$0.01 \pm 2 \cdot s_R = 0.01 \pm 2 \cdot (0.22 \cdot ML) = 0.01 \pm 0.44 \cdot 0.01$ **= 0.01 ± 0.0044 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 below 0.1 mg/kg.

The LOD is estimated as three times the standard deviation of the mean blank ($n \geq 20$ replicates).

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 below 0.1 mg/kg.

The LOQ is estimated as six times the standard deviation of the mean blank ($n \geq 20$ replicates), or two times the LOD.

1.3 The method precision, derived from collaborative method performance studies

The precision should be expressed as the found relative reproducibility standard deviation (RSD_R) obtained from collaborative method performance studies, which is compared to the theoretical relative reproducibility standard deviation (RSD_T)
According to Horwitz, the ratio between the found and the theoretical value should be ≤ 2 (known as the HorRat value), this is also applicable for Thompson equation of $RSD_T = 22\%$:

$$\frac{RSD_R}{RSD_T} \leq 2 \quad \Leftrightarrow \quad RSD_R \leq 2 \cdot RSD_T$$

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
RSD_T (%)	= 22	22	16	11	8	6	4	3	2
RSD_R (%)	≤ 44	≤ 44	≤ 32	≤ 22	≤ 16	≤ 12	≤ 8	≤ 6	≤ 4

RSD_T = theoretical (empirical) 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. Recovery can be defined as the yield of extraction steps in an analytical process divided by the amount of analyte in the original sample.

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:	0.03 mg/kg (= ML - $2s_R$ = 0.05 mg/kg - 0.44 · 0.05 mg/kg). See 1.1.2
LOD:	0.01 mg/kg (= ML · 1/5 = 0.05 mg/kg · 1/5)
LOQ:	0.02 mg/kg (= ML · 2/5 = 0.05 mg/kg · 2/5)
Precision:	For concentration at 0.05 mg/kg, the $RSD_R \leq 44\%$, See 1.1.2
Recovery:	The method procedure does not include an extraction step and hence recovery is of no relevance.
Trueness:	Use of CRM.

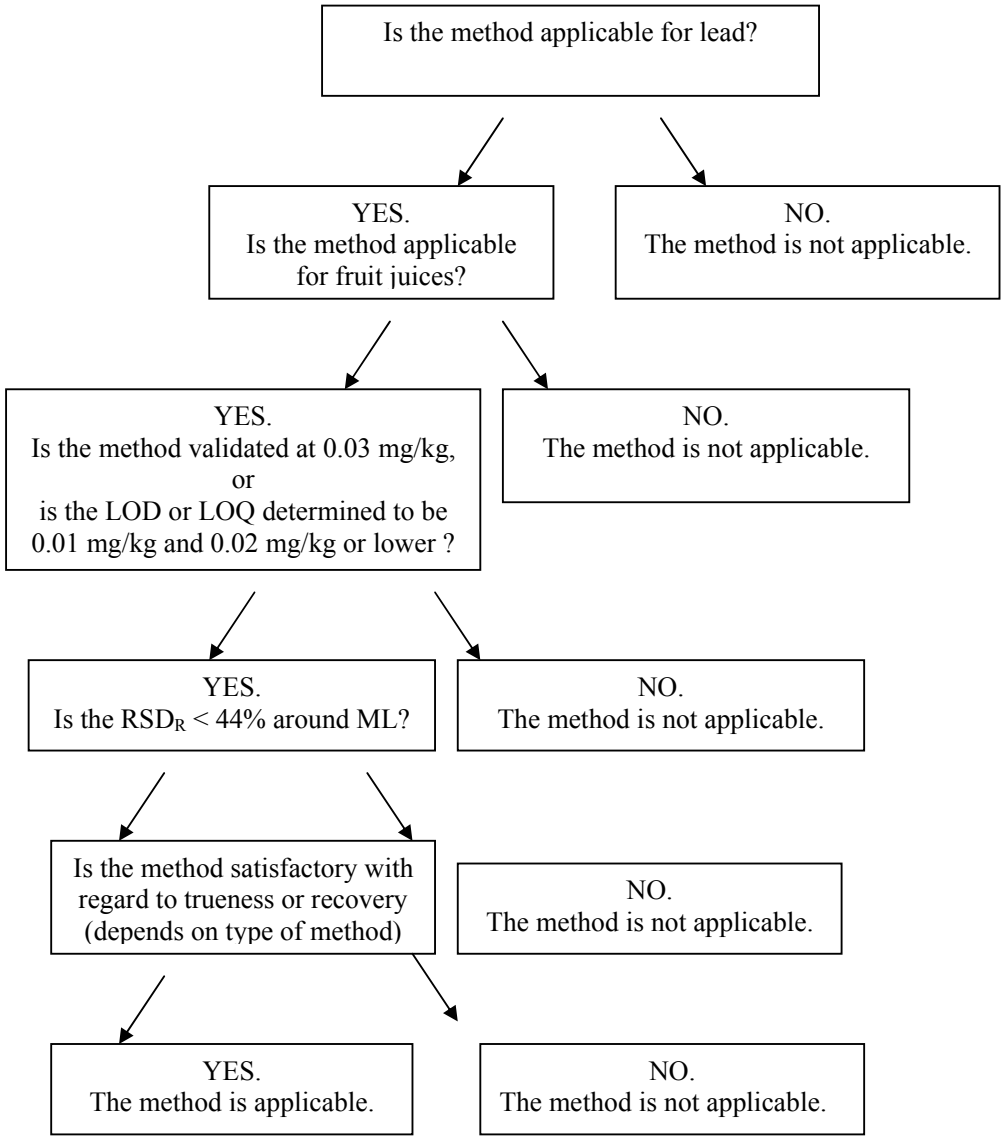
2. HOW TO ELUCIDATE A METHOD'S COMPLIANCE WITH THE CRITERIA.

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 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, RSD_{TR} 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 for the Procedural Manual, the methods' identification is omitted):

Table 4: 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 (Fail step 4)
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 (Fail step 5)
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. (Fail step 2)
5	Natural mineral water	AAS	0.0197-0.977	< 0.01	2.8-4.2	NO The method describes sample prep. for water only. (Fail step 2)
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: The 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.