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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS AND SAMPLING
Twenty-eighth Session
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CONVERSION OF THE METHODS FOR TRACE ELEMENTS INTO CRITERIA
(Prepared by Sweden)

BACKGROUND

At the 25th Session of CCMAS it was agreed to give an example of the application of the criteria approach by converting the methods for trace elements already endorsed Codex general methods into criteria for consideration in the framework of the Agenda Item on Endorsement.

The 26th Session of CCMAS discussed a document prepared by the NMKL. Several delegations supported this work as it could provide clear guidance that would allow a consistent interpretation of the criteria approach across Codex and assist in selecting appropriately validated analytical methods for monitoring compliance with Codex Standards related to trace elements in foodstuffs, so that Commodity Committees would be aware about the quality of methods.

Some delegations drew the attention of the Committee to the need to clarify the validated range of characteristics and precision of the method, definitions and terminology. It was proposed to clarify under which circumstances the lowest validated level should be no more than 3 times the detection limit. It was also noted that recovery might not be appropriate in trace elements analysis, and therefore this should be reflected in the text.

The Delegation of the United Kingdom indicated that in further revision of the document it was necessary to prepare it in a more descriptive way to provide working instructions for the implementation of the criteria approach and conversion of specific methods of analysis to criteria, as described in the Procedural Manual. The Delegation drew the attention of the Committee to the fact that some already adopted Codex methods would not comply with the criteria proposed for trace elements and that this should be stated in the paper.

The 26th Session of CCMAS agreed that the Working Group chaired by Sweden[1], with NMKL as Rapporteur, working electronically would prepare a revised more descriptive version of the document for consideration by the next Session of the Committee, including the status of the document.

At the 27th session the committee concluded that the revised document, introduced by the Swedish delegation, was very useful but that the criteria needed further development prior to the 28th session. It was further emphasized that the document should be considered as a study document for the moment and that at its next session it would decide on whether to keep both the conventional and the criteria approach.

INTRODUCTION

Maximum limits (MLs) are established for the contaminants arsenic, cadmium, lead, mercury and tin [CX/FAC 06/38/1] in a number of commodities. Analysis of these contaminants require analytical methods that are relevant for the purposes. The MLs are then used to identify methods criteria which are

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

assigned specific analytical characteristics/specifications. From these characteristics it is possible to find suitable methods of analysis in e.g. CODEX STAN 228-2001, Rev. 2004 and STAN 234-1999.

According to the procedural manual [15th ed.] *"any Codex Commodity Committee may continue to propose an appropriate method of analysis for determining the chemical entity, or develop a set of criteria to which a method used for the determination must comply"*. Regarding the conversion of specific methods of analysis to methods criteria, the Procedural Manual states that *"When a Codex Commodity Committee submits a Type II or Type III method to CCMAS for endorsement, it should also submit information on the criteria listed below to enable the CCMAS to convert it into suitable generalized analytical characteristics*:

- *accuracy*
- *applicability (matrix, concentration range and preference given to 'general' methods)*
- *detection limit*
- *determination limit*
- *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 collaborative trials 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 Commodity Standard". Analytical methods that have undergone collaborative trials will, however, not automatically be accepted. *"In addition, the CCMAS will identify numeric values for the criteria for which it would wish such methods to comply"*.

METHODS CRITERIA AND CHARACTERISTICS

Table 1 lists the criteria and how to identify numeric values for these, in respect of analysis of trace elements. However, in some sectors, such as the determination of trace elements, not all the criteria are relevant.

Either CCMAS or the Codex Commodity Committee should identify numeric-values for each criterion. It is expected that this will occur most frequently when a number of methods of analysis are available, e.g. for trace elements or mycotoxins.

Table 1. Methods criteria and characteristics for use in trace element analysis.

Criteria	Characteristics
Accuracy:	Based on results from proficiency tests (PT) and/or repeated use of certified reference materials (CRMs). For PT-results a z-score[1] $\leq \pm2$ is satisfactory. Z-scores between ±2 and ±3 are questionable. For CRM-results, when used in a collaborative test of the method, a z-score $\leq \pm2$ is normally satisfactory. Higher z-scores can be accepted, provided that the relative difference between the mean content of the certified material and the experimentally determined mean are within $\pm10\%$.
Bias (trueness):	Applicable when more than one result is available from CRMs and/or PT-programmes. Such data are becoming available from recent collaborative trials.
Applicability:	All foods[2]. Suitable for the food matrix or matrices to be analysed. The validated concentration range should be relevant for the purpose of the analysis and as wide as possible. The lowest validated level should preferably be no more than 3 times the LOD, which approximates to the Quantification Limit as described in the Procedural Manual.
Detection limit[3] (LOD)-1 General:	Three times the standard deviation (σ), for the field blank or a standard solution with a concentration near the LOD. Based on $\geq$ 20 blanks [EN 13804]. (The sample destruction/digestion removes most, or all, of the organic matrix, leaving only the inorganic residue. Certain procedures result in concentrated sample solutions (e.g. dry ashing, due to the large sample weight), whereas others (e.g. microwave digestion, with small sample weights) in rather dilute sample solutions. This factor has an effect on the detection limit.)
Detection limit (LOD)-2 when MLs are defined:	No more than 1/5 of a specified ML below 0.1 mg/kg, and no more than 1/10 of specified ML above 0.1 mg/kg.
Quantification limit:	Six to 10 σ (as described under Detection Limit -1).
Precision of the method: Relative reproducibility standard deviation (RSD$_R$):	The (theoretical) RSD$_{TR}$ equals $2C^{-0.1505}$ for a given concentration provides a basis with which the found RSD$_R$ can be compared (Table 2). The Horwitz ratio, "HorRat" (RSD$_R$/ RSD$_{TR}$) should be ≤ 2 (for some elements the HorRat may be ≤1.5, see table 5) The RSD$_R$ and HorRat values should preferably be estimated from results of collaborative studies.
Precision of the method: Relative repeatability standard deviation (RSD$_r$):	RSD$_r$ should be smaller than RSD$_R$. The usual approximation is that RSD$_r$ is 67% of the RSD$_R$.
Recovery:	100 %, allowing for measurement uncertainty (MU). Recoveries[4] (based on spiked sample solutions) that are systematically lower or higher than 100% $\pm$ MU (usually $\pm10\%$) indicate analytical problems with the method. Such problems must be rectified before further use of the method, or another method selected.
Selectivity:	Matrix, spectral, isobaric and/or polyatomic interferences should be fully compensated for.

SELECTION OF THE VALUES OF THE METHOD CRITERIA

Method criteria should be based on, e.g., a contaminant in a food commodity for which the concentration must be below a stated ML. For example, if a contaminant needs to be controlled against a ML of 1 mg/kg, the criterion precision may call for its characterisation to specify a reproducibility is relevant and should be specified to $\leq16\%$ (Table 2). In the analysis of trace elements most analytical problems are usually encountered at very low concentrations. This is due to contamination and/or analytical interferences. At very

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[1] Z-score is calculated as $z = \dfrac{(\overline{X}_{found} - X_{certified})}{\sqrt{u^2_{found} + \left(\dfrac{U_{certified}}{2}\right)^2}}$ as described in ***NMKL Procedure no. 9, 2007, (Revised)***

[2] Foods with a fat content >30%, or pure fats and oils, may require specific methods.

[3] Determination limit: As for detection limit except that 6 or 10 is required rather than 3.

[4] It is recommended that recovery is used with caution in methods such as the determination of the total amount of a metal and where no extraction step is employed. A recovery may seemingly be acceptable, but on false grounds. Likewise, a recovery that is found to be too low, may also be established but on erroneous grounds.

high concentrations the sample solutions are simply diluted, which reduces problems with both contamination and interferences. In other types of analysis (e.g., pesticide residues) the situation can be very different.

In analytical techniques where one or several extraction steps are included the recovery is an important criterion. Methods for the determination of the total amount of elements usually do not include extraction steps and therefore recovery is not a relevant criterion in this type of analysis.

Table 2. Expected RSD_R at certain concentrations according to the Horwitz equation ($2C^{-0.1505}$)

Concentration ratio	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1
Mass fraction.	0.1 mg/kg	1 mg/kg	10 mg/kg	0.01% 0.1 g/kg	0.1%	1%	10%	100%
RSD_{OR}(%) (=predicted reproducibility)	22	16	11	8	6	4	3	2

NOTE: At a concentration <0,12 ppm the Horwitz value can be generalised to a RSD_R of 22 % in certain applications [Thompson, 2000]

The method performance characteristics, as described in the Procedural Manual, need to be assigned specifications that meet a defined requirement. In cases where a food commodity has been assigned a very low ML the criterion for the limit of detection must be fixed accordingly. In CODEX *[CX/FAC 06/38/19]* the ML for lead in milk is 0.020 mg/kg. This would require a method with a limit of detection of 0.004 mg/kg, which many laboratories would have difficulties to match. Edible offal and crustaceans have a ML for lead of 0.5 mg/kg, at which level the analysis is much less demanding.

Table 3 exemplifies criteria for lead that fill different requirements, from very strict (A), to rather liberal (C). The less stringent the characteristics are the more methods will be found that meet the requirements. After a review of the methods for lead, which are endorsed by CCMAS, and adopted by CAC, it was possible to determine whether or not the methods, used a examples, complies with the proposed numeric values for the characteristics.

Table 3. Examples of method criteria and performance characteristics at three different levels

Methods performance criteria	Methods performance characteristics		
	A	**B**	**C**
Level of control (ML)	*0.02 mg/kg*	*0.2 mg/kg*	*1.0 mg/kg*
Bias[1]	(Z-score: <2)	(Z-score: <2)	(Z-score: <3)
Applicability- Concentration range	0.012 – 0.2 mg/ kg	0.06 - 2 mg/kg	0.3 - 5 mg/kg
Limit of Detection	0.004 mg/kg	0.02 mg/kg	0.1 mg/kg
2. Precision RSD_{OR}	~ 20 - 31%	~ 14 - 24%	~ 13-19%
Methods conforming with the criteria	NMKL 139, AOAC 999.11	NMKL 139, AOAC 999.11 NMKL 161,[2] AOAC 999.10[2]	NMKL 139, AOAC 999.11 NMKL 161,[2] AOAC 999.10[2] AOAC 974.27

[1]Accuracy not applicable in method validation

[2] Only one of the method performance studies has included CRM. Therefore, trueness/accuracy can only be estimated for this method, i.e NMKL 161, AOAC 999.10.

The HorRat value (RSD_r/RSD_{TR}, see table 2) is a well known tool for evaluating the obtained RSD_R. Generally a HorRat value below 2, or even 1.5, is achievable by most methods for determination of metals in food. However, the estimation of the HorRat value is based on a generalisation and therefore not absolutely true. In trace element analysis there may be differences due to type of instrumentation. The determination of e.g. lead by AAS usually results in a higher uncertainty than the determination of cadmium. At levels below 0.12 mg/kg, the applicability of the Horwitz Ratio may depend on analyte. For, e.g., lead it is probably not fully applicable, whereas for, e.g., mycotoxins it is. Applying the Thompson equation which states that for concentrations <0.12 mg/kg the RSD is 22% would probably reduce the number of acceptable methods to a minimum. These factors must be taken into consideration when the HorRat criterion is discussed. Codex has accepted the RSD value of 22% in principle.

Accuracy (trueness) is a crucial criterion, as it is the only factor that gives an objective view of the method bias. This can be established in the collaborative study of a method using CRMs with the concentration of the analyte unknown to the analyst at the time of the analysis. The accuracy can also be estimated from results obtained in PT-programmes, using z-scores. A method for the calculation of the bias, based on the z-score is described in NMKL Procedure No. 9.

It is possible that other analytical techniques may require additional, or other, criteria to those listed in this paper.

A review of the study reports of the trace element–methods referred to in the Codex Standards have been carried out. In Table 4 the Codex methods for trace elements and heavy metals are arranged into groups according to the selected methods performance characteristics and criteria. The methods have also been divided into Complying or Non-complying with basic validation requirements, depending on their methods performance characteristics.

EXAMPLE ON USE OF CRITERIA BASED ON MAXIMUM LIMITS OF ELEMENTAL CONTAMINANTS

If the purpose of identifying methods criteria and characteristics is to find methods for the control of elemental contaminants for compliance with MLs, suitable criteria may be Selectivity (this is an absolute requirement with no numeric value and need not be repeated in each application), Applicability, LOD and Precision, expressed as HorRat. For other purposes, e.g., control of MLs for pesticide residues, it may be necessary to select other/further criteria, such as Recovery.

Table 4 shows the MLs for elemental contaminants in defined foods or food groups. Each specific ML is assigned specific criteria and characteristics. From these parameters defined method of analysis can be identified, in this case from CODEX Stan 228-2001, Rev. 1 2004 and CODEX stan 234-1999. The division of methods into category II and III is connected with certain difficulties due to lack of harmonisation of CODEX Stan 228-2001, Rev. 1 2004 and CODEX stan 234-1999

Table 5 summarises all the methods in the CODEX system that are accepted for the analysis of the elemental contaminants arsenic, cadmium, mercury and lead in food commodities

REFERENCES

Recommended Methods of Analysis and Sampling – Codex standard 234-1999.

General Codex Methods for Contaminants -Codex standard 228-2001, Rev.1 2004.

CODEX [CX/FAC 06/38/19]General Standard for contaminants and toxins in food.

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NMKL Procedure No. 9 (2007, under revision). Evaluation of Results Derived from the Analysis of Certified Reference Materials. The Nordic Committee on Food Analysis.

EN 13804. 2002. Foodstuffs – Determination of trace elements. Performance criteria, general considerations and sample preparation. European Committee for Standardisation. B-1050 Brussels

Thompson, M. (2000) Recent trends in inter-laboratory precision atppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing. Analyst 125:385-386

Table 4. Commodities for which maximum limits (ML) are defined for elemental contaminants and methods criteria and characteristics required for their control. Reference is made to CODEX STAN 228-2001, Rev. 1 2004 and CODEX STAN 234-1999

Commodity	*Provision*	*ML mg/kg*	*Method Criteria and characteristics required[1]*	*Codex methods of type II [Methods in brackets questionable]*	*Codex methods of type III [Methods in brackets questionable]*
Mineral water	Arsenic	0.05	Conc.range: 0.015 - 0.5 mg/kg. RSD_R: 30(23 eller 22% - 10%, HorRat: $\leq$1.5 (May not be valid below 0.1 mg/kg). LOD: 0.005 mg/kg	[AOAC 986.15]	
Fats and oils	Arsenic	0.1	Conc.range: 0.03 - 1 mg/kg RSD_R: 23% - 15%, HorRat: $\leq$1.5 (May not be valid below 0.1 mg/kg) LOD: 0.01 mg/kg	[AOAC 952.13 – surplus] [IUPAC 3.136]	[AOAC 942.17 – surplus] [AOAC 986.15]
Salt	Arsenic	0.5	Conc. range: 0.15 - 5 mg/kg. RSD_R: 23% - 10%, HorRat: $\leq$1.5 . LOD: 0.05 mg/kg.	[ESPA/CN-E/105-1996]	
Mineral water	Cadmium	0.003	Conc. range: 0.002 – 0.1 mg/kg RSD_R: 40% - 15%. HorRat: $\leq$1.5 (May not be valid below 0.1 mg/kg). LOD: 0.0006 mg/kg	ISO 8288-1986	[AOAC 974.27] [AOAC 986.15]
Vegetables, cereals, leafy vegetables	Cadmium	0.05-0.2	Conc. range: 0.01 - 0.5 mg/kg RSD_R: 30% - 15%. HorRat: $\leq$1.5 (May not be valid below 0.1 mg/kg). LOD: 1/5 - 1/10 of ML.	NMKL 139 (1991) AOAC 999.11	NMKL 161 (1998) AOAC 999.10 [AOAC 986.15]
Salt	Cadmium	0.5	Conc. range: 0.1 - 2 mg/kg RSD_R: 40% - 15%. HorRat: $\leq$1.5. LOD: 0.05 mg/ kg.	NMKL 139 (1991) AOAC 999.11	NMKL 161 (1998) AOAC 999.10 [ESPA/CN-E/107-1997 AOAC 986.15]
Mineral water	Lead	0.01	Conc. range: 0.006– 0.05 mg/kg RSD_R: 45% - 20%, HorRat: $\leq$2 (May not be valid below 0.1 mg/kg). LOD: 0.002 mg/kg	ISO 8288-1986	[AOAC 974.27]
Milk	Lead	0.02	Conc. range: 0.012 - 0.2 mg/kg RSD_R: 45% - 20%. HorRat: $\leq$2 (May not be valid below 0.1 mg/kg). LOD: 0.004 mg/kg	NMKL 139 (1991) AOAC 999.11	NMKL 161 (1998) AOAC 999.10 [AOAC 986.15] [IDF 133A:1992]
Fruit juice	Lead	0.05	Conc. range: 0.03 – 0.5 mg/kg RSD_R: 30% − 15%. HorRat: $\leq$2 (May not be valid below 0.1 mg/kg). LOD: 0.01 mg/kg	NMKL 139 (1991) AOAC 999.11	NMKL 161 (1998) AOAC 999.10 [AOAC 986.15] [IDF 133A:1992]
Fats and oils	Lead	0.1	Conc. range: 0.03 – 1 mg/kg RSD_R: 30% − 15%. HorRat: $\leq$2 (May not be valid below 0.1 mg/kg). LOD: 0.01 mg/kg.	AOAC 982.23	IUPAC 63, 1191-1198 AOAC 994.02 IUPAC 2.623 ISO 12193:1994

Commodity	Provision	ML mg/kg	Method Criteria and characteristics required[1]	Codex methods of type II [Methods in brackets questionable]	Codex methods of type III [Methods in brackets questionable]
					[IDF 133A:1992]
Wine	Lead	0.2	Conc. Range 0.06 - 2 mg/kg RSD_R: 45% - 20%. HorRat: $\leq$2 (May not be valid below 0.1 mg/kg). LOD:0.02 mg/kg	NMKL 139 (1991) AOAC 999.11	NMKL 161 (1998) AOAC 999.10 [AOAC 986.15] [IDF 133A:1992]
Vegetables, Meat, Fish, Offal	Lead	0.1-0.5	Conc. range: 0.03 – 1 mg/kg RSD_R: 30% – 15%. HorRat: $\leq$2 (May not be valid below 0.1 mg/kg). LOD: 1/10 of ML.	NMKL 139 (1991) AOAC 999.11	NMKL 161 (1998) AOAC 999.10 [AOAC 986.15] [AOAC 972.25] [IDF 133A:1992]
Canned vegetables	Lead	1.0	Conc. range: 0.3 – 5 mg/kg RSD_R: 25% – 15%. HorRat: $\leq$2. LOD: 0.1 mg/kg.	NMKL 139 (1991) AOAC 999.11	NMKL 161 (1998) AOAC 999.10 [AOAC 986.15] [AOAC 972.25] [IDF 133A:1992]
Salt	Lead	2	Conc. range: 0.5 – 5 mg/kg RSD_R: 15% – 10%). HorRat: $\leq$2. LOD: 0.2 mg/kg.	NMKL 139 (1991) AOAC 999.11	NMKL 161 (1998) AOAC 999.10 [AOAC 986.15] [AOAC 972.25] [IDF 133A:1992] [ESPA/CN-E/108-1994]
Canned food	Tin	50-250	Conc. range: 15 – 500 mg/kg RSD_R: 10% – 8%. HorRat: $\leq$2 . LOD: 1/10 of ML.	AOAC 985.16	
Mineral water	Mercury	0.001	Conc. range: 0.0003-0.001 mg/kg LOD: 0.0001 mg/kg	[ISO5666-3:1984 – withdrawn]	AOAC 977.22
Salt	Mercury	0.1	Conc. range: 0.03 –1 mg/kg (RSD_R: 10% – 8%). HorRat: $\leq$2 (May not be valid below 0.1 mg/kg). LOD: 0.01 mg/kg	[ESPA/CN-E/106-1994]	
Fish	Mercury	0.5-1	Conc. range: 0. 1 –2 mg/kg (RSD_R: 10% – 8%). HorRat: $\leq$2. LOD: 1/10 of ML.		[AOAC 977.22]

[1]Selectivity: All known interferences should be fully compensated for.

Table 5. Analytical methods in Codex CODEX STAN 228-2001, Rev. 1 2004 and CODEX STAN 234-1999 for determination of As, Cd, Hg and Pb in food commodities.

Commodity	Provision	Analytical method[1]	Principe	Comment
Food	Lead	AOAC 934.07		Surplus 1993
Food	Arsenic	AOAC 942.17		Surplus 1993
Food	Arsenic	AOAC 952.13 IUPAC 3.136		Surplus 1993
Food	Lead	**AOAC 972.25**	Open dig. Flame-AAS	Insufficient LOD Validated level too high
Water	Cadmium, Lead	AOAC 974.27	Direct Flame-AAS	Insufficient LOD
Fish	Mercury	AOAC 977.15	Open dig. CV-AAS	
Water	Mercury	AOAC 977.22	Direct CV-AAS	
Food	Cadmium, lead	**AOAC 982.23**	Dry ashing ASV	
Canned food	Tin	AOAC 985.16	Open dig. Flame-AAS	
Human and pet foods	Arsenic, Cadmium, Lead,	**AOAC 986.15**	Pressure dig. HG-AAS, ASV	Limited validation
Fats and oils	Lead	AOAC 994.02 IUPAC 2.623 ISO 12193:1994	Direct GF- AAS	
Sugar	Lead	AOAC 997.15	Open dig. GF-AAS	Narrow valid. range
Foods	Cadmium, lead	**AOAC 999.10 NMKL 161**	Pressure dig. GF-AAS	Conforms with bias criterion
Foods	Cadmium, lead	AOAC 999.11 NMKL 139	Dry ashing GF-AAS	
Fat	Arsenic	IUPAC PAC 63	Colorimetry	
Casein	Lead	IDF 133A:1992	Spectrophotometry	To be checked
Water	Cadmium, lead	ISO 8288:1986	Flame-AAS	Limited validation
Water	Arsenic	ISO 6595:1982	Spectrophotometry	
Water	Mercury	ISO 5666-3/-84	CV-AAS	
Salt	Lead	ESPA/CN E/108-1994	AAS	Horrats >> 2
Salt	Cadmium	ESPA/CN E/107-1997	AAS	Validated level too low
Salt	Arsenic	ESPA/CN E/105-1996	Photometry	Validated level too low
Salt	Mercury	ESPA/CN E/106-1994	CV-AAS	Validated level too low
Sugar	Arsenic	ICUMSA GS2/3-25	Colorimetry	Not collab. validated

[1] Methods in **bold** are general methods in STAN 228-2001, Rev. 1 2004