INFORMATION DOCUMENT ON
CRITERIA APPROACHES FOR METHODS WHICH USE A 'SUM OF COMPONENTS'

INTRODUCTION

1. The Procedural Manual of the Codex Alimentarius Commission provides extensive instructions detailing how a Codex Committee may propose an appropriate method of analysis for determining the analyte and/or develop a set of criteria to which a method used for the determination must comply. In either case the specified maximum / minimum level, any other normative level or the concentration range of interest has to be stated.

2. 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 Committee on Methods of Analysis and Sampling (CCMAS) to "convert" that method into appropriate criteria. The Criteria will then be considered by CCMAS for endorsement and will, after the endorsement, form part of the standard. Methods are evaluated on the characteristics of:

- Selectivity
- Accuracy
- Precision
- Limit of detection
- Sensitivity
- Practicability
- Applicability.

3. It also allows for the establishment of other criteria as required and offers some guidance on choosing between different methods.

4. The Procedural Manual allows for the "Criteria Approach" as an alternative to the endorsement of a specific method (ibid). The Criteria Approach enables the establishment of a set of criteria (numeric values) which must be met by a method in order for the method to be applicable (i.e. "fit for purpose") to a specific standard. The Criteria Approach is applicable to fully validated Type II and III methods, except for methods such as PCR and ELISA; it is not applicable to Type I methods. The Criteria Approach currently requires information on Applicability, Minimum Applicable Range, Limit of Detection and Quantitation, Precision (with requirements for reproducibility relative standard deviation), Recovery and Trueness.

5. 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 above and the second involves the conversion of a specific method to establish numeric criteria. 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.

6. The Guidelines for Establishing Numeric Values for Criteria in the Procedural Manual were developed considering only single analyte determinations and not determinations that involve a sum of components. That is, methods where the concentration of a specific analyte is measured and that determination is assessed against a specification. As such, the approach detailed in the Procedural Manual can be inappropriate for determinations that involve a sum of components i.e. where multiple analytes are determined and summed and the sum is assessed against a specification.

7. This Information Document provides information to Codex Committees and the CCMAS on a variety of (non-exhaustive) issues they may wish to consider when developing numeric method performance criteria for approaches that involve a summation of components.

BACKGROUND

8. There are numerous ways in which methods and limits that involve a sum of components can be converted into numeric method performance criteria. Two example approaches are shown in Annex A but these are not the only approaches available. Approaches taken need to be developed and decided on a case-by-case basis and will be influenced by a number of factors including whether, for example:

- the components are equally or unequally weighted;
- there is a known natural-abundance of the components (e.g. Fumonisins B1 and B2 are determined together where the typical ratio of B1:B2 in naturally contaminated samples is 5:2 but the (maximum limit) ML is a total value of B1+B2);
- measured values for individual components are correlated or uncorrelated. The presence of correlation (for example due to multiple components measured on the same instrument at the same time) can have a substantial effect on the precision of the resulting summed values compared to the precision available when components are measured independently;
- the MLs or methods involving the use of toxic equivalents (TEQs) or toxic equivalent factors (TEFs); or,
- the specification contains multiple MLs for both a single analyte and a sum of components.

9. It is unsurprising that there is currently no single mechanism for converting maximum limits that involve a sum of components into method performance criteria as it is complex. With the assessment of future methods and method developers taking into consideration a 'sum of components' approach, Codex may find future compliance less problematic. Further, as analytical technology capability improves the identification and lower quantitation of multi-components of a provision in a commodity may become feasible when historically this was not the case. Alternatively, individual components may be specified as a 'marker' for the 'total components' e.g. benzo[a]pyrene for polynuclear aromatic hydrocarbons in drinking-water. So some options in the 'sum of components' criteria applied by Codex, plus reviews by Codex Committees in cases where there is a 'sum of components' standard specification, may have to occur together to achieve the best outcome.

TOXIC EQUIVALENT FACTORS

10. For certain commodities or analytes there are specifications where the individual concentrations of multiple analytes are determined by a single method, the concentrations are converted to a "toxic equivalent" using a toxic equivalency factor (TEF) and the specification is a limit based on the sum of equivalents. One example of this approach is the determination of the saxitoxin group in the *Standard for Live and Raw Bivalve Molluscs* (CXS 292-2008). The specification is for the concentration of saxitoxin equivalents which is determined from 12 saxitoxin congeners each multiplied by a TEF and summed. TEFs are also used in other determinations, such as dioxins and dioxin-like PCBs. The current Criteria Approach in the Procedural Manual was not developed considering specifications which use TEF or a sum of toxic equivalents.

RECOMMENDATIONS

1. It is important to note that when developing a Criteria Approach, it is the competent authority (Government, Codex Committee) that is responsible for specifying the range of concentrations for each analyte. Consideration of the ratio of components, toxicity, and properties of matrices (commodities) are outside of the terms of reference of CCMAS, but rather fall under the responsibilities of Codex Commodity Committees or individual Governments.
2. There are numerous ways in which methods and limits that involve a sum of components can be converted into method performance criteria but this should be undertaken with care and also on a case-by-case basis. CCMAS is available to advise Codex Committees if they wish to develop numeric method performance criteria for methods or limits that involve a summation of components.
3. If methods of analysis that employ a summation of components have been collaboratively trialled on a 'sum of components' basis then these can be converted directly into criteria.

11. For MLs that involve use of TEQs/TEFs or other toxicological potencies it is recommended that the MLs themselves are not converted to method performance criteria. In such instances the second approach detailed within the Procedural Manual (i.e. the conversion of a specific method to establish numeric criteria) may be appropriate where numeric criteria may be developed on using untransformed method performance data (i.e. raw data that has not been converted into TEQs) assuming the method has been suitably validated. This was the approach taken when an amendment was made to the *Standard for Live and Raw Bivalve Molluscs* (CXS 292-2008) where un-weighted numerical performance criteria (i.e. TEFs not applied) were established from the various approved methods.

12. For provisions that contain MLs for both single components and also a sum of components, a combination of approaches may be appropriate. For example, using approaches laid down within the Procedural Manual for the single components and a sum of components approach for MLs that involve a summation of components.

13. If the components included in the ML definition are not present in constant ratios and where the inclusion of weighting factors of the individual components results in LOD/LOQ values or minimum applicable range that cannot be validated, ML/n should be used to determine the criterion for LOD (e.g. 1/5*ML/n) and for LOQ (e.g. 2/5*ML/n) or for the minimum applicable range (e.g. $ML/n \pm 2S_R$), with n being the number of components included in the ML definition.

ANNEX A - EXAMPLE APPROACHES

APPROACH 1: THE ML IS A SUM OF COMPONENTS THAT ARE EQUALLY WEIGHTED

For multi-analyte analyses where all components are weighted equal, *n* is the number of components/analytes. The criteria for multi-analyte (and single analyte, *n*=1) would then be as given in Table 1.

Table 1: Guidelines for establishing numeric criteria if the ML is a sum of components that are equally weighted.

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 the individual components[1]:	For ML/*n* ≥ 0.1 mg/kg, [ML/*n* - 3 s_R, ML + 3 s_R] For ML/*n* < 0.1 mg/kg, [ML/*n* - 2 s_R, ML + 2 s_R] NB: the upper level is above the ML for the individual components.			
Limit of Detection (LOD) for the individual components:	For ML/*n* ≥ 0.1 mg/kg, LOD ≤ ML/*n* · 1/10 For ML/*n* < 0.1 mg/kg, LOD ≤ ML/*n* · 1/5			
Limit of Quantification (LOQ) for the individual components:	For ML/*n* ≥ 0.1 mg/kg, LOQ ≤ ML/*n* · 1/5 For ML/*n* < 0.1 mg/kg, LOQ ≤ ML/*n* · 2/5			
Precision for the individual components:	For ML/*n* ≥ 0.1 mg/kg, HorRat value ≤ 2 For ML/*n* < 0.1 mg/kg, the RSD_R < [44%]. RSD_R = relative standard deviation of reproducibility.			
Recovery (R) for the individual components:	**Concentration**	**Ratio**	**Unit**	**Recovery (%)**
	100	1	100% (100 g/100g)	98-102
	≥10	10^{-1}	≥10% (10 g/100g)	98-102
	≥1	10^{-2}	≥1% (1 g/100g)	97-103
	≥0.1	10^{-3}	≥0.1% (1 mg/g)	95-103
	0.01	10^{-4}	100 mg/kg	90-107
	0.001	10^{-5}	10 mg/kg	80-110

[1] For multi-analyte analyses where all components are weighted equal, *n*=number of components/analytes.

	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.			

Worked Example

Substance X, consisting of 4 analytes, x_1, x_2, x_3 and x_4, in matrix Y.

The ML (i.e. $x_1 + x_2 + x_3 + x_4$) = 20 µg/kg,

As there are 4 analytes, *n* = 4,

ML/*n* = 20/4 µg/kg = 5 µg/kg

Using the NMKL Excel spreadsheet. The following are established:

Minimum Applicable Range for the individual components:	0.003* - 0.029** mg/kg = 3 - 29 µg/kg *corresponding to ML/*n* = 5 µg/kg **corresponding to ML = 20 µg/kg
Limit of Detection (LOD) for the individual components:	1 µg/kg
Limit of Quantification (LOQ) for the individual components:	2 µg/kg
Precision for the individual components:	$RSD_R \leq 44\%$
Recovery for the individual components (R):	40-120%

Issues for consideration

1. It is important to note that throughout this approach the actual ML (for compliance purposes) remains unchanged.
2. The concept of minimum applicable range is clear and can be applied for testing compliance with a specification. However, it might be misinterpreted in cases of food contaminants where the analytical results are used for assessment of exposure to the substances analysed and consumers' risk (e.g. mycotoxins, dioxins PCBs, etc.). For this purpose, the results of measurements of low concentrations at or above the technically achievable LOQ are important. Especially for the most toxic analytes of the sum to be determined.
3. Using this approach the LOD and LOQ criteria may be too strict; especially when "*n*" is large (e.g. *n* >> 5). In such instances the developers of numeric method performance criteria need to consider the manner in which it considers methods that involve the summation of multiple components (e.g. sterols and PAHs) but where there is only ever likely to be a few components actually present. In such instances the calculated LOD/LOQ

may be far too strict for practical purposes and an alternative approach may be more appropriate. For example, in such instances it may be appropriate for *n* to equal the number of analytes of 'interest' rather than the total number of components. Alternatively, it may be appropriate to leave the individual minimum applicable range, the LODs and LOQs if already stipulated without taking into account the number of congeners or components of the sum.

APPROACH 2: THE ML IS A SUM OF COMPONENTS WHERE THERE IS A KNOWN NATURAL ABUNDANCE/RATIO OF COMPONENTS.

For multi-analyte analyses where there is a known natural abundance/ratio of components, *f* is the ratio factor. The criteria for multi-analyte (and single analyte, *f*=1) would then be as given in Table 2.

Table 2: Guidelines for establishing numeric criteria if the ML is a sum of components where there is a known natural abundance/ratio of components.

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 <u>the individual components</u>:**	For ML · *f* ≥ 0.1 mg/kg, [ML · *f* - 3 s_R , ML + 3 s_R] For ML · *f* < 0.1 mg/kg, [ML · *f* - 2 s_R , ML + 2 s_R] s_R = standard deviation of reproducibility			
Limit of Detection (LOD) for <u>the individual components</u>:	For ML · *f* ≥ 0.1 mg/kg, LOD ≤ ML · *f* · 1/10 For ML · *f* < 0.1 mg/kg, LOD ≤ ML · *f* · 1/5			
Limit of Quantification (LOQ) for <u>the individual components</u>:	For ML · *f* ≥ 0.1 mg/kg, LOQ ≤ ML · *f* · 1/5 For ML · *f* < 0.1 mg/kg, LOQ ≤ ML · *f* · 2/5			
Precision for <u>the individual components</u>:	For ML · *f* ≥ 0.1 mg/kg, HorRat value ≤ 2 For ML · *f* < 0.1 mg/kg, the RSD_R < [44%] RSD_R = relative standard deviation of reproducibility.			
Recovery (R) for <u>the individual components</u>:	**Concentration**	**Ratio**	**Unit**	**Recovery (%)**
	100	1	100% (100 g/100g)	98-102
	≥10	10^{-1}	≥10% (10 g/100g)	98-102
	≥1	10^{-2}	≥1% (1 g/100g)	97-103
	≥0.1	10^{-3}	≥0.1% (1 mg/g)	95-103

	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.			

Worked Example

Substance X, consisting of 2 analytes, x_1 and, x_2, in matrix Y. It is known that analytes x_1 and x_2 are typically found in a ratio of 5:3 in naturally-contaminated samples.

The ML = 5000 µg/kg,

As the 2 analytes are normally found in the ratio of 5:3

$f_1 = 5/8 = 0.625$ and,

$f_2 = 3/8 = 0.375$

For analyte x_1

ML · f_1 = 5000 · 0.625 µg/kg = 3125 µg/kg and,

For analyte x_2

ML · f_2 = 5000 · 0.375 µg/kg = 1875 µg/kg

Using the NMKL Excel spreadsheet[2] the following are established:

Analyte x_1

Minimum Applicable Range for Analyte x_1:	1.862* - 6.883** mg/kg = 1860 - 6880 µg/kg *corresponding to ML · f = 3125 µg/kg **corresponding to ML = 5000 µg/kg
Limit of Detection (LOD) for Analyte x_1:	313 µg/kg
Limit of Quantification (LOQ) for Analyte x_1:	625 µg/kg
Precision for Analyte x_1:	$RSD_R \leq 27\%$
Recovery (R) for Analyte x_1:	80-110%

Analyte x_2

Minimum Applicable Range for Analyte x_2:	1.056* - 6.883** mg/kg = 1060 - 6880 µg/kg *corresponding to ML · f = 1875 µg/kg **corresponding to ML = 5000 µg/kg
Limit of Detection (LOD) for Analyte x_2:	188 µg/kg
Limit of Quantification (LOQ) for Analyte x_2:	375 µg/kg
Precision for Analyte x_2:	$RSD_R \leq 29\%$
Recovery (R) for Analyte x_2:	80-110%

Issues for consideration

It is important to note that throughout the above process the actual ML (for compliance purposes) remains unchanged.

[2] www.nmkl.org under "How to get method criteria based on ML"