

CODEX ALIMENTARIUS COMMISSION



Food and Agriculture
Organization of the
United Nations



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Organization

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Agenda Item 4

CX/MAS 17/38/4

March 2017

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON METHODS OF ANALYSIS SAMPLING

Thirty-eighthth Session
Budapest, Hungary, 8 - 12 May 2017

GUIDANCE ON THE CRITERIA APPROACH FOR METHODS WHICH USE A 'SUM OF COMPONENTS' (Prepared by the EWG led by the United Kingdom)

1. At CCMAS37 the Delegation of the United Kingdom, as chair of both the electronic working group (eWG) and physical working group (pWG), introduced the reports of the eWG and pWG. The Delegation reminded the Committee of the decision of CCMAS36 for the work to continue with the mandate as outlined in CX/MAS 16/37/5, paragraph 4. The pWG had looked at examples, and concluded that there was no single mechanism for determining numeric method performance criteria for methods and that performance criteria should be addressed on a case-by-case basis.
2. The Delegation further noted that the current procedures in the Procedural Manual are for single analytes only, and an amendment might be necessary to indicate that the process was not always suitable for 'sum of components'.
3. The Delegation reported that the pWG had considered the report of the eWG and discussed the way forward. The Delegation clarified that the document did not address toxic equivalency factors (TEFs), analyte weighting or situations where maximum levels involve both a single component and multi-component analysis and that the pWG were of the opinion that should the work proceed, then those examples where performance criteria have already been generated should be included.
4. The Delegation concluded that guidance was needed from the Committee on whether work should proceed, and if so, what the format of this work would be, i.e. what type of document was needed.
5. There was general agreement that further work was needed, as it was clear that the current procedures were not necessarily fit for purpose. Discussion was held on whether it should be an internal procedure for Codex use, or a Codex guidance directed at governments.
6. There was also support to amend the Procedural Manual to clarify that the procedures were not always suitable for a "sum of components". Concerns were raised on the complexity of the issue and that the type of document that would result, would not be suitable for inclusion the Procedural Manual.
7. The Secretariat clarified that if the procedure was developed for use by CCMAS and other Codex committees, then it was a procedural matter and it would not be appropriate to have it as a document outside of Codex. This would not preclude governments from consulting the Codex procedure. The Committee should proceed with the work and a decision could be taken a later stage on how to make it available for use in Codex.
8. The Committee agreed to amend the *General Criteria for the Selection of Methods of Analysis* section of the Procedural Manual and to send it to the 30th Session of the Committee on General Principles (CCGP) for endorsement and adoption by the 39th Session of the Commission.
9. The Committee noted that Codex Committees should consider seeking guidance from CCMAS if they wish to develop numeric values for method criteria where a sum of components is required.
10. The Committee agreed to re-establish the eWG led by the United Kingdom and working in English. The mandate of the re-establish eWG was to:
 - i. develop a document in the style of guidance to Codex committees and CCMAS;
 - ii. concentrate on chemical methods of analysis only;

- iii. use CX/MAS 16/37/5 as a starting point, the eWG will continue to develop guidance on how MLs and methods of analysis which involve a sum of components could potentially be converted to method performance criteria;
 - iv. note that the guidance, to be used on a case-by-case basis, will contain some of the current potential approaches available;
 - v. include examples of where approaches have already been successfully undertaken and cover methods with TEQs/TEFs, analyte weighting and instances where an ML includes both a single analyte and sum of components; and
 - vi. investigate the existence of practical examples of sum of components outside the Codex framework.
11. The next session of the Committee will take a decision on how to take this work forward.
12. The eWG chair (United Kingdom) prepared a draft paper during mid/late 2016 and distributed this for comment to eWG members in early 2017. Comments were received from a number of delegations and many of these have been addressed in the revised document given in Appendix I. The eWG had over 45 participants. The list of participants and affiliations is attached as Appendix II to this document.
13. Whilst no delegation disagreed with the recommendations proposed a number of comments were raised during the consultation process, summarised below, which require further attention/discussion and if necessary addressed within a revised text:
- i.) One delegation suggested that the most logical location for this document is the Codex Procedural Manual, but that there will be some pressure to reduce the text from its current six pages.
 - ii.) Another delegation mentioned the examples provided in Annex A assumed the ratio of components to be fixed and therefore did not consider situations where the analytes determined may be present in varying ratios.
 - iii.) A Delegation highlighted that an important factor in using the criteria approach is the ability of the Competent Authority (government, commodity committee) to be able to specify the range of concentrations for each analyte.

Recommendations

14. Given the complexity of the issue concerning the criteria approaches for methods which use the sum of components, the approach taken for each method needs to be assessed individually on a case by case basis. The Committee is therefore invited to:
- i.) consider the draft Guidance paper on the criteria approaches in Appendix I; and
 - ii.) consider the next step of the eWG to undertake further work if required.

Appendix I

DRAFT GUIDANCE PAPER ON CRITERIA APPROACHES FOR METHODS WHICH USE A 'SUM OF COMPONENTS'**INTRODUCTION**

1. The Procedural Manual of the Codex Alimentarius Commission establishes General Criteria for the Selection of Methods of Analysis. Methods are evaluated on the characteristics of selectivity, accuracy, precision, limit of detection, sensitivity, practicability and applicability. It also allows for the establishment of other criteria as required and offers some guidance on choosing between different methods. The Procedural Manual also 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, but 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 criteria for reproducibility relative standard deviation), Recovery and Trueness.

2. Two approaches for establishing criteria have been 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.

3. Although it is not specifically stated in the Procedural Manual, the *Guidelines for Establishing Numeric Values for Criteria* 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.

BACKGROUND

4. There are numerous ways in which methods and maximum limits that involve a sum of components can be converted into 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 weighted;
- there is a known natural-abundance of the components (e.g. Fumonisin B1 and B2 are determined together where the typical ratio of B1:B2 in naturally contaminated samples is 5:2 but the 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 measured values are independent;
- the MLs or methods involving the use of toxic equivalents (TEQs) or toxic equivalent factors (TEFs); or,
- the ML includes both a single analytes and a sum of components.

5. It is unsurprising that there is currently no single mechanism for converting maximum limits that involve a sum of components into method performance criteria. 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-individual 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 commodity committees in cases where there is a 'sum of components' standard specification, may have to occur together to achieve the best outcome.

6. There are currently a number of multi-analyte methods being standardised by various standardisation bodies that involve the determination of single substances and/or substance groups in the same analytical run. For example, CEN/TC327 - Animal Feeding Stuffs is currently developing a Technical Specification detailing

a criteria approach for methods of analysis for mycotoxins in order to support the standardisation work of Working Group 5 (Natural Toxins). CEN/TC 327 - Animal Feeding Stuff WG 4 (Methods of sampling and analysis) is also developing a Technical Specification on performance criteria for single laboratory validated and ring-trial validated methods of analysis for the determination of heavy metals.

TOXIC EQUIVALENT FACTORS

7. 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* (CODEX STAN 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.

8. The use of a TEF to determine a "toxic equivalent" requires a calculation, and if this calculation is part of the method, then historically CCMAS would consider such methods as Type I. Even if the analytical procedure to determine the value prior to conversion was rational (Type II/III), the final determination is Type I because the calculation is empirical. A possible alternative to including the TEFs in the method would be to include them in the standard.

CONCLUSIONS

1. There are numerous ways in which methods and maximum limits that involve a sum of components can be converted into method performance criteria but this should be undertaken with care by analysts who fully understand the methodology employed and also on a case-by-case basis.
2. 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.
3. 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 that that has not been converted into TEQs) assuming the method has been suitably validated. This was the approach taken when amended *Standard for Live and Raw Bivalve Molluscs* (CODEX STAN 292-2008) where un-weighted numerical performance criteria (i.e. TEFs not applied) were established from the various approved methods.
4. For provisions that contain MLs for both single substances and also a sum of components (e.g. CODEX STAN 33-1981 *Standard for olive oils and olive pomace oils*) a combination of approaches may be appropriate. For example, using approaches laid down within the Procedural Manual for the single substances and a sum of components approach for MLs that involve a summation of components.

REFERENCES

- i.) CODEX STAN 292-2008: *Standard for live and raw bivalve molluscs*
- ii.) CODEX STAN 33-1981: *Standard for olive oils and olive pomace oils*

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 <u>the individual components</u>¹:	For $ML/n \geq 0.1$ 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 <u>the individual components</u>:	For $ML/n \geq 0.1$ mg/kg, $LOD \leq ML/n \cdot 1/10$ For $ML/n < 0.1$ mg/kg, $LOD \leq ML/n \cdot 1/5$			
Limit of Quantification (LOQ) for <u>the individual components</u>:	For $ML/n \geq 0.1$ mg/kg, $LOQ \leq ML/n \cdot 1/5$ For $ML/n < 0.1$ mg/kg, $LOQ \leq ML/n \cdot 2/5$			
Precision for <u>the individual components</u>:	For $ML/n \geq 0.1$ 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 <u>the individual components</u>:	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)	95-103
	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 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.			

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

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 $\mu\text{g}/\text{kg}$,

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

$\text{ML}/n = 20/4 \mu\text{g}/\text{kg} = 5 \mu\text{g}/\text{kg}$

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

Minimum Applicable	0.003* - 0.029** mg/kg = 3 - 29 $\mu\text{g}/\text{kg}$
Range for <u>the individual components</u>:	*corresponding to $\text{ML}/n = 5 \mu\text{g}/\text{kg}$ **corresponding to $\text{ML} = 20 \mu\text{g}/\text{kg}$
Limit of Detection (LOD) for <u>the individual components</u>:	1 $\mu\text{g}/\text{kg}$
Limit of Quantification (LOQ) for <u>the individual components</u>:	2 $\mu\text{g}/\text{kg}$
Precision for <u>the individual components</u>:	$\text{RSD}_R \leq 44\%$
Recovery (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 to be too strict; especially when “ n ” is large (e.g. $n \gg 5$). In such instances the developers of method performance criteria needs 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.

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 $ML \cdot f \geq 0.1$ mg/kg, $[ML \cdot f - 3 s_R, ML + 3 s_R]$ For $ML \cdot f < 0.1$ mg/kg, $[ML \cdot f - 2 s_R, ML + 2 s_R]$ s_R = standard deviation of reproducibility			
Limit of Detection (LOD) for <u>the individual components</u>:	For $ML \cdot f \geq 0.1$ mg/kg, $LOD \leq ML \cdot f \cdot 1/10$ For $ML \cdot f < 0.1$ mg/kg, $LOD \leq ML \cdot f \cdot 1/5$			
Limit of Quantification (LOQ) for <u>the individual components</u>:	For $ML \cdot f \geq 0.1$ mg/kg, $LOQ \leq ML \cdot f \cdot 1/5$ For $ML \cdot f < 0.1$ mg/kg, $LOQ \leq ML \cdot f \cdot 2/5$			
Precision for <u>the individual components</u>:	For $ML \cdot f \geq 0.1$ mg/kg, HorRat value ≤ 2 For $ML \cdot f < 0.1$ 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}	$\geq 10\%$ (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)	95-103
	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 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.			

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 $\mu\text{g}/\text{kg}$,

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

$\text{ML} \cdot f_1 = 5000 \cdot 0.625 \mu\text{g}/\text{kg} = 3125 \mu\text{g}/\text{kg}$ and,

For analyte x_2

$\text{ML} \cdot f_2 = 5000 \cdot 0.375 \mu\text{g}/\text{kg} = 1875 \mu\text{g}/\text{kg}$

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

Analyte x_1

Minimum Applicable Range for Analyte x_1 :

1.862* - 6.883** mg/kg = 1860 - 6880 $\mu\text{g}/\text{kg}$

*corresponding to $\text{ML} \cdot f = 3125 \mu\text{g}/\text{kg}$

**corresponding to $\text{ML} = 5000 \mu\text{g}/\text{kg}$

Limit of Detection (LOD) for Analyte x_1 :

313 $\mu\text{g}/\text{kg}$

Limit of Quantification (LOQ) for Analyte x_1 :

625 $\mu\text{g}/\text{kg}$

Precision for Analyte x_1 :

$\text{RSD}_R \leq 27\%$

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 $\mu\text{g}/\text{kg}$

*corresponding to $\text{ML} \cdot f = 1875 \mu\text{g}/\text{kg}$

**corresponding to $\text{ML} = 5000 \mu\text{g}/\text{kg}$

Limit of Detection (LOD) for Analyte x_2 :

188 $\mu\text{g}/\text{kg}$

Limit of Quantification (LOQ) for Analyte x_2 :

375 $\mu\text{g}/\text{kg}$

Precision for Analyte x_2 :

$\text{RSD}_R \leq 29\%$

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.

Appendix II

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