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CRITERIA APPROACH FOR METHODS WHICH USE A "SUM OF COMPONENTS"

(Prepared by the Electronic Working Group led by the United Kingdom)

BACKGROUND

1. At CCMAS36 the Delegation of United Kingdom introduced the report[1] of the in-session Working Group on criteria approach for methods, which use a "sum of components" and the recommendations as presented in CRD 22[2]. The Delegation indicated that the in-session WG had not discussed the discussion paper (and comments), in detail, but had focused its discussion on proposals for a way forward.

2. The Committee generally supported further work on the criteria approach for methods which use a sum of components, and noted that such work should focus on chemical methods only, and should also not overlap with the work on equivalency to Type I methods. The Committee also noted that clarification was needed on the purpose of the work and who it was aimed at. Delegations expressed the view that while criteria might be useful for use within Codex, in particular by the Committee, that there might also be value in providing guidance to member countries.

3. The Committee therefore agreed that work should continue and re-established the eWG, led by the United Kingdom, and working in English.

4. The mandate of the re-established eWG was to:

   i.) Concentrate on chemical methods of analysis only.

   ii.) Undertake an analysis of CODEX STAN 234-1999 and individual methods in relevant commodity standards, to determine the extent to which methods of analysis that use a sum of components approach are cited and used; and try to identify potential methods that could be considered by the Committee for future conversion to method performance criteria.

   iii.) Develop potential options for establishing criteria approaches for methods that are sum of components using CX/MAS 14/35/5[3] and CX/MAS 15/36/61 as a starting point.

   iv.) Evaluate the options identified within recommendation iii) to ascertain fitness for purpose.

   v.) Based on the outcome of recommendations i) to iv), consider the need to either amend the General Criteria for the Selection of Methods of Analysis section of the Procedural Manual and/or for development of a Guideline Document for governments.

5. The eWG chair (United Kingdom) prepared a draft paper during mid/late 2015 and distributed this for comment to eWG members. 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 60 participants. The list of participants and affiliations is attached as Appendix II to this document.

6. Whilst no delegation disagreed with the tentative 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.

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[1] CX/MAS 15/36/6 Discussion Paper on Criteria Approach for Methods Which Use a 'Sum of Components'
[2] ftp://ftp.fao.org/codex/meetings/CCMAS/CCMAS36/CRDs/MAS36_CRD22x.pdf
[3] CX/MAS 14/35/5 Discussion Paper on Considering Procedures for Establishing Criteria

i.) Some delegations questioned the legality of the tentative recommendations because they considered the approaches proposed might indicate a change of the maximum limit (ML) values given within the referenced Codex documents. The eWG chair wishes to make it clear that the intention is NOT to change the ML and amendments have been made to the text to clarify this point.

ii.) The concept of minimum applicable range is clear and can be applied for testing compliance with 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. Therefore, in such cases the Option 2-2A could be recommended. It might be stated under Table 3.

iii.) A number of delegations were concerned that the tentative recommendations have been made on the assumption that all the analyte components included within a sum or components approach are equally weighted in terms of risk and the recommendations do not take into account instances where one (or more) analytes included within such an approach are 'more important' than the others. The issue of how to take into account 'analyte weighting' needs to be discussed and agreed by the eWG/CCMAS.

iv.) A number of delegations commented that whilst the generation and formulation of the tentative recommendations has involved a retrospective analysis of current methods and MLs where a sum of components is required, it is not surprising that a single mechanism has not been identified that will fulfil the criteria for all the endorsed examples identified. With assessment of future methods and method developers taking into consideration a 'sum of components' criteria, CCMAS 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 CCMAS, 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.

v.) Some delegations considered the LOD and LOQ criteria to be very strict; especially when "n" is large (e.g. n >> 5). In such instances the eWG/CCMAS 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. It 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.

vi.) A number of delegations suggested that what might actually be required is a document which sets down the general principles and example options available when considering the generation of method performance criteria, for methods/MLs that involve a sum of components, but where the approaches taken at a practical level would need to be assessed and implemented by CCMAS on a case-by-case basis.

Appendix I

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 (24th Ed. 2016, English Version, p 73). 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 is summarized in Table 1. The second involves the conversion of a specific method to establish numeric criteria for the parameters listed in Table 1. 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.

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 ≥ 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[4] = standard deviation of reproducibility	
Limit of Detection (LOD):			For ML ≥ 0.1 mg/kg, LOD ≤ ML · 1/10 For ML < 0.1 mg/kg, LOD ≤ ML · 1/5	
Limit of Quantification (LOQ):			For ML ≥ 0.1 mg/kg, LOQ ≤ ML · 1/5 For ML < 0.1 mg/kg, LOQ ≤ ML · 2/5	
Precision:	For ML ≥ 0.1 mg/kg, HorRat value ≤ 2 For ML < 0.1 mg/kg, the RSD_{TR} < 22% RSD_R[5] = relative standard deviation of reproducibility. $RSD_R \leq 2 \cdot PRSD_R$			
Recovery (R):	**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

[4] 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.

[5]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_R from an appropriate method performance study.

	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.			

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 Table 1 is inappropriate for determinations that involve a sum of components.

4. The aim of this discussion paper is to:

- undertake an analysis of CODEX STAN 234-1999 and individual methods in relevant commodity standards, to determine the extent to which methods of analysis that use a sum of components approach are cited and used; and try to identify potential methods that could be considered by the Committee for future conversion to method performance criteria;
- develop potential options for establishing criteria approaches for methods that are sum of components using CX/MAS 14/35/53 and CX/MAS 15/36/61 as a starting point;
- evaluate the options identified within recommendation iii) to ascertain fitness for purpose; and,
- consider the need to either amend the General Criteria for the Selection of Methods of Analysis section of the Procedural Manual and/or for development of a Guideline Document for Governments and other Codex Committees.

SPECIFICATIONS REQUIRING A COMBINATION OF COMPONENTS

5. An extensive analysis of CODEX STAN 234-1999, and individual methods in Codex Commodity Standards and Guideline Documents, indicates there to be a number of Codex specifications that stipulate MLs which are a sum of components or which require the analysis of multiple components. However, although there are a number of MLs that involve a summation of components the approach is limited to relatively few analyte groups:

- Antioxidants (e.g. BHA, BHT, tocopherols)
- Colours (e.g. carotenoids, synthetic dyes)
- Thickeners (e.g. distarch phosphate, hydroxypropyl starch, guar gum, gum arabic)
- Acidity Regulators (e.g. sodium hydroxide, potassium carbonate)
- Emulsifiers (e.g. mono- and diglycerides, acetic and fatty acid esters of glycerol)
- Flavouring Agents (e.g. vanillin and ethyl vanillin)
- Anticaking Agents (e.g. calcium carbonate, magnesium carbonate)
- Desmethylsterols (e.g. cholesterol, campesterol, β-sitosterol)
- Tocopherols (e.g. α-tocopherol, β-tocopherol, γ-tocopherol, α-tocotrienol)
- Mycotoxins (e.g. aflatoxins $B_1 + B_2 + G_1 + G_2$)
- Polychlorinated Biphenyls (e.g. in natural mineral water)
- Polycyclic Aromatic Hydrocarbons (e.g. in natural mineral water)
- Organochlorine Pesticides (e.g. in natural mineral water)
- Scoville Units (e.g. total capsaicinoids)
- Shellfish Toxins (e.g. saxitoxin (STX) group, okadaic acid (OA) group)

6. A number of Codex Commodity Standards stipulate some MLs (especially for the analytical groups given above) on either an individual component or a sum of components basis. For example, '*25 mg/kg (singly or in combination)*'. There are relatively few Codex Commodity Standards that stipulate MLs on a sum of components basis only (e.g. aflatoxins in peanuts, halogenated solvents in (olive) oils and fats).

7. A number of Codex Guideline Documents infer a sum of components approach for some analytes but do not stipulate MLs. For example, the *Guidelines on Nutrition Labelling (CAC/GL 2-1985)* refers to the analysis/determination of saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids which by definition implies a sum of components approach to be taken. The *Code of Practice for the Prevention and Reduction of Dioxin and Dioxin-like PCB Contamination in Foods and Feeds (CAC/RCP 62-2006)* refers to the analysis of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (PCBs) which again by definition implies a sum of components approach to be taken.

8. Table 2 shows a number of Codex Standards that stipulate MLs which involve a summation of analytical components.

Table 2: Example Codex Standards that stipulate MLs which involve a summation of analytical components.

Codex Standard	Title	Analyte	Maximum Level	STAN 234 Methods
STAN 19-1981	Standard For Edible Fats And Oils Not Covered By Individual Standards	Antioxidants Any combination of gallates, BHA, BHT, and/or TBHQ.	200 mg/kg but limits above not to be exceeded	AOAC 983.15; or AOCS Ce-6-86
STAN 33-1981	Standard For Olive Oils And Olive Pomace Oils	Desmethylsterol composition (% total sterols) • Cholesterol • Brassicasterol • Campesterol • Stigmasterol • Delta-7-stigmastenol • β-sitosterol + δ-5-avenasterol + δ-5,23-stigmastadienol + clerosterol + sitostanol + δ-5,24-stigmastadienol	Minimum value for total sterols Virgin olive oils Refined olive oil Olive oil 1,000 mg/kg Refined olive-pomace oil 1,800 mg/kg Olive-pomace oil 1,600 mg/kg	COI/T.20/Doc. no. 10 or ISO 12228 or AOCS Ch 6-91
STAN 193-1995	General Standard for Contaminants and Toxins in Foods and Feeds (GSCTFF)	Aflatoxins (Total)	Peanuts, maximum ML = 15 µg/kg for total aflatoxins (B_1 + B_2 + G_1 + G_2)	AOAC 991.31 AOAC 993.17 AOAC 975.36 EN 12955 ISO 16050
STAN 193-1995	GSCTFF	Fumonisins	Maize flour, ML = 2000 µg/kg (B_1 + B_2)	None
STAN 193-1995	GSCTFF	Inorganic arsenic (As(III)+As(V))	Polished rice, 0.2 mg/kg*	none

POTENTIAL OPTIONS FOR ESTABLISHING CRITERIA APPROACHES FOR METHODS THAT ARE SUM OF COMPONENTS

9. 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. CCMAS paper CX/MAS 14/35/5 indicates the approaches detailed for single analytes in the Procedural Manual to be unsuitable for establishing criteria for specifications requiring the determination of a combination of components. For example, aflatoxins in nuts in CODEX STAN 193-2005 where the specification is for the concentration of total aflatoxin, which is determined as the sum of B_1, B_2, G_1, and G_2. Paper CX/MAS 14/35/5 extensively describes a number of possible options, each with benefits and drawbacks for establishing criteria in these situations. Namely,

- Option 2-1: Use the specification (sum of components) as the specified level (maximum/minimum limit) and develop numeric criteria based on this limit and the parameters listed in Table 1.
- Option 2-2: Choose a suitable method and convert it into criteria using the guidelines currently listed in the Procedural Manual.
  - Option 2-2A: The numeric criteria are established from the approved method for each of the individual components.
  - Option 2-2B: The numeric criteria are established based on the specification and on the method performance for individual components.
- Option 2-3: Numeric criteria established based on the ML and the number of components.

EVALUATION OF OPTIONS

10. A major problem central to all the options detailed within CX/MAS 14/35/5 and CX/MAS 15/36/6 is the determination of the predicted relative standard deviation ($PRSD_R$) criterion. The Horwitz/Thompson Equation was originally derived based from data associated with 'individual' analytes and is not directly applicable to determining the $PRSD_R$ of a 'sum of components.' Therefore, the Horwitz/Thompson Equation or HorRat cannot be used to establish a numeric value for the precision. If one were to attempt to apply the Horwitz/Thompson Equation to the 'sum of components' it could produce a situation where the precision of one or more individual component would need to exceed 100%. During discussions of CX/MAS 14/35/5 and CX/MAS 15/36/6 it was widely agreed that it was inappropriate to calculate the $PRSD_R$ value for summed component specifications from the ML value itself because in multi-component analysis the individual analyte measurements are correlated and therefore not independent[6]. If the Horwitz/Thompson equation is used then it should be restricted to individual analyte measurements.

11. In reality, for the majority of measurements undertaken for 'contaminant' specifications which are summed components and the concentrations concerned fall into the Thompson $PRSD_R$ = 22% range so any method that does as well as 22% for the individual analyte should have acceptable precision. For higher levels (e.g. emulsifiers, thickeners, antioxidants, etc.) the Horwitz value is likely to be the criterion.

12. A general question also raised within CX/MAS 14/35/5 was whether it is "permitted" within Codex to establish criteria for analytes that do not have associated specifications? Whilst this is a valid question this discussion paper takes the view that if individual analytes are specified (as is the case for aflatoxins in CODEX STAN 193-2005) then by default they are linked to the total specification and criteria can therefore be established.

13. Although not explicitly stated, papers CX/MAS 14/35/5 and CX/MAS 15/36/6 indicated the most pragmatic approach, at that time, to be Option 2-3 where numeric criteria established are based on the ML and the number of components.

14. The Procedural Manual guidelines for establishing numeric values for LOQ are as follows:

Limit of Quantification (LOQ):	For ML ≥ 0.1 mg/kg, LOQ ≤ ML · 1/5 For ML < 0.1 mg/kg, LOQ ≤ ML · 2/5

15. This is valid for analysing one component. When the ML is based on a sum of components, the LOQ for the individual component should theoretically be correspondingly low. When summing two components, the LOQ for each component should be the half for each component, and if summing three components; the

[6]RSC AMC Technical Brief No. 30, 2008. The standard deviation of the sum of several variables (http://www.rsc.org/Membership/Networking/InterestGroups/Analytical/AMC/TechnicalBriefs.asp)

LOQ for each component should be 1/3 of the LOQ. **It is important to note that throughout this process the actual ML itself remains unchanged.**

16. Based on this, the following criteria for LOQ were suggested:

Limit of Quantification (LOQ):	For ML ≥ 0.1 mg/kg, LOQ ≤ ML · 1/5 · 1/*n* For ML < 0.1 mg/kg, LOQ ≤ ML · 2/5 · 1/*n* Where *n* = number of components

17. 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 3.

Table 3: 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 the individual components[7]:			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):	**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.			

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

Example A:

Aflatoxin, consisting of 4 analytes, B_1, B_2, G_1 and G_2, in peanuts.

The ML = 15 µg/kg,

As there are 4 analytes, n = 4,

ML/n = 15/4 µg/kg = 3.75 µg/kg

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

Minimum Applicable Range for the individual components:	0.002* - 0.022** mg/kg = 2 - 22 µg/kg *corresponding to ML/*n* = 3.75 µg/kg **corresponding to ML = 15 µg/kg
Limit of Detection (LOD) for the individual components:	0.75 µg/kg
Limit of Quantification (LOQ) for the individual components:	1.5 µg/kg
Precision for the individual components:	$RSD_R \leq 44\%$
Recovery (R):	40-120%

Examples on methods fulfilling the criteria:

AOAC 999.07 Immunoaffinity Column LX with post column derivatization

AOAC 2005.08 LC with Post-column photochemical derivatization

Examples on methods not fulfilling the criteria:

AOAC 975.36 (Romer mini-column method) applicable for ≥ 10 µg/kg

AOAC 990.34 (Enzyme Linked Immunosorbent (ImmunoDot Screen Cup) Screening Assay ≥ 20 µg/kg

AOCS–AOAC 970.45, AOCS –AOAC 998.03. AOAC 993.17 Thin Layer Chromatography

Example B:

Antioxidant in Oils – Propyl gallate [PG], 2- and 3-tert-butyl-4-hydroxyanisole [BHA], 3,5-di-tert-butyl-4-hydroxytoluene [BHT] and tert-butylhydroquinone [TBHQ].

The ML = 200 mg/kg,

As there are 4 analytes, n = 4,

ML/n = 200/4 mg/kg = 50 mg/kg

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

Minimum Applicable Range for the individual components:	37* - 243** mg/kg *corresponding to ML/*n* = 50 mg/kg **corresponding to ML = 200 mg/kg
Limit of Detection (LOD) for the individual components:	5 mg/kg
Limit of Quantification (LOQ) for the individual components:	10 mg/kg
Precision for the individual components:	$RSD_R \leq 17.8\%$
Recovery (R):	80-110%

Examples on methods fulfilling the criteria:

AOAC Official Method 983.15 - Phenolic Antioxidants in Oils, Fats, and Butter Oil

Examples on methods not fulfilling the criteria:

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Example C:

Fumonisins in Maize Flour, consisting of 2 analytes B1 and B2.

The ML = 2000 µg/kg,

As there are 2 analytes, n = 2,

ML/n = 2000/2 µg/kg = 1000 µg/kg

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

Minimum Applicable Range for the individual components:	830* - 2300** µg/kg *corresponding to ML/*n* = 1000 µg/kg **corresponding to ML = 2000 µg/kg
Limit of Detection (LOD) for the individual components:	100 µg/kg
Limit of Quantification (LOQ) for the individual components:	200 µg/kg
Precision for the individual components:	$RSD_R \leq 11.3\%$
Recovery (R):	80-110%

Examples on methods fulfilling the criteria:

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Examples on methods not fulfilling the criteria:

BS EN 14352:2004 - Foodstuffs. Determination of fumonisin B1 and B2 in maize based foods. HPLC method with immunoaffinity column clean up. Whilst BS EN 14352:2004 is fit for purpose in terms of LOD and LOQ the RSD_R determined through the collaborative trial is too high.

18. Option 2-2A of CX/MAS 14/35/5 and CX/MAS 15/36/6 describes how numeric criteria may be established from the approved method for each of the individual components. Whilst reservations were expressed in CX/MAS 14/35/5 about Option 2-2A this approach has already successfully been used to develop numeric performance criteria for various biotoxins groups detailed in the *Standard for Live and Raw Bivalve Molluscs* (CODEX STAN 292-2008).

19. Alternatively, it is feasible to directly convert an approved sum of components method into numeric criteria if the method performance statistics cited within the approved method are determined on a sum of components basis and not simply an individual component basis. For example, BS EN 14123:2007[8] and COI/ T.20/ Doc. no.10[9] both report method performance statistics on an individual component and a total (sum of) components basis so it should be feasible to convert these data into sum of component method performance criteria. A potential hybrid approach is given in Table 4.

[8] BS EN 14123:2007 Foodstuffs. Determination of aflatoxin B1 and the sum of aflatoxin B1, B2, G1 and G2 in hazelnuts, peanuts, pistachios, figs, and paprika powder. High performance liquid chromatographic method with post-column derivatisation and immunoaffinity column cleanup.

[9] COI/ T.20/ Doc. no.10/ Rev. 1 2001 - Determination of the Composition and Content of Sterols by Capillary-Column Gas Chromatography.

Table 4: Hybrid approach for establishing numeric values for the criteria using collaborative trial data that have been determined on a sum of components basis.

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 (the sum of components s_R is taken from the published collaborative trial data):	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] NB: the upper level is above the ML for the individual components.			
Limit of Detection (LOD) for <u>the individual components</u> (if this is not already stated within the approved method):	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 <u>the individual components</u> (if this is not already stated within the approved method):	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:	Sum of components RSD_R to be taken from published collaborative trial data.			
Recovery (R):	**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.			

<u>Example D:</u>

Desmethylsterol Composition of Olive Oil, consisting of 15 analytes but where results are expressed as a sum of components.

The ML (on a sum of components basis) = 1000 mg/kg,

The LOD/LOQ is not given within the approved method[10] so for LOD and LOQ calculations ML/n = 1000/15 mg/kg = 67 mg/kg

Reported s_R and RSD_R values for total sterols in extra virgin olive oil[10] are 34 mg/kg and 2.2%, respectively.

Using selected calculations (LOD and LOQ) from the excel spreadsheet on www.nmkl.org under "how to get method criteria based on ML", the following are established:

Minimum Applicable Range:	898 - 1102 mg/kg
Limit of Detection (LOD) for <u>the individual components</u>:	67/10 = 7 mg/kg
Limit of Quantification (LOQ) for <u>the individual components</u>:	67/5 = 13 mg/kg
Precision for <u>the sum of components</u>:	RSD_R ≤ 2%
Recovery (R):	95-105%

Examples on methods fulfilling the criteria:

?

Examples on methods not fulfilling the criteria:

?

RECOMMENDATIONS

20. From the examples above it is clear that there is no single mechanism for determining numeric method performance criteria for methods and MLs that are wholly, or partially, a sum of components.

21. Owing to the complexity of the issue CCMAS should consider the need to develop a document detailing the various options available when developing numeric criteria for methods and MLs that are a sum of components (with worked examples) and also typical information required when such an approach is being considered.

22. Method performance criteria for methods and MLs that are a sum of components may potentially be determined using one of the following options where a decision on which option to use should be made on a case-by-case basis:

- Option A: Select an approved method and convert it into numeric criteria using a hybrid approach (Table 3). This method is applicable if method performance data supporting the approved method are already calculated on a sum of components basis. This option may be more appropriate for Codex Guideline Documents that infer a sum of components approach for some analytes (e.g. polyunsaturated fatty acids, dioxins, etc) but do not stipulate MLs.
- Option B: Numeric criteria are established from the approved method for each of the individual components. This approach has already been adopted for shellfish biotoxins in the *Standard for Live and Raw Bivalve Molluscs* (CODEX STAN 292-2008).
- Option C: Numeric criteria are established from the ML and the number of components (Table 2). This approach has merit but is unlikely to be applicable if the number of components is large (say >>5) because the target LOD and/or LOQ may become analytically unachievable

23. The *General Criteria for the Selection of Methods of Analysis* section of the Procedural Manual should be amended to indicate that the process based upon the ML value is only suitable for single-analyte analyses.

Appendix II

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