
Analize živil - Biotoksini - Kriteriji analiznih metod mikotoksinov

Food analysis - Biotoxins - Criteria of analytical methods of mycotoxins

Lebensmittel - Biotoxine - Kriterien für Mycotoxin-Analyseverfahren

Produits alimentaires - Biotoxines - Criteres des méthodes d'analyse des mycotoxines

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Food analysis - Biotoxins - Criteria of analytical methods of mycotoxins

Produits alimentaires - Biotoxines - Critères des méthodes
d'analyse des mycotoxines

Lebensmittel - Biotoxine - Kriterien für Mycotoxin-
Analyseverfahren

This CEN Report was approved by CEN on 23 December 1998. It has been drawn up by the Technical Committee CEN/TC 275.

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Foreword

This report has been drawn up by Technical Committee CEN/TC 275 - 'Food analysis - Horizontal methods'.

Introduction

The working group CEN/TC 275/WG 5 - 'Biotoxins' selects and elaborates methods of analysis for mycotoxins that are to become European Standards. The Standards can be used for mycotoxins which are subject to legal limits. When used for this purpose, the main functions of the Standards are to enable food manufacturers to determine with reasonable certainty whether a production batch may be put on the market and to enable regulatory authorities to determine equitably whether foodstuffs on the market comply with legal limits.

The CEN working group 'Biotoxins' decided to first establish some criteria to guide in the selection between several methods of analysis. The performance criteria laid down in this CEN Report are based on published data, collected from official reports on European interlaboratory studies on the respective mycotoxins (certification studies, method validation studies). [See annex A Bibliography]. Where these performance characteristics were absent or limited in availability, the criteria were estimated based on the experiences and opinions of the experts of the CEN working group 'Biotoxins'. The selection criteria may need updating in future revisions of this document, if newer or more accurate data on method performance characteristics become available. This document gives information concerning method performance, which can be expected from experienced analytical laboratories.

It may contain useful information for example for CEN members, the European Commission, the EFTA secretariat or other governmental agencies or outside bodies.

The criteria in this CEN report are used as guidance in the CEN working group 'Biotoxins' selection of methods of analysis to be standardized.



1 Scope

This CEN Report gives criteria for the selection of methods of analysis for mycotoxins. Criteria covered are the repeatability, reproducibility, recovery, extraction solvents, applicability and food types. Performance criteria are included for aflatoxin B₁, total of aflatoxins B₁, B₂, G₁ and G₂, aflatoxin M₁, ochratoxin A, patulin, fumonisin B₁, fumonisin B₂, deoxynivalenol, nivalenol, HT-2 toxin, T-2 toxin and zearalenone.

2 Definitions

For the purposes of this CEN Report, the following definitions apply:

- 2.1 **precision:** The closeness of agreement between independent test results obtained under stipulated conditions [ISO 5725-1:1994, [1]].
- 2.2 **recovery:** (Measured concentration in fortified material - measured concentration in unfortified material) x 100 % / (known increment in concentration). Recovery is expressed in %.

NOTE 1: The amount added should be a substantial fraction of, or more than, the amount present in the unfortified material. Ideally the unfortified material should contain no measurable level of the analyte under test.

NOTE 2: A true or assigned value is known only in cases of spiked or fortified materials, certified reference materials, or by analysis by another (presumably unbiased) method. The concentration in the unfortified material is obtained by direct analysis or by the method of additions. In other cases, there is no direct measure of bias, and consensus values derived from the collaborative study itself often can be used for the reference point.

- 2.3 **repeatability:** Precision under repeatability conditions [ISO 5725-1:1994].
- 2.4 **repeatability conditions:** Conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time [ISO 5725-1:1994].
- 2.5 **reproducibility:** Precision under reproducibility conditions [ISO 5725-1:1994].
- 2.6 **reproducibility conditions:** Conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment [ISO 5725-1:1994].
- 2.7 **RSD_r:** (Relative within- laboratory standard deviation): This precision characteristic relates to the within- laboratory error of a method. It is the standard deviation, divided by the mean of the test results, obtained with the same method on identical test material, under the same conditions (same operator, apparatus, laboratory and within short intervals of time).
- $$RSD_r = \frac{SD_r}{\text{mean}} \times 100 \%$$
- 2.8 **RSD_R:** (Relative between-laboratory standard deviation): This precision characteristics relates to the between-laboratory error of a method. It is the standard deviation, divided by the mean of the test results, obtained with the same method on identical test material, but under different conditions (different operators, apparatus, laboratories and different times).

3 Criteria for analytical methods for mycotoxins

3.1 Lowest validated levels

The lowest validated levels for matrices are those established in collaborative studies.

3.2 Performance characteristics

3.2.1 General

The performance characteristics given in 3.2.2 are based on published data collected from official reports on European interlaboratory studies on the respective mycotoxins. Where possible references are given to relevant published collaborative studies [see annex A - Bibliography]. Values are rounded off and were considered reasonable and realistic by the experts of CEN/TC 275/WG 5 'Biotoxins'.

3.2.2 Performance characteristics

The following performance characteristics are meant as guidelines. It is the responsibility of the analyst to select the method of analysis to suit their purposes.

Table 1: Performance characteristics for aflatoxin B₁ [2], [3]

Level μg/kg	B ₁		
	RSD _r %	RSD _R %	Recovery %
< 1	≤ 40	≤ 60	50 to 120
1-10	≤ 20	≤ 30	70 to 110
> 10	≤ 15	≤ 20	80 to 110

Table 2: Performance characteristics for aflatoxins B₁, B₂, G₁ and G₂ [2], [3]

Level μg/kg	Total (B ₁ + B ₂ + G ₁ + G ₂)		
	RSD _r %	RSD _R %	Recovery %
< 1	--	--	50 to 120
1-10	≤ 40	≤ 60	70 to 110
> 10	≤ 30	≤ 50	80 to 110

Table 3: Performance characteristics for aflatoxin M₁ [4]

Level ng/l	M ₁		
	RSD _r %	RSD _R %	Recovery %
10-50	≤ 30	≤ 50	60 to 120
> 50	≤ 20	≤ 30	70 to 110

Table 4: Performance characteristics for ochratoxin A [5], [6], [7], [8]

Level μg/kg	Ochratoxin A		
	RSD _r %	RSD _R %	Recovery %
< 1	≤ 40	≤ 60	50 to 120
1-10	≤ 20	≤ 30	70 to 110

Table 5: Performance characteristics for patulin [9], [10], [11]

Level $\mu\text{g/l}$	Patulin		
	RSD _r %	RSD _R %	Recovery %
20-50	≤ 20	≤ 30	70 to 105
> 50	≤ 15	≤ 25	75 to 105

Table 6: Performance characteristics for fumonisin B₁ or B₂ [12]

Level $\mu\text{g/kg}$	Fumonisin		
	RSD _r %	RSD _R %	Recovery %
< 500	≤ 30	≤ 60	60 to 120
500-5000	≤ 20	≤ 30	70 to 110

Table 7: Performance characteristics for deoxynivalenol and nivalenol, [13], [14], [15], [16], [17]

Level $\mu\text{g/kg}$	Deoxynivalenol and nivalenol		
	RSD _r %	RSD _R %	Recovery %
> 100	≤ 20	≤ 40	70 to 110

Table 8: Performance characteristics for HT-2 toxin [16]

Level $\mu\text{g/kg}$	HT-2 toxin		
	RSD _r %	RSD _R %	Recovery %
100-200	≤ 40	≤ 60	60 to 120
> 200	≤ 30	≤ 50	60 to 110

Table 9: Performance characteristics for T-2 toxin [16]

Level $\mu\text{g/kg}$	T-2 toxin		
	RSD _r %	RSD _R %	Recovery %
50-250	≤ 40	≤ 60	60 to 120
> 250	≤ 30	≤ 50	60 to 110

Table 10: Performance characteristics for zearalenone [17], [18]

Level $\mu\text{g/kg}$	Zearalenone		
	RSD _r %	RSD _R %	Recovery %
> 100	≤ 25	≤ 40	70 to 100

3.3 Extraction solvents used in the method of analysis

The choice of extraction solvent should be that which gives the most efficient extraction of mycotoxins from a food matrix. This should be evaluated by means of naturally contaminated foodstuffs, where possible. The safety regulations concerning restricted use of solvents e.g. chloroform should be taken into account.

3.4 Applicability of the method of analysis

Methods should be preferred which can easily be performed by many users, have been validated in collaborative studies and can be accomplished with common laboratory equipment.

3.5 Standard preparation

One substantial source of variation can be the use of different standard toxin preparations. The purity and nominal weight or concentration can vary and ought to be checked carefully before use.

4 Food types

4.1 General

In 4.2 to 4.8 products are mentioned in which the mycotoxin has been found that is mentioned in the heading of the subclause. The CEN working group 'Biotoxins' considers it desirable to have methods of analysis for the mycotoxins in these matrices.

4.2 Aflatoxins B₁, B₂, G₁ and G₂

Nuts (including peanuts) and nut products, dried fruits (especially figs), oilseeds (only cold pressed oils), cereals (e.g. buckwheat), cocoa mass, desiccated coconut, crude oils, spices.

4.3 Aflatoxin M₁

Milk and milk products.

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4.4 Ochratoxin A

Cereals, pulses, vegetables, fruits, vegetable and fruit products, coffee, pig kidneys.

4.5 Patulin

Fruit products.

4.6 Fumonisin

Maize and maize products.

4.7 Trichothecenes

Cereals and cereal products.

4.8 Zearalenone

Maize, maize products, corn

Annex A (informative)

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