

## JRC TECHNICAL REPORT



# Progress in processing and certification of new mycotoxin matrix reference materials for mycotoxin analysis

*ERM-BD285 (pistachios), ERM-BD286 (paprika powder)  
and ERM-BC473 (barley)*

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**Abstract**

This report describes the processing and certification of a set of new mycotoxin reference materials for the determination of the aflatoxin content in pistachio powder (ERM BD285), the determination of the aflatoxin and ochratoxin A content in paprika powder (ERM BD286) as well as the determination of ochratoxin A content in barley (ERM BC473) to improve the quality of analytical data in the field of mycotoxins in food and to support EU legislation.

An overview is given regarding the base materials, project planning, homogeneity, short-term stability and characterisation studies of the above mentioned materials. Furthermore, achievements as well as the currently on-going studies, the recruitment of competent laboratories and other planned activities are described.

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## **Executive summary**

This report describes the processing and certification of a set of new mycotoxin reference materials for the determination of the aflatoxin content in pistachio powder (ERM-BD285), the determination of the aflatoxin and ochratoxin A content in paprika powder (ERM-BD286) as well as the determination of ochratoxin A content in barley (ERM-BC473) to improve the quality of analytical data in the field of mycotoxins in food and to support EU legislation.

An overview is given regarding the base materials, project planning, homogeneity, short-term stability and characterisation studies of the above mentioned materials. Furthermore, achievements as well as the currently on-going studies, the recruitment of competent laboratories and other planned activities are described.

# 1 ERM-BD285 – Aflatoxins in pistachios

## 1.1 Introduction

The problem of the contamination of nuts and nut products with aflatoxins (AFs) is well known for many years. Maximum levels for certain mycotoxins have been introduced in the European Union since 1997 [1-4]. Numerous Rapid Alert System for Food and Feed (RASFF) notifications [5], as well as publications [6-8] confirm that contamination with AFs of peanuts and pistachios appears to be the most prevalent among foodstuffs. Every year 20 to 25 % of all mycotoxin-related notifications reported in RASFF concern particularly AFs in pistachios, while no certified reference materials (CRMs) for pistachios exist.

Since the Institute for Reference Materials and Measurements (IRMM) intends to extend its range of CRMs for mycotoxin analysis, it was decided to start the aforementioned project. In line with the IRMM's quality assurance procedures a project planning form (PPF) was drafted, discussed and approved (PP-D-00189/2).

## 1.2 Objectives

Since the base materials for this candidate reference material were already purchased and information on approximate concentrations of target analytes obtained, the material was processed, pre-checked, filled in bottles and gamma-irradiated (see previous year's report for details on mentioned actions) [9]. The following main objectives were set:

1. Planning of homogeneity study
2. Planning of short- and long-term stability studies
3. Planning of characterisation study as well as the recruitment of competent laboratories

## 1.3 Achievements

- The homogeneity study indicated that the material is sufficiently homogenous ( $u_{bb}^* = 2.61$  and  $2.65$  % for aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) and aflatoxin B<sub>2</sub> (AFB<sub>2</sub>), respectively). See results of homogeneity study in Table 1 ( $s_{wb}$  – within-unit standard deviation;  $s_{bb}$  – between-unit standard deviation;  $u_{bb}^*$  – standard uncertainty related to a maximum between-unit heterogeneity that could be hidden by method repeatability;  $u_{bb}$  – standard uncertainty related to a possible between-unit heterogeneity).

**Table 1:** Results of homogeneity study

Analyte	$s_{wb}$ [%]	$s_{bb}$ [%]	$u_{bb}^*$ [%]	$u_{bb}$ [%]
AFB <sub>1</sub>	8.90	5.17	2.61	5.17 <sup>1)</sup>
AFB <sub>2</sub>	9.04	1.59	2.65	2.65 <sup>1)</sup>

<sup>1)</sup> Higher value of  $u_{bb}$  or  $s_{bb}$  was taken as a contribution of heterogeneity

No trends in the filling sequence were visible. However one outlier for AFB<sub>1</sub> at 99 % confidence level (single Grubbs test) was detected in both cases, individual result as well as unit mean (approximately 35 % higher than mean value of the study).

- The short-term stability study (STS) confirmed stability of all analytes at +4 °C and +18 °C. Uncertainties of degradation ( $u_{sts}$ ) during dispatch are 0.99 % and 1.36 % for AFB<sub>1</sub> and AFB<sub>2</sub>, respectively (estimated from the +4 °C study describing the possible change during dispatch at +4 °C for 1 week). A shipment with cooling elements is recommended.
- When the STS study results were tested for homogeneity, one outlier at 99 % confidence level (single Grubbs test) was detected for AFB<sub>1</sub> and similar as stated above, approximately 35 % higher than mean value of the study.

However, the analysis of additional samples ("around" the outlier: few bottles before and after the outlying bottle) did not fully proof heterogeneity. Only one out of 5 tested bottles (each in triplicate; 15 results in total) showed a mean result approximately 25 % higher than the means of homogeneity and short-term stability studies). Nevertheless, in order to prevent possible heterogeneity due to possible "hot-spots", it was decided to re-process the material, re-assess homogeneity, short- and long-term stability (on-going).

- Recruitment of laboratories for characterisation study is performed (49 laboratories invited; 25 agreed to participate; only 16 sent their offers and 15 were selected for the characterisation study).
- The characterisation study is on-hold until new data on homogeneity and short-term stability of the material are available.

## 2 ERM-BD286 – Aflatoxins and ochratoxin A in paprika powder

### 2.1 Introduction

According to the RASFF annual reports [5], every year mycotoxins are the hazard category with the highest number of notifications. Among others, a significant group of foodstuffs prone to mycotoxin contamination are spices. The number of notifications (between 2008-2014) related to aflatoxins (AFs) and ochratoxin A (OTA) in herbs and spices accounted for about 15-20 % of all notifications were due to mycotoxin contaminations [5]. According to the current EC Regulations [1-4] and numerous surveys reported in the literature [10-13] major contributors to the AFs and OTA exposure are dried fruits of *Capsicum* spp. (whole or ground, including chillies, chilli powder, cayenne and paprika), white and black pepper, nutmeg, ginger, turmeric and liquorice. The interest in analytical quality assurance in this field is significant and the demand for appropriate CRMs for AFs and OTA in *Capsicum* spp. is high.

Taking the above into consideration, it was decided to launch a new project aiming at the development of a reference material for the determination of AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, AFG<sub>2</sub> and OTA in paprika powder. A project plan was discussed, drafted and approved (PP-D-00248/1). Appropriate base materials contaminated with the target analytes were purchased and the levels of mycotoxins in the purchased materials were determined. Furthermore, a new combined analytical method for the determination of aflatoxins and ochratoxin A in paprika powder was developed and validated (VAL-D-00145/1) to be able to perform the necessary measurements for the development of a new paprika powder reference materials (see last year's report [9]).

### 2.2 Objectives

Since the base materials for this candidate reference material were already purchased and information on approximate concentrations of target analytes obtained, the materials were processed, pre-checked, filled in bottles and gamma-irradiated. The following main objectives were set:

1. Planning of homogeneity study
2. Planning of short- and long-term stability studies
3. Planning of characterisation study as well as the recruitment of competent laboratories
4. Drafting certification report and certificate

### 2.3 Achievements

- The homogeneity study was performed. No heterogeneity was detected for any of the tested analytes (see Table 2;  $s_{wb}$  – within-unit standard deviation;  $s_{bb}$  – between-unit standard deviation;  $u_{bb}^*$  – standard uncertainty related to a maximum between-unit heterogeneity that could be hidden by method repeatability;  $u_{bb}$  – standard uncertainty related to a possible between-unit heterogeneity).

**Table 2:** Results of homogeneity study

Analyte	$s_{wb}$ [%]	$s_{bb}$ [%]	$u_{bb}^*$ [%]	$u_{bb}$ [%]
AFB1	10.6	n.c. <sup>1)</sup>	3.10	3.10 <sup>2)</sup>
AFG1	8.63	n.c. <sup>1)</sup>	2.53	2.53 <sup>2)</sup>
OTA	5.45	n.c. <sup>1)</sup>	1.60	1.60 <sup>2)</sup>

<sup>1)</sup> n.c. – cannot be calculated as  $MS_{within} < MS_{between}$  (from analysis of variance)

<sup>2)</sup> Higher value of  $u_{bb}^*$  or  $s_{bb}$  was taken as a contribution of heterogeneity

- The short-term stability study (STS) confirmed stability of all analytes at +4 °C and +18 °C. In Table 3 uncertainties of degradation ( $u_{\text{sts}}$ ) during dispatch are shown (estimated from the +18 °C study describing the possible change during dispatch at +18 °C for 1 week). A shipment with cooling elements is recommended. The long-term stability (LTS) study is still on-going (results to be expected in March 2015).

**Table 3:** Estimated uncertainties of stability during dispatch and storage

Analyte	$u_{\text{sts}}$ [%]	$u_{\text{lts}}$ [%]
AFB <sub>1</sub>	1.12	on-going
AFG <sub>1</sub>	1.27	on-going
OTA	0.75	on-going

- The recruitment of laboratories for the characterisation study was successful. It was aimed to obtain at least 15 valid data sets from the participants. Initially, about 146 laboratories were selected from the IRMM internal database as possible candidates for characterisation study. Information and questions about organisation of characterisation study were sent to all laboratories. Only 49 laboratories were interested or eligible (based on quality requirements and provided quality information) to participate in the study. Out of these 49 laboratories, only 26 fully agreed to participate in the study, but only 19 of them sent official offers in response to the invitation to tender. Finally, 15 laboratories were selected for the characterisation study based on quality and price requirements.
- The characterisation campaign resulted in 10 accepted datasets for AFB<sub>1</sub>, 9 accepted datasets for AFG<sub>1</sub> and OTA. In total, 6 out of 15 sets of data for AFG<sub>1</sub> and OTA; and 5 out of 15 sets of data for AFB<sub>1</sub> were rejected/cancelled or withdrawn for technical reasons.
- For AFG<sub>2</sub> it is considered to state an indicative value (~ 0.2 µg/kg). Chromatographic peaks were detected in all cases and results on mass fractions in almost half or the cases. For AFB<sub>2</sub> only few results were received, but all below the limit of quantification (LOQ). Therefore, it was decided not to state any value. More details on certification can be found in the certification report.
- The drafts of certification report and certificate are prepared and can be finalised after addition of long-term stability data when the long-term stability study will be completed.



### **3 ERM-BC473 – Ochratoxin A in barley**

#### **3.1 Introduction**

The problem of the contamination of grains and grain products with ochratoxin A is well known for many years. Maximum levels for certain mycotoxins have been introduced in the European Union since 1997 [1-4]. As mentioned in the previous sections, numerous RASFF notifications [5], as well as publications [14-16], confirm that contamination of barley with ochratoxin A (OTA) as well as with other mycotoxins appears to be the most prevalent among foodstuffs, while no CRM for OTA in barley exists.

Since the IRMM intends to extend the range of the produced CRMs for mycotoxin analysis, it was decided to start the aforementioned project.

In line with IRMM's quality assurance procedures a project planning form (PPF) was drafted and discussed during the project initiation meeting held in September 2014. According to the outcome of the project initiation meeting a PPF has been finalised, approved and released.

#### **3.2 Objectives**

The following main objectives were set:

1. Screening for potential contamination with other "regulated" (maximum limits defined in legislation) and "non-regulated" (maximum limits are not defined in legislation) mycotoxins and their conjugates
2. Purchase of additional batch of a blank barley to "dilute" (1:1) the existing batch, which contains approximately 20 µg/kg of OTA (about 400 % of legal limit) to reach a mass fraction of OTA of about 200 % of the legal limit
3. Processing planning (suitable parameters and conditions for milling, mixing and filling, optimal sample size, etc.)
4. Planning of homogeneity, short- & long-term stability studies
5. Planning of characterisation study as well as recruitment of competent laboratories

#### **3.3 Achievements**

- Screening performed for potential contamination with other regulated and non-regulated mycotoxins and their conjugates (about 78 different mycotoxins and plant toxins were detected; 32 of them in significant concentrations; 6 out of 32 detected mycotoxins belong to the group of regulated mycotoxins and another 2 are candidates for becoming regulated).
- It was decided that the material should be certified only for mass fraction of OTA with the possible extension to other important mycotoxins in the future.
- The project plan was prepared, discussed, approved and released (PP-D-0287/1).

The following actions planned to be performed in the next year:

- Material processing, pre-check, filling and gamma-irradiation of the material.
- Homogeneity, short- and long-term stability studies.
- Recruitment of laboratories for characterisation study. The data base of laboratories who participated in the characterisation of ERM-BD286 (paprika powder) and who will participate in the characterisation of ERM-BD285 (pistachios) may be used as a starting point.

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