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## Mycotoxin metrology: Gravimetric production of zearalenone calibration solution

E C P Rego<sup>1</sup>, M E Simon<sup>2</sup>, Xiuqin Li<sup>3</sup>, Xiaomin Li<sup>3</sup>, A Daireaux<sup>4</sup>, T Choteau<sup>4</sup>, S Westwood<sup>4</sup>, R D Josephs<sup>4</sup>, R I Wielgosz<sup>4</sup>, V S Cunha<sup>1</sup>

<sup>1</sup> INMETRO, Chemical and thermal metrology division, Duque de Caxias, Brazil

<sup>2</sup> INTI Argentina, Toxicology and nutrition laboratory, Agro-food center, Buenos Aires, Argentina

<sup>3</sup> NIM China, Division of chemistry metrology and analytical science, Beijing, China

<sup>4</sup> BIPM, Department of chemistry, Paris, France

E-mail: ecrego@inmetro.gov.br

**Abstract.** Food safety is a major concern for countries developing metrology and quality assurance systems, including the contamination of food and feed by mycotoxins. To improve the mycotoxin analysis and ensure the metrological traceability, CRM of calibration solution should be used. The production of certified mycotoxin solutions is a major challenge due to the limited amount of standard for conducting a proper purity study and due to the cost of standards. The CBKT project was started at BIPM and Inmetro produced gravimetrically one batch of zearalenone in acetonitrile ( $14.708 \pm 0.016 \mu\text{g/g}$ ,  $k=2$ ) and conducted homogeneity, stability and value assignment studies.

**Keywords:** zearalenone; mycotoxin; traceability; gravimetric preparation; Certified Reference Material (CRM).

### 1. Introduction

Zearalenone (ZEN) is a fungal mycotoxin produced by *Fusarium spp.* and present in several types of food but especially in maize and wheat. It is a non-steroid estrogenic compound which can cause changes in reproductive organs and fertility loss and has also been shown to have several other toxic effects [1]. Zearalenone analyses are a matter of health and food safety for many countries around the globe which produce or consume large quantities of corn grains and wheat.

In 2016 the Capability Building and Knowledge Transfer (CBKT) project on Safe Food and Feed in Developing Economies was started at BIPM to allow NMIs (National Metrology Institutes) to work together to strengthen mycotoxin metrology infrastructure; provide knowledge transfer to scientists developing capabilities in this area, including periods as visiting scientists at the BIPM; and enable NMIs to provide mycotoxin calibrant and matrix (C)RMs and PT materials to support mycotoxin testing laboratories within their countries [2]. Inmetro took part in this project and prepared gravimetrically one batch of calibration solution of Zearalenone, and conducted the homogeneity, stability and value assignment studies of this solution, following requirements of ISO 17034 [3] and



ISO Guide 35 [4]. The ZEN stock solution batch used to prepare the calibration solution batch was previously studied by BIPM, that conducted the purity assessment of ZEN powder standard.

## 2. Methods

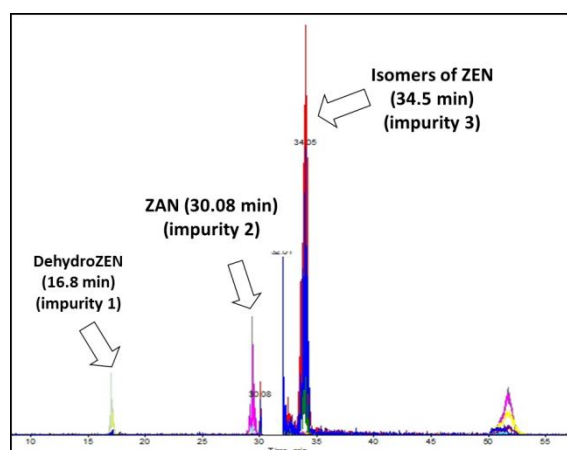
### 2.1. Gravimetric preparation and ampouling

One ZEN calibration solution (named OGP.026A) at about 15  $\mu\text{g/g}$  in acetonitrile was prepared in February of 2017 by dilution of BIPM stock solution OGP.025. The preparation of the solution was carried out using a gravimetric procedure. The final concentration and its uncertainty were calculated. The solution was filled in 10 mL clear glass ampoules with 4 mL of appropriate solution. A batch of 64 ampoules was produced. All the ampoules of OGP.026A were stored at  $-20^\circ\text{C}$ . The homogeneity, stability and value assignment of the batch was tested by use of UV-Visible Spectrophotometer (Perkin Elmer Lambda 650) and LC-MS/MS system (Applied Q-trap 4000).

### 2.2. Homogeneity Testing

The first procedure to determine the homogeneity of the main component Zearalenone (ZEN) was based on the UV spectrophotometric measurements at three wavelengths (235 nm, 274 nm and 314 nm).

A second procedure to determine the homogeneity of the impurities of ZEN was based on the LC-MS/MS method. Three impurities were identified: Dehydrozearalenone (dehydroZEN), Zearalanone (ZAN) and Isomers of Zearalenone (isomerZEN) (figure 1). An LC-DAD method was also carried out to determine the main compound concentration (ZEN).



**Figure 1.** ZEN impurities on OGP.026A batch.

The response data obtained from each method were evaluated by ANOVA to get information concerning the homogeneity of main component and its impurities in the calibration solution.

### 2.3. Stability Testing

Stability testing is performed to confirm the stability of the material. The study is normally conducted under storage conditions (long-term stability) and under transport conditions (short-term stability).

The stability testing only considers the possible impact of temperature, time and light.

The batch was also submitted to stability testing for ZEN and its related impurities using UV-Visible spectrophotometry and LC-DAD-MS/MS methods.

The stability study of ZEN calibration solutions of ZEN (OGP.026A) followed the isochronous design, under repeatability conditions, with a reference temperature of  $-20^\circ\text{C}$  (dark) and study temperatures of  $4^\circ\text{C}$  (dark) and  $22^\circ\text{C}$  (with light) during 4 weeks. For each condition/time were used two ampoules.

The response data obtained from each method were tested for trends using linear regression evaluation.

#### 2.4. Value Assignment

Two samples from the OGP.026A batch were analysed directly without dilution. One ampoule of BIPM OGP.024 (ZEN calibration solution) was used as standard for UV measurements and one ampoule of BIPM OGP.025 (ZEN stock solution) was used to prepare standards for LC measurements.

Three independent replicates were analyzed in each method for major compound (ZEN). Two methods were used: UV spectrophotometric method with temperature fixed at 25 °C (Scan and fixed wavelength 235 nm, 274 nm and 314 nm) and LC-DAD method using bracketing quantification.

### 3. Results and discussion

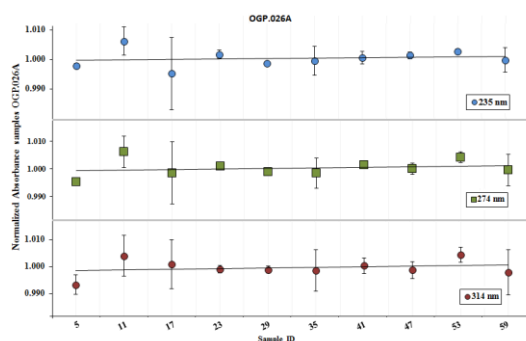
#### 3.1. Gravimetric preparation and ampouling

The gravimetric value for ZEN in OGP.026A is  $14.708 \pm 0.016 \mu\text{g/g}$  ( $k=2$ ). The sources of uncertainties considered in the calculation were: weighing, buoyancy correction, evaporation correction and ZEN stock solution OGP.025 uncertainty (that includes purity of ZEN powder).

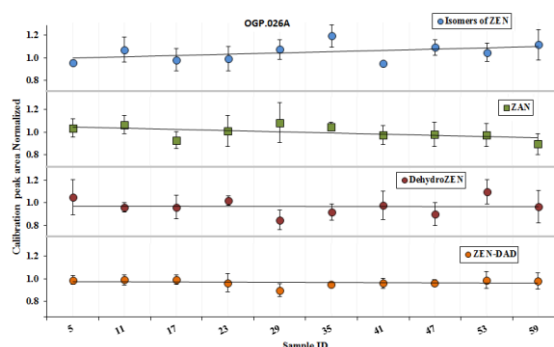
#### 3.2. Homogeneity Testing

No trends for the filling sequence were observed for ZEN at 235nm, 274 nm and 314 nm for UV measurements (figure 2).

No trends for the filling sequence were observed for ZEN and its impurities on LC-MS/MS and LC-DAD methods (figure 3).



**Figure 2.** Homogeneity Testing by UV.

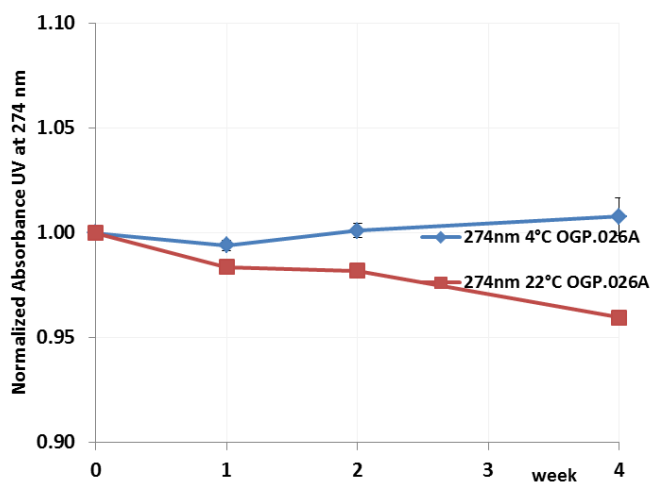


**Figure 3.** Homogeneity Testing by LC-MS/MS-DAD.

No trends for the analysis sequence were observed. No differences in the within- and between-sample variances could be detected by the F-tests at the 95 % confidence level for Zearalenone (main compound/ZEN-DAD) and its three impurities (dehydroZEN, ZAN and isomerZEN). The material could be regarded as homogeneous. The homogeneity uncertainty for ZEN by UV is 0.16% (274 nm) and 0.27% by LC-DAD. The minimum sample intake is 30  $\mu\text{L}$  for UV and 10  $\mu\text{L}$  for LC-DAD.

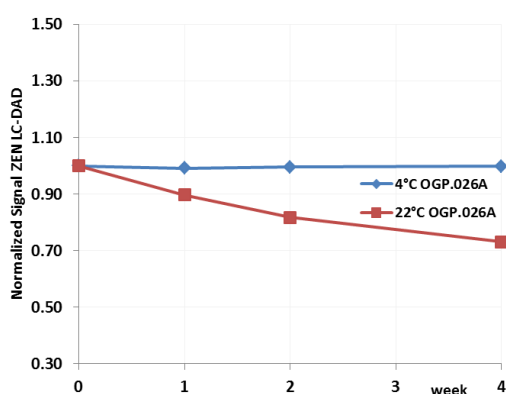
#### 3.3. Stability Testing

A significant negative trend at 22°C with light exposure was observed for all wavelengths (235nm, 274 nm and 314 nm) by UV method. The wavelength 274 nm was chosen as it is the most specific wavelength (figure 4).

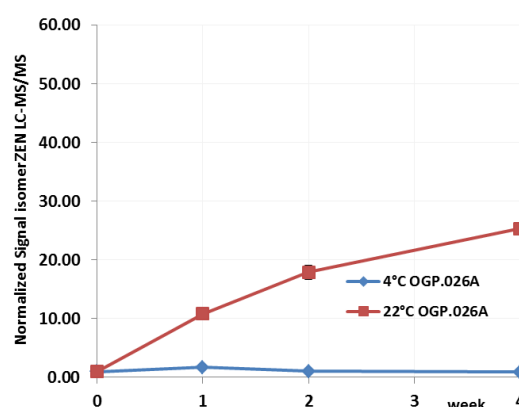


**Figure 4.** Stability Testing by UV.

A significant decrease of ZEN by LC-DAD and a significant increase of the IsomerZEN impurity were observed at 22 °C with light exposure (figures 5 and 6). No trends could be identified for 4 °C and 22 °C (with light) for the 7'DehydroZEN and ZAN impurities.



**Figure 5.** ZEN degradation at 22 °C with light.



**Figure 6.** Increase of the IsomerZEN impurity during stability study at 22 °C with light.

It was concluded that 22 °C and light exposure conditions should be avoided during shipment and storage of this material. The batch showed stability at 4 °C without light exposure for ZEN and its impurities.

### 3.4. Value Assignment

The value assignment for ZEN studied by two methods (UV and LC-DAD), and the analytical values agreed with gravimetric value (table 1).

**Table 1.** Value assignment of ZEN calibration solution OGP.026A.

|                           | Gravimetric | UV - using standard | UV – using molar absorptivity | LC-DAD |
|---------------------------|-------------|---------------------|-------------------------------|--------|
| Value ( $\mu\text{g/g}$ ) | 14.708      | 14.461              | 15.156                        | 14.645 |
| U ( $k=2$ )               | 0.081*      | 0.50                | 1.5                           | 0.13   |

\*Including homogeneity ( $u_{bb}$ ) from LC-DAD

There are no significant differences between the gravimetric value and each analytical result, using normalized error ( $E_n \leq 1$ ).

#### 4. Conclusion

One batch of ZEN calibration solution in acetonitrile was successfully produced and studied.

The batch OGP.026A was submitted to homogeneity study and showed sufficient homogeneity, with  $u_{bb}$  less than 0.27% for ZEN. No evidence of statistically significant trend was observed for filling sequence. The stability study showed that 22 °C and light exposure conditions should be avoided during shipment and storage due to a degradation of ZEN in these conditions. The ZEN showed stability at 4 °C without light exposure during 4 weeks (conditions for transportation of the batch). The mass fraction of zearalenone in OGP26A obtained gravimetrically ( $14.708 \pm 0.016 \mu\text{g/g}$ ,  $k=2$ ) was checked by two methods during value assignment study (UV and LC-DAD) and the analytical values agreed with gravimetric value.

This work was successful and the knowledge acquired at the BIPM will be implemented at Brazil to improve the production of new calibration solutions CRMs and to develop capabilities in mycotoxin metrology.

#### Acknowledgments

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#### References

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- [2] BIPM website, CBKT project, <http://www.bipm.org/en/cbkt/safe-food.html>, accessed on June 2017
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