**APMP-APAC joint PT (APAC T112)**

Non-polar analytes in high carbohydrate food matrix:

trans-Zearalenone in Maize Powder

（Coordinated by National Institute of Metrology, China NIM）

Technical Protocol

**Introduction**

Zearalenone (ZEN) is a fungal mycotoxin produced by Fusarium spp. and present in several types of food, especially in maize and wheat. It is a non-steroid estrogenic compound which may cause changes in reproductive organs, fertility loss and several other toxic effects. Zearalenone analysis is a matter of health and food safety for many countries around the world, especially those with high production or consumption of corn grains and wheat. Worldwide regulatory limits of ZEN in maize and its products are from 20 μg/kg to 350 μg/kg.

Testing of the core competencies of laboratories that deliver measurement services of non-polarity analytes and low mass fraction of analyte (III >1µg/kg, <1mg/kg) in high carbohydrate food material has not been covered for many years. An agreement was reached at the OAWG meeting held in October 2019 in Italy to conduct the trans-zearalenone in maize as the next Track A matrix comparison. This comparison fits into the 10-year strategy for the CCQM OAWG Track A comparisons which covers a range of different types of food matrices which map against the different types of capabilities needed.

This APMP-APAC Joint Proficiency Testing Programme (Non-polar analytes in high carbohydrate food matrix: trans-Zearalenone in Maize Powder) is coordinated by the National Institute of Metrology, China (NIM). It will be run in parallel with key comparison CCQM-K168 of Bureau International des Poids et Mesures (BIPM), assisting in building the measurement capabilities of analytical field laboratories through better linkages between the NMIs/DIs worldwide and the PT participants.

**Study Material**

The matrix, maize, is a high carbohydrate and low protein, low fat matrix that falls within Sector 5 of the AOAC International food triangle. Maize material was screened from a local supermarket, and pulverized, sieved and homogenized at room temperature. The indicative range for the mass fractions of the trans-Zearalenone is 10-500 μg/kg. Aflatoxin B1, Ochratoxin A, and Fumonisin B1 etc. are also detected in the material as potential interferences. The homogenized maize powder was separately vacuum-packed in polyethylene bags to give about 500 packets, with content of 90 g each. Then, each packet was sealed in aluminum foil bag. Long term storage of the material at NIM is at about -20ºC.

**Measurand**

The measurand of this study is trans-zearalenone in maize powder. The measurand is trans-zearalenone (or E-Zearalenone) with information listed as follows.

**Table 1 Information of trans-zearalenone**

|  |  |  |
| --- | --- | --- |
| Name | Trans-Zearalenone (trans-ZEN) |  |
| CAS | 17924-92-4 |
| Molecular formula | C18H22O5 |
| *MW* | 318.36 |
| *pKow* | -3.83 |

**Methods**

The study will require extraction, clean-up, analytical separation, and selective detection of the analyte in maize. Participants are anticipated to perform measurements by liquid chromatography-mass spectrometry (LC-MS/MS), isotope-dilution liquid chromatography-mass spectrometry (LC-IDMS/MS), HPLC-FLD etc.

**Homogeneity**

All samples were kept at the storage condition of -20ºC by NIM. 19 packets of samples were taken randomly, and triplicate sub-samples (5.0g) were determined using LC-MS/MS method. One-way ANOVA with F-test in accordance with the requirements as stipulated in ISO Guide 35 [1] was used to test whether there were significant between-packet differences in the concentration of the measurand. The value of the relevant F-test ratios 1.06 is smaller than critical value 1.88 at 95% confidence level, which indicates that the inhomogeneity of the study material was insignificant.

**Stability**

The long-term and short-term stability testing of ZEN in the maize samples have been performed. Samples were stored at 60ºC for 0, 3, 7 and 14 days for the short-term stability with three packets being analyzed at each time point. This study was designed to test the material stability under transportation conditions. Similarly, five samples were selected randomly at the storage condition of -20ºC for testing at the 0, 1, 3, 6, 12, 18 months points for the long-term stability study. The trend-analysis technique proposed by ISO Guide 35[1] was applied to assess the stability. The effect of time on the stability was evaluated using a linear approximation model by fitting linear regression lines to the data set (Y=*β0*+*β1*X). The statistical results indicated that no significant trend at 95% confidence level was detected for long-term and short-term stability testing.

**Reference Standards Available**

Pure and Solution CRMs are available from NIM and NMIA, which were listed in Table 2. Isotope labeled (carbon-13) ZEN for use as internal standard is commercially available from LGC and ROMER listed in Table 3. The use of CRM and isotope labeled ZEN is not mandatory.

 **Table 2 List of ZEN pure and solution CRMs available as calibrants**

|  |  |  |  |
| --- | --- | --- | --- |
| **Item** | **Item No.** | **Value** | **producer** |
| **Zearalenone (Trans-)** | GBW10157 | (99.5±0.4)% | NIM |
| **Zearalenone (Trans-)** | NMIAP1787 | (99.9±0.3)% | NMIA |
| **Zearalenone in acetonitrile (Trans-)** | GBW10160 | 11.0 (1±0.02) μg/mL  | NIM |
| **Zearalenone in acetonitrile (Trans-)** | GBW10161 | 1.10 (1± 0.03) μg/mL  | NIM |
| **Zearalenone in acetonitrile (1 mL)** | CRM0005 | (126.4 ± 3.2) μg/g | NMISA |

**Table 3 List of isotopically labeled ZEN internal standards**

|  |  |  |  |
| --- | --- | --- | --- |
| **Producer** | **Isotope** | **Concentration** | **Item No.** |
| **Romer labs** | U-[13C18]-Zearalenone in acetonitrile | 25.1 μg/mL  | ILM009 |
| **LGC** | U-[13C18]-Zearalenone in acetonitrile | 25.2 μg/mL  | B-MYC0610 |

**Study Guidelines**

Each participant will receive 1 packet, each containing about 90 g of maize powder. A minimum sample intake of 5.0 g is recommended. The test sample should be stored at -20ºC in the dark. The samples should be equilibrated to room temperature (at 20 ± 5 °C and relative humidity <60 %) before opening for analysis. After taking sub-samples, users are reminded to seal the sachets and store them in a freezer at about -20 °C.

**Note：**In sunlight or ultraviolet light, some of the analyte can change from trans to cis isomer. Therefore, solutions containing the measurand should be placed in a brown container, and care should be taken to avoid sunlight or ultraviolet light.

**Reporting of Results**

At the time of sample dispatch, a sample receipt form will be provided electronically to all participants and must be filled in and returned to the study coordinator on receipt of the shipments. The results reporting form will be provided to each participant and must be completed and returned to the study coordinator before the submission deadline.

The results should be reported in the unit of µg/kg for trans-ZEN in maize on a dry mass basis and should include standard and expanded uncertainties (95 % level of confidence) for the mean of the replicate determinations (n=3). Results of all replicate determinations should be included in the Results Report Form. The analysis should be conducted with a recommended sample size of at least 5.0 g. Information on the measurement procedure (extraction, clean-up, column and conditions, quantification approach), calibration standards, internal standards, any quality control materials, number of replicates, the means of calculation of the results and the estimation of measurement uncertainty should be included.

Participants are required to carry out a dry mass correction. Dry mass correction should be carried out at the same time as the test sample portion is to be analyzed in the same package of sample. Due to the relatively high moisture content of maize powder, the moisture content should be measured using the following drying method. Take a flat glass container with cap, which has first been dried until no change in mass. A minimum of three subsamples (recommended sample size of 5.0 g each) of the maize powder should be put in the containers, spread flat to a thickness of not more than 10 mm, put it into the oven at 130 ℃, and support the cap obliquely on the side of the container. After drying for 4 h, take out the glassware out of the oven, cover it, place it in a desiccator with anhydrous calcium sulphate as the desiccant, cool it to room temperature, and weigh it. Moisture content of sample is calculated by weight reduction method, and the formula is as follows:

 $Mois.=\left（\frac{M\_{0}-M\_{1}}{M\_{0}}\right）\*100$

 *Mois., is the moisture content in sample, g/100g;*

 *M0, is the mass of sample before drying, g；*

 *M1， is the mass of sample after drying for 4 hours, g;*

**Evaluation of Results**

This program will demonstrate participants’ capabilities in determining low-polarity analytes (*pKow* < -2) with molecular mass range from 100 to 500 g/mol at mass fraction levels of 1 to 1000 µg/kg in a high carbohydrate food matrix. It is a paralleled study with the track A key comparison of CCQM-K168 (Non-polar analytes in high carbohydrate food matrix: trans-Zearalenone in Maize Powder). The key comparison reference value will be used as the assigned value for this proficiency test.

The performance of the participating laboratories will be assessed using z-score, which is calculated as follows [2]:

$$z\_{i}=\frac{x\_{i}-x\_{pt}}{σ\_{pt}}$$

Where

*xi* : the participant’s result

*xpt* : the assigned value\*

*σpt*: the standard deviation for proficiency assessment estimated from the Horwitz equation [2]

\* Note: The Key Comparison Reference Values (KCRVs) obtained from CCQM key comparison CCQM-K168 will be used as the assigned values for evaluating the performance of participants. This is in accordance with the ISO/IEC 17043 recommendations on the determination of assigned values for proficiency testing schemes [3].

z-score is commonly interpreted as:

1. |z| ≤ 2.0 Satisfactory
2. 2.0 < |z| < 3.0 Questionable
3. |z| ≥ 3.0 Unsatisfactory

Laboratories having a |z| score equal to or larger than 3.0 shall thoroughly investigate their results for the discrepancy and those having a z-score in the range 2.0 < |z| < 3.0 are also encouraged to review their results.

For reference purpose, the performance of the participating laboratories will be assessed using zeta-score (ζ), which is calculated as follows [2]:

$$ζ\_{i}=\frac{x\_{i}-x\_{pt}}{\sqrt{u\_{(x\_{i})}^{2}+u\_{(x\_{pt})}^{2}}}$$

Where

*xi*: the participant’s result

*xpt*: the assigned value (KCRV)

*u(xi)*: the participant’s own estimate of the standard uncertainty of its result *xi*.

*u(xpt)*: the standard uncertainty of the assigned value *xpt*

ζ-scores are interpreted as in the same way as z-scores using the same critical values of 2.0 and 3.0. ζ-scores may be used in conjunction with z-scores, as an aid for improving the performance of laboratories as follows. If a laboratory obtains |z| scores that exceed 3.0, they may find it of value to examine their test procedure step by step and derive an uncertainty budget for that procedure. The uncertainty budget will identify the steps in the procedure where the largest uncertainties arise, so that the laboratory can see where to expend effort to achieve an improvement. If their |ζ| scores also exceed the critical value of 3.0, it implies that their uncertainty budget does not include all significant sources of uncertainty. Laboratories are encouraged to review their uncertainty budget.

**Confidentiality**

The proficiency testing programme is conducted in the belief that participants will perform the analysis and report results with scientific rigour. Collusion and falsification of results are clearly against the spirit of the proficiency testing programme.

The concerned parties (APMP, APAC and NIM) strive to maintain strict confidentiality with respect to composition of the proficiency test samples distributed and the performance of all participating laboratories. To preserve the confidentiality, participants receive report(s) giving all results for assessment but without identifying individual laboratories.

In general, all information on participant performance shall not be disclosed to any third party unless prior agreement with the concerned participants has been obtained or applicable laws or regulations stipulate such disclosure. NIM, the proficiency testing provider for this proficiency testing programme, shall also take into consideration local regulatory requirements for the disclosure of confidential information. NIM may disclose any relevant information to China National Accreditation Service for accreditation purposes, with the consent/agreement obtained from participating laboratories through completion of the Registration Form/Sample Receipt Form / Result Proforma for this proficiency testing programme.

Invitation and selection of participants

APAC members and APMP Developing Economies’ Committee (APMP DEC) members will be invited to participate in this program. Once this proposal is approved by the APAC Proficiency Testing Committee, invitations will be sent to all APAC members through their accreditation bodies, and to APMP DEC members by APMP DEC Chairs.

Total number of participants for this Joint PT programme will be 65.

Laboratories nominated by the APMP DEC are about 15.

Laboratories nominated by APAC accreditation bodies and non-APAC accreditation bodies are about 50.

Participants should investigate the particular local customs and quarantine requirements for the samples to be sent to their countries. If special permits are required, please inform the organizer. The organizer will not be responsible for any charges such as import taxes or related charges for the importation of the samples.

**Proposed study schedule**

* + Call for participants：1 May, 2021
	+ Deadline for registration：31 May, 2021
	+ Distribution of samples：1 July, 2021
	+ Submission of results：1 September, 2021
	+ Release of the draft final report for comment：2022

**Contact information**

For enquiries, participants may wish to make contacts as follows:

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Ms. Jiao Hui, NIM, jiaohui@nim.ac.cn

References:

[1] ISO Guide 35:2017, Reference materials — Guidance for characterization and assessment of homogeneity and stability. International Standards Organization, Geneva, Switzerland, 2017.

[2] ISO 13528:2015, Statistical methods for use in proficiency testing by interlaboratory comparison. International Standards Organization, Geneva, Switzerland, 2015.

[3] ISO/IEC 17043:2010, Conformity assessment — General requirements for proficiency testing. International Standards Organization, Geneva, Switzerland, 2010.