APMP-APLAC joint PT (APLAC T108)

Benzo[a]pyrene in Olive Oil

（Coordinated by National Institute of Metrology, China NIM）

Technical Protocol

Introduction

Benzo[a]pyrene (BaP) is a category of polycyclic aromatic hydrocarbon (PAH) that is toxic and carcinogenic to humans. It also has some mutagenic properties as described by the World Health Organization, which makes its presence in food a health concern. BaP is one of the markers for the occurrence of PAHs in foods, for which maximum residue limits are enforced in many countries. Edible oil and fats are the main source of human PAH intake. BaP may form in edible oils by pyrolytic processes, such as incomplete combustion of organic substances. Worldwide regulatory limits of BaP in edible fats and oils are from 2.0 μg/kg to 10 μg/kg. This project aims to improve member economies' PAH in oil sample measurement capabilities, support the mutual recognition of measurements and enhance international partnerships for the benefits trade in the APEC region.

This APMP-APLAC Joint Proficiency Testing Programme (Benzo[a]pyrene in Olive Oil) is coordinated by the National Institute of Metrology, China (NIM). It will be run in parallel with key comparison CCQM-K146, assists in building the measurement capabilities of analytical field laboratories through better linkages between the NMIs/DIs worldwide and the PT participants.

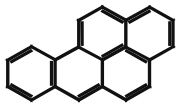
Study Material

The matrix, olive oil, is a high fat and low protein, low carbohydrate matrix that falls within Sector 1 of the AOAC International food triangle. Olive oil was purchased from a local supermarket, spiked with BaP, and homogenized by vibro-mixing at room temperature for 24 hours. The indicative range for the mass fractions of the analyte is 0.1-100 μg/kg. Benzo(a)anthracene, benzo(b)fluoranthene, and chrysene are also spiked into the material as potential as interferences. The homogenized oil was separately dispensed into aluminum bottles under nitrogen atmosphere to give about 500 bottles, with content of 30 g each. Packing was in vacuum-sealed- aluminium foil bags. Long term storage of the material at NIM is at about 25ºC.

Measurand

The measurand of this study is benzo(a)pyrene in olive oil.

Table 1 Information of BaP

Benzo(a)pyrene	
CAS	50-32-8
Molecular formula	$C_{20}H_{12}$
M_W	252.31
pK_{ow}	-6.35
Structure	
Mass fractions	0.1-100 μg/kg

Methods

The study will require extraction, clean-up, analytical separation, and selective detection of the analyte in olive oil. Participants are anticipated to perform measurements by isotope-dilution gas chromatography-mass spectrometry (IDGC-MS); however, other techniques such as liquid chromatography (LC) may be used.

Homogeneity

All samples were kept at the storage condition of 25ºC by NIM. 15 bottles of samples were taken randomly, and analysis of triplicate sub-samples was carried out using an ID-GC-MS/MS method and results indicated that the inhomogeneity of the study material was insignificant.

Stability

The long-term and short-term stability testing of BaP in the olive oil samples have been performed. Samples were stored at 50ºC for 0, 5, 8, 12, 20 and 30 days for the short-term stability with two bottles being analyzed at each time point. This study was designed to test the material stability under transportation conditions. Similarly, duplicate samples were selected randomly at the storage condition of 25ºC for testing at the 0, 1, 3, 6, 12, 16 month time points for the long-term stability study. Duplicate sub-samples were taken from each bottle and analyzed using the ID-GC-MS/MS method. The trend-analysis technique proposed by ISO Guide 35 was applied to assess the stability. The statistical results indicated that no significant trend at 95% confidence level was detected. Hence, the instability of the material was insignificant at the study temperature over the study period.

Reference Standards Available

Solution CRMs are available from NIM (GBW(E)080476 benzo[a]pyrene in methanol), NIST (SRM 2260a PAHs in Dichloromethane) and SRM 1647f (Priority Pollutant PAHs in Acetonitrile), and NMIJ (CRM 4313 benzo[a]pyrene in 2,2,4-trimethylpentane). Isotopically-

labeled (deuterium or carbon-13) BaP for use as internal standard is commercially available from a number of sources.

Study Guidelines

Each participant will receive 1 bottle, each containing 30 g of olive oil. The bottle is intended for both method development and for determination of the final results. Samples can be stored at room temperature. A minimum sample intake of 0.5 g is recommended.
International standard for the determination of BaP in oil are listed in table 2 for reference. Participants may use their preferred laboratory procedures. Participating laboratories should use their accredited test methods (or if not accredited their normal test methods) which are normally used to test their customer samples. Participants are requested to perform at least three independent measurements of the sample and to determine the mass fractions of the analyte.

Table 2 International standards for the determination of BaP in Oil

Number	Title
ISO 15302:2017	Animal and vegetable fats and oils -- Determination of benzo[a]pyrene -- Reverse-phase high performance liquid chromatography method

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^{-1} for BaP and should include standard and expanded uncertainties (95 % level of confidence) for the mean of the replicate determinations. Information on the measurement procedure (extraction, clean-up, column and conditions, quantification approach), the calibration standards, the internal standard, any quality control materials, number of replicates, the calculation of the results and the estimation of measurement uncertainty should be included.

Evaluation of Results

This program will demonstrate participant's capabilities in determining the mass fraction of low-polarity analytes (pKow < -2) with molecular mass range from 100 to 500 g mol^{-1} at levels of 0.1 to 100 μg/kg in a high fat, low protein, low carbohydrate food matrix. It is a paralleled study with the track A key comparison of CCQM-K146 (Low-Polarity Analyte in high fat food: Benzo[a]pyrene in Olive Oil). 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 [1,2]:

$$z_i = \frac{x_i - x_{pt}}{\sigma_{pt}}$$

Where x_i : the participant's result
x_{pt} : the assigned value*
σ_{pt} : the standard deviation for proficiency assessment estimated from the Horwitz equation [2, 3]

* Note: The Key Comparison Reference Values (KCRVs) obtained from CCQM key comparison CCQM-K146 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:

(i)	$\|z\| \leqslant 2.0$	Satisfactory
(ii)	$2.0 < \|z\| < 3.0$	Questionable
(iii)	$\|z\| \geqslant 3.0$	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 [4]:

$$\zeta_i = \frac{x_i - x_{pt}}{\sqrt{u_{(x_i)}^2 + u_{(x_{pt})}^2}}$$

Where x_i:the participant's result
x_{pt}:the assigned value (KCRV)
$u_{(xi)}$:the participant's own estimate of the standard uncertainty of its result x_i.
$u_{(xpt)}$:the standard uncertainty of the assigned value x_{pt}

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 [4]. 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, APLAC 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

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

Total number of participants for this Joint PT programme will be 100.
Laboratories nominated by the APMP DEC are about 15.
Laboratories nominated by APLAC accreditation bodies and non-APLAC accreditation bodies are about 85.

Proposed study schedule

- Call for participants and deadline for registration Nov. 2017
- Distribution of samples Nov.- Dec. 2017
- Submission of results March 2018
- Release of the draft final report for comment 2018

Contact information

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

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