



APMP-APAC Joint Proficiency Testing Programme
(APAC T113)
Benzoic Acid in Fish Sauce



**Asia Pacific Metrology Program (APMP) -
Asia Pacific Accreditation Cooperation (APAC)
Joint Proficiency Testing Programme
<Study No. T113 >**

Benzoic Acid in Fish Sauce

Protocol

Coordinated by
Government Laboratory of Hong Kong, China (GLHK)

August 2021



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1. Introduction

Fish sauce is widely used as a liquid condiment or seasoning, it is made by the fermentation of fish or krill from months to years. Many cultures use fermented fish sauce in cooking, and fish sauce is particularly popular in some parts of Europe, China, Indonesia, Laos, Malaysia, Philippines, Thailand, and Vietnam. The safe use of preservatives in fish sauce is thus of growing concern in the Asia Pacific region. Commercial fish sauces might contain different levels of preservatives. Benzoic acid is one of the preservatives commonly found in condiments and beverages for preventing and inhibiting the growth of yeasts and moulds in food. According to the assessment by WHO [1], the acute toxicity of benzoic acid is low. However, it is known to cause non-immunological contact reactions (pseudo allergy) in sensitive person when lower dose of benzoic acid is consumed [1]. For the general population, the main route of exposure to benzoic acid is likely via foodstuffs, which contain the substances naturally or added as antimicrobial agents. Food safety regulations have been set by various jurisdictions for limiting the amount of benzoic acid in sauces, seasonings and condiments. The Codex Alimentarius Commission (CAC) sets maximum levels of food additives in food and feed to protect public health [2]. In the case of benzoic acid, the maximum level in sauces and like products – clear sauces (e.g. fish sauce) is set at 1000 mg/kg. The European Union has also set maximum levels for preservatives in food, where the maximum level of benzoic acid is set at 1000 mg/kg in seasonings and condiments [3].

With the aim of enhancing the quality and traceability of measurements in various economies of the Asia-Pacific region through a better scientific infrastructure, the Asia-Pacific Metrology Programme (APMP) and the Asia Pacific Laboratory Accreditation Cooperation (APLAC) agreed to strengthen cooperation. At the APMP General Assembly and Related Meetings held in Taipei in November 2013, the APMP-APLAC Joint Proficiency Testing Working Group (PTWG) was established as an entity to provide proficiency testing programmes with metrologically traceable reference values for performance evaluation purpose.

To echo this new initiative, the Government Laboratory of Hong Kong, China (GLHK) first proposed a new joint PT study on the determination of benzoic acid in fish sauce at the 2018 meeting of the Joint PT Working Group that supported the intent of carrying out a joint PT programme on the measurement of benzoic acid in



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fish sauce. This joint PT programme will be organised by GLHK under the auspices of the APMP-APAC Joint PTWG ^{Note 1}. The purpose of this study is to demonstrate the capability of participating laboratories in measuring benzoic acid in aqueous-based condiments such as fish sauce.

The reference value for benzoic acid provided by GLHK will be used as the assigned value for evaluating the performance of the participants in this PT programme. The relevant Calibration and Measurement Capabilities (CMCs) of GLHK on benzoic acid is registered in the BIPM Key Comparison Database (KCDB). The use of reference values traceable to the International System of Units (SI) as the PT assigned value for this PT programme will allow more rigorous evaluation of the accuracy of participants' results. It will not only enhance the quality of the study but also help build the measurement capabilities of the participants through a better linkage between the metrology institutes and the analytical laboratories at large.

2. Objectives

The purposes of the study are (i) to enable participating laboratories to demonstrate their capabilities on the measurement of the mass fractions of the benzoic acid at mg/kg levels in aqueous-based condiments (e.g. fish sauce); and (ii) to identify opportunities for further improvement. Mass fraction of analyte as received will be used for comparability purposes.

3. Organisers of the joint PT Programme

GLHK (Address: 7/F, Homantin Government Offices, 88 Chung Hau Street, Homantin, Kowloon, Hong Kong, China) is the Designated Institute (DI), in the field of metrology in chemistry for Hong Kong, China, under the Mutual Recognition Arrangement of the International Committee for Weights and Measures (CIPM MRA). GLHK, which will follow ISO/IEC 17043:2010 [4], is the provider of this proficiency testing programme and takes responsibility for all tasks in the development and operation of the proficiency testing programme, including preparation and distribution

Note 1: The Asia Pacific Accreditation Cooperation (APAC) was established on 1 January 2019 by the amalgamation of two former regional accreditation cooperations – the Asia Pacific Laboratory accreditation Cooperation (APLAC) and the Pacific Accreditation Cooperation (PAC).



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of proficiency test samples, data analysis and evaluation of results, preparation of interim and final reports, and communications with participants.

4. Fee for participation

Free of charge.

5. Call for participation

Due to the limited number of proficiency test samples available, the total number of participants for the PT programme will be restricted to 100. Invitations will be sent to institutes/laboratories through the APMP network. The number of laboratories shall be preferably limited to 20. Invitations will also be sent to APAC accreditation bodies and non-APAC accreditation bodies. Participating accreditation bodies will be asked to nominate laboratories for participation and to indicate the accreditation status of the nominated laboratories for the test. The number of laboratories shall be preferably limited to 80. APAC members are invited to nominate up to a maximum of 4 laboratories/member body. Non-APAC members are invited to nominate up to a maximum of 2 laboratories/member body. When enrolment exceeds the limit, the number of participating laboratories from each accreditation body may be reduced upon discussion with APMP and APAC.

The participation will be confirmed within one week after the deadline of the nomination with the assigned laboratory codes.

6. Test sample

GLHK followed the procedures for preparation of proficiency test samples for the previous PT programmes. A batch of about 20 litres of fish sauce was purchased from the local market. Preliminary analysis of the fish sauce has been conducted to confirm that the level of the target analyte is fit for the intended purpose. The fish sauce was thoroughly homogenised in a mixer for 2 hours. The material was then be packed into pre-cleaned and nitrogen-flushed amber glass bottles, each of about 80 mL. Finally, each bottle was vacuum-sealed in a polypropylene bag. All finally packaged samples are stored at about 4 °C prior to distribution or use.

The homogeneity and stability studies of the proficiency test samples are performed in accordance with ISO13528:2015 [5] and carried out using isotopic



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dilution liquid chromatography tandem mass spectrometry (ID-LC-MS/MS) method. The results of homogeneity and stability studies will be checked and confirmed to be satisfactory before distribution of proficiency test samples.

7. Reporting and submission of results

Participants should complete the Result Report. The instructions on the manner of reporting test results are as follows:

- Result: the mean value of at least three independent measurements, the expanded uncertainty of the mean value and the coverage factor (which gives a level of confidence of approximately 95 %)
- Units of measurement: the mass fraction of the analyte and associated uncertainty in mg/kg;
- Number of significant figures: report the test results to 3 significant figures;
- Report of test results: “as received” basis; and
- Test methods: participants should provide information on the methods of analysis.

Participants should be aware that any submitted results are considered final, and such results and units should be thoroughly checked before submission. Participants should submit the Result Report electronically to the coordinator of the proficiency testing programme on or before the deadline. Results submitted after the deadline will not be accepted. Participants are reminded that the ability to report results in the specified unit and within the given time scale are part of the proficiency test.

8. Measurement uncertainty

Measurement uncertainty is best estimated within the individual laboratory environment. An estimate of measurement uncertainty is normally based on the combination of a number of influencing parameters (components of uncertainty). As stipulated in ISO Guide to the Expression of Uncertainty in Measurement [6], the uncertainty of each individual parameter should be quantified and expressed numerically as a standard uncertainty. These values are then combined according to the rules of the propagation of uncertainty and the combined standard uncertainty is multiplied by a coverage factor to produce an expanded uncertainty at the 95 % level of confidence.



9. Evaluation of performance of participants

The performance of the participating laboratories will be assessed using z-score, which is calculated as follows [4][5]:

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

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 [$\sigma_{pt} = 0.02c^{0.8495}$, where c is the assigned value of the analyte expressed as a dimensionless mass ratio (e.g. 1 mg/kg = 1 ppm = 10^{-6})] [7]

* Note: The reference value determined by GLHK will be used as the assigned value which is based on the relevant CMCs claims of GLHK as listed in the Key Comparison Database (KCDB). This is in accordance with the ISO/IEC 17043 recommendations on the determination of assigned values for proficiency testing schemes [4].

z-Score is commonly interpreted as:

- | | | |
|-------|-------------------|----------------|
| (i) | $ z \leq 2.0$ | Satisfactory |
| (ii) | $2.0 < z < 3.0$ | Questionable |
| (iii) | $ z \geq 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 [5]:

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



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where

x_i	:	the participant's result
x_{pt}	:	the assigned value
$u(x_i)$:	the participant's own estimate of the standard uncertainty of its result x_i .
$u(x_{pt})$:	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 $|\zeta|$ scores also exceed the critical value of 3.0, it implies that their uncertainty budget does not include all significant sources of uncertainty [5]. Laboratories are encouraged to review their uncertainty budget.

10. Issue of reports

An interim report will be issued to the participants for checking of transcription errors made by the PT provider. The draft final report will be submitted to the APMP-APAC Joint PTWG for comments. The revised version will then be sent to the APAC PT Subcommittee for further comments and approval. An electronic copy of the final report will be distributed to the participants and their respective accreditation bodies.



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11. Programme schedule (proposed)

The proposed time schedule for the various phases of the proficiency testing programme is as follows:

Time schedule	Phase
August 2021	Call for Participation
30 September 2021	Deadline for Registration
October 2021	Distribution of samples
28 February 2022	Deadline for submission of results
April 2022	Issue of the interim report
July 2022	Issue of the final report

12. Confidentiality

The concerned parties (APMP, APAC and GLHK) strive to maintain strict confidentiality of the characteristic properties of the proficiency test samples distributed and the performance of all participating laboratories. To preserve the confidentiality, participants receive reports giving all results for assessment but without identifying individual laboratories. The code number assigned to a participating laboratory in the proficiency testing programme will be provided only to the contact person/authorized person of the participating laboratory and/or the respective accreditation body.

13. Contact

For more information on the proficiency testing programme, please contact the coordinator by e-mail (E-mail: apac.fishsaUCE@govtlab.gov.hk).

If you have any query or comment on the protocol, please send it to the following contact point:

Dr. Wai-hong FUNG (Email: whfung@govtlab.gov.hk)

Dr. Jasmine Po-kwan LAU (Email: pklau@govtlab.gov.hk)



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14. References

- [1] WHO, Concise International Chemical Assessment Document 26 “Benzoic acid and sodium benzoate”, 2000, World Health Organization.
- [2] CODEX STAN 192-1995 “General standard for food additives”, 2019, CODEX Alimentarius.
- [3] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives, updated on 25 Mar 2020.
- [4] ISO/IEC 17043:2010 “Conformity assessment – General requirements for proficiency testing”, 2010, Geneva, Switzerland.
- [5] ISO 13528:2015 “Statistical methods for use in proficiency testing by interlaboratory comparison”, 2015, Geneva, Switzerland.
- [6] ISO/IEC Guide 98-3:2008 “Uncertainty of measurement -- Part 3: Guide to the expression of uncertainty in measurement (GUM:1995)”, 2008, Geneva, Switzerland.
- [7] W. Horwitz, *Anal. Chem.* 1982, **54**, 67A-76A “Evaluation of analytical methods used for regulations of food and drugs”.