



## **PROPOSAL**

### **Asia Pacific Laboratory Accreditation Cooperation (APLAC) Proficiency Testing Programme (APLAC PT T103)**

#### **Determination of Acesulfame Potassium and Sucralose in Cake Mix Flour**

Coordinated by

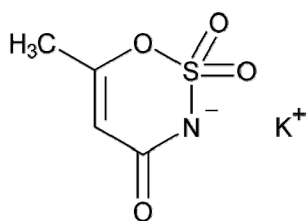
Health Sciences Authority  
&  
Singapore Accreditation Council

Singapore

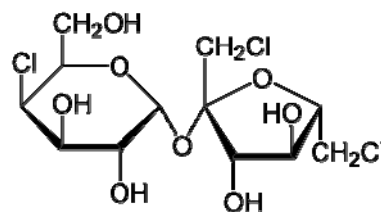
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## 1. BACKGROUND

Artificial sweeteners are extensively used to substitute sugar as excessive consumption of sugar would lead to obesity and the possible development of other health conditions. However, animal studies have shown that artificial sweeteners can also cause headaches, respiratory difficulties, seizures, bladder cancer and other health hazards [1-3]. Hence, regulatory controls on the maximum amount of some artificial sweeteners in food are put in place and their concentration levels are routinely analysed in food testing laboratories worldwide. Acesulfame potassium and sucralose are two such commonly used artificial sweeteners in confectionary products, e.g. cake mixes.



Acesulfame potassium



Sucralose

Commercial Proficiency Testing (PT) programmes for the determination of artificial sweeteners are available. The majority of such programmes make use of consensus results to evaluate the performance of the participating laboratories. In this proposed PT programme, assigned values which are traceable to the International System of Units (SI) will be used to evaluate the performance of the participating laboratories. These assigned values will be determined by the Chemical Metrology Laboratory (CML) of the Health Sciences Authority (HSA)<sup>1</sup> using high accuracy isotope dilution mass spectrometry (IDMS). In addition, pure substance certified reference materials (CRMs) of acesulfame potassium and sucralose will be provided to the participating laboratories for use as calibrants. The proposed PT programme would thus enable participating laboratories to compare their results against metrologically traceable assigned values.

<sup>1</sup> HSA is a Designated Institute for chemical metrology in Singapore. One of its designated areas covers food safety. CML HSA is accredited by the Singapore Accreditation Council (SAC) as a Proficiency Testing Provider since August 2013, in accordance with the requirements of ISO/IEC 17043:2010.

The development and operations of this proposed PT programme are carried out following the requirements of ISO/IEC 17043:2010 [4].

## **2. OBJECTIVES**

The PT programme focuses on the determination of two commonly used artificial sweeteners, acesulfame potassium and sucralose, in a cake mix flour sample. The objectives of the programme are to:

- (a) enable participating laboratories to compare their results against metrologically traceable assigned values;
- (b) enable participating laboratories to demonstrate their competence on the analysis of artificial sweeteners in a cake mix flour by various analytical techniques; and
- (c) identify problems and opportunities for further improvement.

## **3. COORDINATING INSTITUTES AND RESPONSIBILITIES**

The Singapore Accreditation Council (SAC) is the proposer of this APLAC PT programme and is responsible for submitting the proposal for approval by the APLAC PT Committee. It acts as the contact point for APLAC, HSA CML, the Accreditation Bodies (ABs) and the participating laboratories. In addition, SAC also takes on other logistic responsibilities such as sending invitations to ABs, assigning confidential laboratory codes, dispatching PT samples to ABs, receiving results, as well as preparing, issuing and sending the Reports.

HSA CML is responsible for preparing and packaging the PT samples, performing homogeneity and stability tests on the samples, determining the assigned values and the associated measurement uncertainties, compiling and conducting statistical evaluation on the participating laboratories' results, as well as preparing and issuing the Reports.

The ABs of the respective economies are responsible for nominating the laboratories and dispatching the PT samples to them.

#### **4. CONTACT DETAILS OF COORDINATOR**

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#### **5. APPLICATION FEE**

There is no participation fee for this proposed PT programme for the participating laboratories nominated by the ABs.

#### **6. SELECTION OF PARTICIPATING LABORATORIES**

APLAC members, as well as non-APLAC members, will be invited to participate in this PT programme. Each AB who is an APLAC member may nominate a maximum of two laboratories to participate in the programme. An AB who is a non-APLAC member can nominate a maximum of one laboratory to participate in the programme. SAC will accept the nomination forms from the accreditation bodies until the end of the nomination period. However, due to the limited number of PT samples available, the total number of participating laboratories for this PT programme will be restricted to about 80.

SAC will notify the ABs and the laboratories on the acceptance of their participation in the PT programme after the end of the nomination period. SAC will, as far as possible, accept at least one laboratory from each AB to participate in the PT programme. Priority will be given to accredited laboratories. The participating laboratories will then be selected on a first-come, first-served basis. A Registration Form For Participating Laboratory will be sent to the laboratories for completion, and must be returned to SAC before the deadline for registration. The participating laboratories are allowed to register for one or both of the analytes, depending on their services.

## 7. DOCUMENTS FOR PT PROGRAMME

The participating ABs will receive the following documents from SAC at various stages of the PT programme:

<b>Stage</b>	<b>Documents</b>
Call for nomination	Invitation letter to AB
	Nomination Form For Accreditation Body
	Instructions For Accreditation Bodies
	Instructions For Participating Laboratories
	Result Proforma
Acceptance of nomination	Registration Form For Participating Laboratory
Acknowledgement of registration	Acknowledgement letter to AB and participating laboratory (bearing confidential laboratory code assigned to the participating laboratory)
Sample pre-distribution	Instructions For Accreditation Bodies
	Instructions For Participating Laboratories
	Sample Receipt Form For Accreditation Body
	Sample Receipt Form For Participating Laboratory
	Result Proforma

## 8. PREPARATION OF PT SAMPLES

A suitable source of 'blank' cake mix flour was obtained from local supermarkets and screened for the presence of acesulfame potassium and sucralose using liquid chromatography coupled with mass spectrometry (LC-MS). The cake mix flour was fortified with the sweeteners to achieve the desired mass fraction values of the two analytes. The fortified cake mix flour was then adequately homogenised by mixing the flour in a drum mixer over 14 days before being distributed into polyethylene pouches under an inert atmosphere and controlled conditions (temperature and humidity). About 140 packets of the PT samples were prepared and stored between 18 °C and 25 °C. The mass fraction ranges of acesulfame potassium and sucralose are expected to be 500 – 1400 mg/kg and 500 – 1200 mg/kg, respectively.

## 9. HOMOGENEITY STUDIES

The homogeneity of the PT samples was established using LC-MS following the guidelines in ISO 13528:2005 [5]. Homogeneity testing was performed on 11 packets, with two subsamples taken from each packet. These 11 packets were selected using stratified random sampling approach. Each subsample with a minimum sampling size of 5 g was taken and analysed under repeatability conditions. The uncertainty due to between-packet inhomogeneity for acesulfame potassium and sucralose was 1.5 % and 1.1 %, respectively. The between-packet standard deviation was compared against 0.3\*standard deviation estimated from the Horwitz Equation [5-6]. The samples were found to be sufficiently homogeneous in accordance with the recommendations of ISO 13528:2005 [5].

## **10. STABILITY STUDIES**

The stability of the PT samples stored between 18 °C and 25 °C was established using LC-MS. The stability study was performed on three packets, with two subsamples (5 g each) taken from each packet. The difference in results after a storage period of about 3 months and homogeneity data was compared against 0.3\*standard deviation estimated from the Horwitz Equation [5-6]. The study showed that the samples were sufficiently stable. The stability of the PT samples under the storage condition will be re-established using LC-MS following the guidelines in ISO 13528:2005 [5] before dispatch and after dispatch.

The stability of the PT samples under the maximum allowable transportation condition of 40 °C for a period of 1 month was also established using LC-MS. The difference in results between samples not exposed to 40 °C and those exposed to 40 °C for 1 month was compared against 0.3\* standard deviation estimated from the Horwitz Equation [5-6]. The study showed that the samples were sufficiently stable.

## **11. ASSIGNED VALUES AND ASSOCIATED UNCERTAINTIES**

The assigned values of acesulfame potassium and sucralose in the PT samples will be determined by HSA CML using the IDMS method. The assigned values will be traceable to the SI through the use of CRMs of acesulfame potassium and sucralose from HSA and reference weights calibrated by the National Metrology Center (NMC), Agency for Science, Technology and Research (A\*STAR), Singapore. The associated uncertainties of the assigned values will be estimated to include all possible uncertainties in the characterisation, homogeneity and stability of the PT samples.

## **12. THE PT PACKAGE**

Each participating laboratory will be provided with one packet of the PT sample (about 50 g). In addition to the PT sample, pure substance CRMs of acesulfame potassium

and sucralose (about 250 mg each) from HSA will also be provided. The CRMs should be used as calibrants for the determination of the analytes in the cake mix flour. The purity value and associated expanded uncertainty of the pure substance CRMs have been established as shown below.

Pure substance CRM	CRM Code	Certified mass fraction value (mg/g)*	Expanded uncertainty (mg/g) at approx. 95% confidence level with coverage factor, k = 2)
Acesulfame potassium	HRM-1012A	999.2	5.0
Sucralose	HRM-1015A	985.4	4.8

\*A certified mass fraction value of 1,000 mg/g is equivalent to a purity value of 100 %.

### 13. TRANSPORT, HANDLING AND STORAGE

The PT packages will be delivered to the ABs under non-controlled temperature conditions. A temperature strip will be pasted on each package to enable the recipient to check if it has been exposed to a high temperature. The AB should then promptly acknowledge receipt of the PT packages to SAC by returning the Sample Receipt Form For Accreditation Body. The AB should not break the seal to remove any items from the PT package.

The PT samples and CRMs should be stored between 18 °C to 25 °C. The ABs should refer to the Instructions For Accreditation Bodies for advice on the appropriate handling and storage conditions for the PT packages in the interim if they are not immediately distributed to the nominated participating laboratories.

Upon receipt and checking of the condition of the PT packages, the participating laboratories should return the completed Sample Receipt Form For Participating Laboratory to SAC. The participating laboratories will be advised on the appropriate handling and storage conditions of the PT packages in the Instructions For Participating



Laboratories distributed to them prior to sample distribution. After opening the PT sample and CRMs, the polyethylene pouch and bottles should be resealed and recapped tightly.

#### **14. PARTICIPATING LABORATORIES' METHODS/PROCEDURES**

The participating laboratories are expected to apply the methods/procedures which they would normally use for routine analysis to determine the analytes in the PT samples. The participating laboratories will be requested to take a minimum of three subsamples from a minimum sample size of 5 g each for analysis. It is essential for the participation laboratories to use the CRMs provided as calibrants to ensure that their results are included for performance evaluation. The laboratories may re-analyse the PT sample using their own standards as calibrants. However, the results will only be used for comparison purposes and not for performance evaluation.

#### **15. REPORTING AND SUBMISSION OF RESULTS**

The participating laboratories are expected to report the mass fraction of acesulfame potassium and sucralose using a Result Proforma provided to them. All results should be reported in mg/kg.

The uncertainty of measurement should be reported with a confidence level of approximately 95 %. The expanded uncertainty is to be reported to 2 significant figures and the results rounded off to the same number of decimal place(s) as the expanded uncertainty.

The participating laboratories are required to provide technical details of their methodologies in the Result Proforma. The completed Result Proforma must be returned to SAC by the stipulated deadline. Results received after the reporting deadline will not be evaluated. Re-submission of the Result Proforma due to modification of any information will also not be accepted.

## 16. EVALUATION OF PERFORMANCE OF PARTICIPATING LABORATORIES

The performance of the participating laboratories will be primarily assessed using the z-score. For laboratories which report the uncertainties of their results, the  $\zeta$ -scores will also be calculated. The calculations of z-score and  $\zeta$ -score are as follows [5]:

(a) z-score: 
$$z = \frac{x - X}{\sigma_{PT}}$$

where, x is participating laboratory's result, X is the assigned value determined by HSA CML and  $\sigma_{PT}$  is the standard deviation for proficiency assessment.

A z-score with absolute value of:

- $|z| \leq 2.0$  is satisfactory
- $2.0 < |z| < 3.0$  is questionable
- $|z| \geq 3.0$  is unsatisfactory

(b)  $\zeta$ -score: 
$$\zeta = \frac{x - X}{\sqrt{u_x^2 + u_X^2}}$$

where,  $u_x$  is the standard uncertainty of the participating laboratory's result x and  $u_X$  is the standard uncertainty of the assigned value X determined by HSA CML.

A  $\zeta$ -score with absolute value of:

- $|\zeta| \leq 2.0$  is satisfactory
- $2.0 < |\zeta| < 3.0$  is questionable
- $|\zeta| \geq 3.0$  is unsatisfactory

The  $\zeta$ -score provides an indication of whether a participating laboratory's estimate of uncertainty is consistent with the observed deviation from the assigned value. It is complementary to the z-score in the assessment of laboratory performance.

Both z-score and  $\zeta$ -score for the reported result(s) obtained using the CRMs provided and the participating laboratories' own standards will be calculated. However, only the results obtained from the use of the CRMs provided will be used to evaluate the performance of the participating laboratories.

## 17. STANDARD DEVIATIONS FOR PROFICIENCY ASSESSMENT

The  $\sigma_{PT}$  for both analytes will be determined using the robust standard deviation of the results reported by all the participating laboratories, calculated using Algorithm A in ISO 13528:2005 [5].

## 18. SCHEDULE

Activity	Timeline	Responsibility
Preparation of PT samples	March 2015	HSA
Homogeneity testing on PT samples	April 2015	HSA
Submission of proposal to APLAC PT Committee for approval	June 2015	SAC
Stability testing on PT samples	May 2015 to July 2015	HSA
Update to APLAC PT TC Chair on stability on PT samples. Submission of finalised proposal, all relevant instructions and forms to APLAC PT TC Chair for approval.	July 2015	SAC
Call for nomination	August 2015	SAC
Deadline for submission of Nomination Form For Accreditation Body to SAC	October 2015	ABs
Distribution of Registration Form For Participating Laboratory to nominated laboratories	October 2015	SAC

Deadline for submission of Registration Form For Participating Laboratory to SAC	November 2015	Nominated laboratories
Distribution of Instructions For Accreditation Bodies, Sample Receipt Form For Accreditation Body and confidential lab codes of nominated laboratories to ABs.  Distribution of confidential lab codes, Instructions to Participating Laboratories, Sample Receipt Form For Participating Laboratory and Result Proforma to participating laboratories.	December 2015	SAC
Preparation of PT packages for ABs	January 2016	HSA
Dispatch of PT packages to ABs	January 2016	SAC
Dispatch of PT packages to participating laboratories	January 2016	ABs
Determination of assigned values for PT samples	January 2016 to April 2016	HSA
Collection of completed Result Proforma from participating laboratories	April 2016	SAC
Data analysis and performance evaluation	May 2016	HSA
Preparation of Interim Report	May 2016	HSA / SAC
Distribution of Interim Reports to ABs and participating laboratories, and collation of feedbacks/complaints/appeal	June 2016	SAC
Preparation of Final Report	July to August 2016	HSA / SAC
Submission of Final Report for approval by APLAC TC Committee	August to September 2016	SAC
Distribution of Final Report to ABs and participating laboratories	September to October 2016	SAC

## 19. CONFIDENTIALITY

Each participating laboratory will be assigned a unique and confidential laboratory code. Both the identity and the code will only be known to staff of SAC involved in the PT programme and the AB of the participating laboratory.

## 20. REFERENCES

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2. Chattopadhyay, S., Raychaudhuri, U., Chakraborty, R.; Artificial sweeteners – a review. *Journal of Food Science and Technology*, 2014, 51(4), 611-621.
3. Whitehouse, C.R., Boullata, J., McCauley, L.A.; The potential toxicity of artificial sweeteners. *Journal of the American Association of Occupational Health Nurses*, 2008, 56(6), 251-259.
4. ISO/IEC 17043: 2010 Conformity assessment – General requirements for proficiency testing, International Standards Organisation, Switzerland.
5. ISO 13528:2005 Statistical methods for use in proficiency testing by interlaboratory comparisons, International Standards Organisation, Switzerland.
6. Thompson M.; Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing. *Analyst*, 2000, 125, 385-386.