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Matrix reference materials development for food safety applications in Philippine products

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ABSTRACT
The Philippine Industrial Technology Development Institute (ITDI) – Department of Science and Technology pioneered the development of reference materials (RMs) as part of its vision to establish metrological traceability and to support the RM needs of chemical testing laboratories in the country. Food safety of Philippine products were prioritised in this activity as these could affect the health of consumers as well as trade issues. The focus of this study was on contaminants like histamine and benzoic acid in canned tuna and mango juice, respectively. The materials were prepared and characterised, and they were found to be homogeneous and stable for at least six months. Higher order methods like the isotope dilution technique, coupled with liquid chromatography–triple quadrupole mass spectrometry, were used to assign reference values to the RMs, and the results were cross-checked using gravimetric techniques for high-performance liquid chromatography analysis. Certified RMs purchased from metrology institutes were also used to further support traceability of measurements. These steps resulted in RMs for histamine and benzoic acid in Philippine products, and ITDI disseminated the measurement traceability to local laboratories through proficiency testing schemes organised and conducted for the analytes.

Section: RESEARCH PAPER

Keywords: Reference Material; Food Contaminant; Histamine; Benzoic Acid; Food Safety

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1. INTRODUCTION

The need to ensure comparable and traceable chemical measurements is important for a country engaging in global trade. This need requires the establishment of a national chemical measurement organisation tasked with improving the capability for accurate measurements throughout the country. Its sub-tasks could include the provision of certified reference materials (CRMs) and general reference materials (RMs), the conduct of proficiency testing (PT) schemes and the accreditation for ISO/IEC 17025. The organisation could guarantee the quality of chemical measurements performed within the country and increase the confidence in such measurements abroad.

As part of a vision to establish such an organisational infrastructure for chemical metrology in the Philippines, the Industrial Technology Development Institute (ITDI) – Department of Science and Technology (DOST) pioneered the development of RMs to support the needs of chemical testing laboratories in the country. Food safety issues were prioritised in this activity since food chemicals could affect the health of consumers and the level of some substances in food has been a reason for detention of some Philippine export products abroad.

This study focused on the development of RMs for measuring histamine in canned tuna and benzoic acid in mango juice. These two chemicals are vital criteria for export food products as other countries have based their import approval decisions on the levels of these chemicals. Further, the monitoring of these two chemicals are essential for quality control and food safety in general. CRMs for benzoic acid have been developed for orange juice [1], soy sauce [2] and quasi-drug drinks [3]; however, currently, there are no Philippine RMs or PT schemes available for benzoic acid in mango juice, nor are there RMs or PT schemes for histamine levels in canned tuna available. CRMs for histamine in fish products have not been reported. Included in this study are homogeneity and stability studies, RM characterisation, and the process for conducting PT schemes for these substances.

2. EXPERIMENTAL

2.1. Chemicals and Materials

The calibration standards for benzoic acid were SRM 350b Benzoic Acid, 99.9994% (National Institute of Standards and Technology (NIST, USA) and HRM-1002A Benzoic Acid (Health Science Authority (HSA), Singapore). The CRMs for benzoic acid were HRM-1001A Benzoic Acid in orange juice (HSA) and isotopically labelled analogue benzoic acid ring-D5 (Cambridge Isotope Laboratory, CIL). Mass spectrometer (MS)-grade solvents and high-performance liquid chromatography (HPLC)-grade solvents were used. For histamine, the calibration standards were histamine standard (Sigma Aldrich, Singapore) and histamine dihydrochloride (National Institute of Metrology China). Isotopically labelled analogue histamine dihydrochloride-D4 (CIL) was used as an internal standard. Brij-35, mercaptoethanol, o-phthaldialdehyde (OPA), and 1-octanesulfonic acid sodium salt - used in the HPLC analysis - were all obtained from Sigma Aldrich.

2.2. Measurement of benzoic acid and histamine by HPLC

For benzoic acid analysis, the HPLC (Shimadzu Prominence LC-20A with UV - VIS Detector, set at 234 nm) with Waters C18 Sunfire (3.5 µm, Ø 4.6 mm × 150 mm) column was used following a validated, in-house-developed method [4].

For histamine analysis, HPLC with Waters C18 Sunfire column and fluorescence detector (340 nm excitation and 445 nm emission) was utilised after post-column derivatisation with OPA [5].

2.3. Preparation of reference material

The RM for benzoic acid in mango juice was prepared using locally made commercial mango juice drinks, all manufactured on the same date. The juice was mixed, centrifuged, filtered, spiked with benzoic acid and pasteurised. The hot juice was poured into 100-mL amber bottles, which were then sealed, labelled, shrink wrapped and stored at 4 °C.

The RM for histamine was prepared from canned tuna which was homogenised until a paste-like consistency was obtained. The tuna sample was spiked with a set amount of histamine and it was thoroughly mixed. The spiked tuna sample (80 mg) was packed into a 100-ml amber bottle with screw cap. The packed samples were properly labelled and sterilised at 115.6 ºC for about 3 hours and stored at 4 °C after cooling.

2.4. Assessment of homogeneity and stability

The assessment of homogeneity was carried out after 10 bottles from the batch were selected by stratified random sampling. Two subsamples were taken from each bottle for measurement. Statistical evaluation was based on the IUPAC (International Union of Pure and Applied Chemistry) Harmonised Protocol [6], and the stability of RMs was also considered. A short-term stability test using isochronous design was carried out for three weeks at three temperatures: 4 °C, 30 °C and a simulated transport temperature of about 40 °C. Two randomly selected bottles were transferred from the control temperature to each temperature setting. After the study period, all bottles were collected and analysed under repeatability conditions. For long term stability, three bottles were analysed at designated intervals: one, three and six months. The statistical evaluation was based on ISO Guide 35 [7].

2.5. Value assignment of reference material

Liquid chromatography-isotope dilution mass spectrometry (LC-IDMS) with gravimetric sample preparation was used for value assignment of the prepared RMs. The mass fraction of the measurand was calculated based on the equation below [8]:

$$C = \frac{m_{\text{is-sp,sample}} \cdot m_{\text{std}} \cdot AR_{\text{sample}} \cdot C_{\text{stock,std}}}{m_{\text{sample}} \cdot AR_{\text{std}} \cdot m_{\text{is-sp,std}}} \quad (1)$$

where C is the concentration of the analyte in the sample (mg/kg), $m_{\text{is-sp, sample}}$ is the mass of isotopically labelled analyte solution spiked into the sample (g), m_{std} is the mass of the analyte standard solution added into the calibration blend solution (g), AR_{sample} is the observed response ratio of the of the analyte/isotope for the sample solution, $C_{\text{stock, std}}$ is the concentration of the analyte standard solution (mg/kg), m_{sample} is the mass of sample taken for analysis (g), AR_{std} is the observed response ratio of the of the analyte/isotope in the calibration blend solution and $M_{\text{is-sp, std}}$ is the mass of isotopically labelled analyte solution added into the calibration blend solution (g).

Liquid chromatography–triple quadrupole mass spectrometry (Agilent 1290 LC system) coupled to a 6460 LC/MS Triple Quadrupole (Agilent Technologies, Inc.) with Agilent Zorbax Eclipse Plus C18 column (3.5 µm, Ø 4.6 mm × 100 mm) were employed for the benzoic acid. The sample was diluted with a mobile phase solution after the addition of the benzoic acid ring-D5 solution. For histamine, the separation was achieved by using the Agilent Poroshell 120 HILIC column (2.7 µm, Ø 2.1 mm × 100 mm). Sample extraction was based on Agilent Application Note [9]. Histamine dihydrochloride-D4 was used as the internal standard.

3. RESULTS AND DISCUSSION

3.1. Method Validation

The HPLC and LC-IDMS methods for benzoic acid and histamine with gravimetric sample preparation were validated, and the results are tabulated in Table 1 and Table 2, respectively. The use of CRM HRM-1001A Benzoic Acid in Orange Juice for benzoic acid analysis established the metrological traceability to the International System of Units (SI). The results are within the criteria set by AOAC for repeatability, trueness (% recovery) and linearity for the concentration range of 5-100 mg/kg [10]. The LOD and LOQ obtained were lower than the ranges of sample.

Table 1. Summary of validation results obtained by HPLC method for the matrix RMs.

Parameter	Benzoic acid (n = 10)	Histamine (n = 10)
Repeatability, % RSD	1.08	2.1
Trueness, % recovery	99.3	101.6
Linearity, r	0.9999	0.9997
LOD, mg/kg	0.0161	0.0537
LOQ, mg/kg	0.0537	0.0503

Table 2. Summary of validation results obtained by LC-IDMS.

Parameter	Benzoic acid (n = 10)	Histamine (n = 10)
Repeatability, % RSD	1.21	1.35
Accuracy, % recovery	100.5	104.7
Linearity, r	0.997	0.997
LOD, mg/kg	0.0292	0.0014
LOQ, mg/kg	0.0974	0.0046

Table 3. Summary of statistical analysis for homogeneity of benzoic acid in mango juice.

Criteria	Critical Value	Obtained Result	Remarks
Cochran's Test, C	0.602	0.403	Passed
Test for analytical precision of the method	< 0.5	0.125	Passed
Test for sufficient homogeneity	417.75	0.437	Passed
Test for adequate homogeneity	14.25	0.661	Passed

Table 4. Summary of statistical analysis for homogeneity of histamine in canned tuna.

Criteria	Critical Value	Obtained Result	Remarks
Cochran's Test, C	0.602	0.356	Passed
Test for analytical precision of the method	< 0.5	0.473	Passed
Test for sufficient homogeneity	42.451	33.395	Passed
Test for adequate homogeneity	0.602	0.356	Passed

3.2. Homogeneity and Stability

The RMs prepared was found to be sufficiently homogeneous for benzoic acid and histamine studies. The results of the statistical evaluation for the homogeneity test of the benzoic acid and histamine RMs are shown in Table 3 and Table 4, respectively. Trend analyses for both RMs - used for short-term stability evaluation - confirms that no significant instability of the materials were observed during transport at 4 °C, 30 °C and 40 °C for a period of three weeks. The long-term storage of the RMs at 4 °C was determined to be about 6 months for benzoic acid and 12 months for histamine. Figure 1 shows the plot of the long-term stability test for histamine in canned tuna.

3.3. Reference value assignment

The reference values assigned for benzoic acid in mango juice and histamine in canned tuna listed in Table 5 are the mean values of duplicate measurements from the three bottles from the batch, analysed by the LC-IDMS method. As part of quality assurance, the acceptable percentage recovery of the CRM HRM-1001A and control materials was achieved for benzoic acid and histamine, respectively.

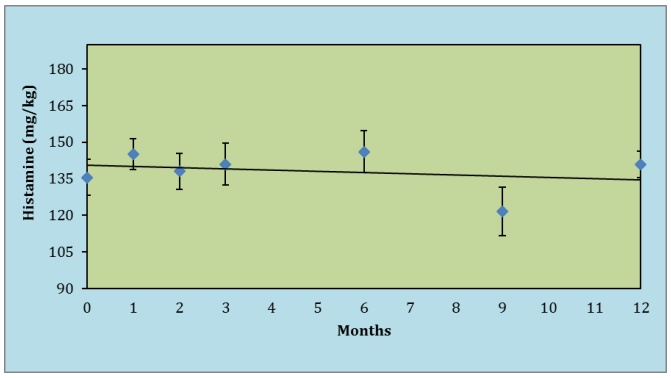

Figure 1. Long-term stability for histamine in canned tuna.

Table 5. Assigned value for the developed reference material.

Reference Material	Mass Fraction (mg/kg)		*n*
	Assigned Value	Uncertainty (*k* = 2)	
Benzoic acid in mango juice	774	42	6
Histamine in canned tuna	148	14	6

For benzoic acid, MS was operated in electrospray ionisation in negative mode. Benzoic acid and its isotope analogue were detected by selectively monitoring the collision-induced dissociation channels of $[M\text{-}H]^- \rightarrow [M\text{-}CO_2H]^-$ at m/z 121.1 → 77.2 and m/z 126.1 → 82.1, respectively. For histamine, MS was operated in electrospray ionisation in positive mode. Histamine and its isotope analogue were detected by selectively monitoring the collision-induced dissociation channels of $[M+H]^+ \rightarrow [M+H\text{-}NH_3]^+ \rightarrow [M+H\text{-}CH2CH2NH2]^+$ at m/z 112.1 → 95.1 → 68.2 and m/z 116.1 → 99.1.→ 85.2, respectively.

3.4. Measurement of uncertainty

The expanded uncertainties of the reference values of the RMs produced presented in Table 5 were estimated based on the ISO GUM approach [11]. The highest contribution to the expanded uncertainty of the reference values of RM benzoic acid (94.6%) was obtained from the peak area ratio of sample-isotope blend, while homogeneity gave the highest contribution (97.67%) for RM histamine in canned tuna. The remainder of the uncertainty sources included masses of samples, standards and solutions, and purity of the calibration standard.

3.5. Participation in International supplementary comparison

In 2014, in support of the reference value assignment capability, the Standards and Testing Division (STD) of ITDI - the designated institute for metrology in chemistry in the country - participated in a supplementary comparison, APMP.QM-S8: Determination of mass fraction of benzoic acid, methyl paraben and n-butyl paraben in soy sauce [2]. This was coordinated by the Health Sciences Authority of Singapore under the auspices of the Organic Analysis Working Group (OAWG) of the Comité Consultatif pour la Quantité de Matière (CCQM). In this comparison, ITDI-STD demonstrated its competence for benzoic acid analysis in soy sauce.

3.6. Conduct of Proficiency Test (PT) Scheme

The RMs developed in this study were also used for accuracy-based PT schemes, organised to assess the performance of local testing laboratories based on ISO/IEC 17043 [12]. The reference value obtained by a candidate higher order method was used as the assigned value for the PT scheme. The z-score was based on the reference values of the RMs.

Government and private testing laboratories, research institutes and the academe participated in the PT schemes by using routine methods of analysis. In the PT round for benzoic acid in mango juice (MiCPT-14-01), most of the participants used HPLC, as shown in Figure 2. Nine (82 %) out of 11 laboratories obtained satisfactory performance, and two (18 %) obtained unsatisfactory results. The unsatisfactory results were observed to be due to computation error and overestimation of results due to matrix effects.

Furthermore, two different analytical techniques based on fluorometry and ELISA were used by twelve participants for the PT round for histamine (MiCPT-14-04). However, only 50 % satisfactory performance was achieved. Results for this PT round

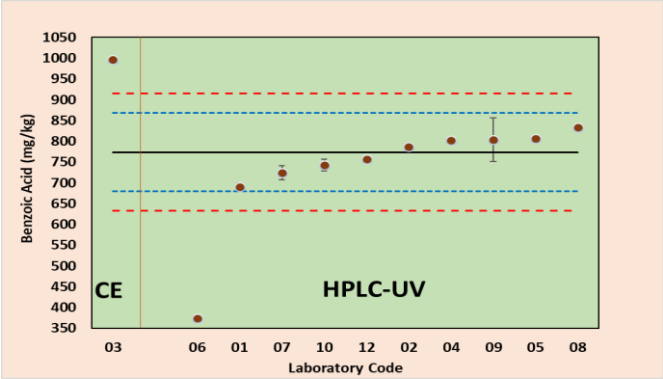

Figure 2. Participant results for MICPT-14-01: Benzoic acid in Mango Juice using capillary electrophoresis and high-performance liquid chromatography with UV detector.

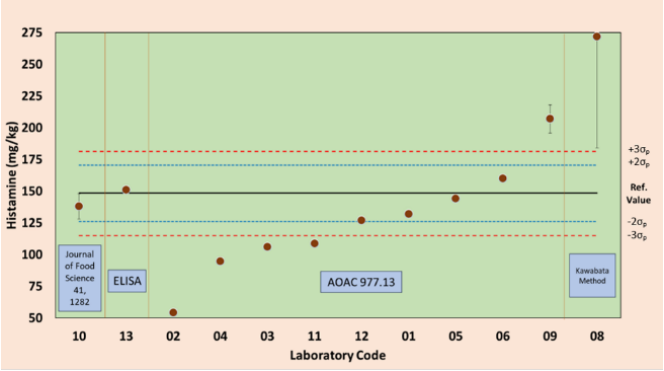

Figure 3. Participant results for MICPT-14-04: Histamine in canned tuna using fluorometer detection with different sample preparation.

are displayed in Figure 3, with reference to the sample preparation used prior to fluorometric detection. Under estimation of results were observed from four laboratories using the AOAC 977.13 method. The relative standard deviation of the participating laboratories with satisfactory performance is approximately 9%. Different parameters for the extraction techniques and the need for validation of the method were identified problems in this PT scheme.

4. CONCLUSIONS

In this work, the capability to developed RMs for benzoic acid in mango juice and histamine in canned tuna, the provision of reference value to RMs using a candidate higher order method (LC-IDMS), and the conduct of PT schemes were demonstrated in the country through ITDI-STD. The prepared RMs were homogeneous and stable and were made available for the first time to local chemical testing laboratories. Through the PT exercises conducted using these RMs, an assessment for better performance by local laboratories for benzoic acid than for histamine analysis was observed. Likewise, this served as a means for our institute to disseminate the traceability of measurement to SI standards. The continuous development and use of RMs, PT schemes and method validation by the institute, along with local laboratories participating in PT schemes, are regarded as essential to further improving laboratory performance in the country and facilitating fair trade in food globally.

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