

P72: MATRIX REFERENCE MATERIALS DEVELOPMENT FOR FOOD SAFETY APPLICATION IN PHILIPPINE PRODUCTS

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Abstract – Reference materials (RMs) were developed for contaminants such as histamine in canned tuna and benzoic acid in mango juice. The RMs were found homogeneous and stable for at least six (6) months. For the reference value assignment, a candidate higher order method like isotope dilution (ID) technique - Liquid Chromatography-Triple Quadrupole Mass Spectrometry (LCMSMS) was applied. These characterized matrix RMs relevant to food safety issues were also used in PT schemes organized locally to help in the dissemination of measurement traceability to international system (SI) of units.

Keywords: reference material, food contaminant, histamine, benzoic acid, food safety

1. INTRODUCTION

The need to ensure comparable and traceable chemical measurement is highly important in global trading. This would require the establishment of a national chemical measurement infrastructure in the country which involve using available certified reference materials (CRMs) and reference materials (RMs), participation in proficiency testing (PT) schemes, and accreditation to ISO 17025 among others. These will help improve the quality of chemical measurements in the Philippines and thus make them acceptable everywhere.

The development of RMs was pioneered by the Industrial Technology Development Institute - Department of Science and Technology (ITDI-DOST) as part of its vision to establish metrology in chemistry infrastructure and support the RMs needs of chemical testing laboratories in the country. Food safety issues for chemical testing of Philippine products were prioritized in this activity as these could affect the health of consumers as well as local and foreign trade issues. Additional criteria for the choice of analytes includes detention

cases of Philippine export products and capability of local testing laboratories. Currently, there is no local reference material and PT scheme for benzoic acid in fruit juice nor histamine in canned tuna available in the country.

This study entails the development of reference materials, RM characterization and conduct of proficiency testing schemes for histamine in canned tuna and benzoic acid in fruit juice.

2. EXPERIMENTAL

2.1. Chemicals and Materials

SRM 350b Benzoic acid (99.9994%) and HRM-1002A Benzoic acid purchased from the National Institute of Standards and Technology (NIST) and Health Science Authority (HSA, Singapore) were used as calibration standards. HRM-1001A Benzoic acid in orange juice (HSA) and isotopically labelled analog benzoic acid ring D5 (Cambridge Isotope Laboratory (CIL) were used as CRMs. MS grade solvents and HPLC grade solvents were used. Histamine standard (Sigma Aldrich), histamine dihydrochloride from the National Institute of Metrology China were used as calibration standards. Isotopically labelled analog histamine dihydrochloride-D4 (CIL), formic acid (97%), Brij-35, mercaptoethanol, o-phthalaldehyde (OPA) and 1-octanesulfonic acid sodium salt (Sigma Aldrich) were used in the experiments.

2.2. Equipment

For benzoic acid analysis, the HPLC (Shimadzu Prominence LC-20A with UV - VIS Detector, set at 234 nm) with Waters C18 Sunfire (150 mm x 4.6 mm, 3.5 µm) and LC-MSMS (Agilent 1290 LC system coupled to a 6460 LC/MS Triple Quadrupole (Agilent Technologies, Inc.) with Agilent Zorbax Eclipse Plus C18 column (4.6 x 100 mm x 3.5 µm) were employed. For histamine analysis, HPLC with

Waters C18 Sunfire column and fluorometric detector (340 nm excitation and 445 nm emission) and LCMSMS with Waters Poroshell 120 HILIC column (2.7 μm , 2.1 mm x 100 mm) were utilized.

2.3. Reference material preparation

Reference material (RM) for benzoic acid in mango juice was prepared using commercial mango juice drinks locally available and manufactured on the same date. The juice was mixed, centrifuged, filtered, spiked with benzoic acid and pasteurized. This was followed by hot filling in amber bottles (100 ml), sealing, labelling, shrink wrapping and storing at 4°C. On the other hand, RM for histamine in canned tuna was done by using homogenized canned tuna sample until paste like consistency was obtained. Known amount of histamine was spiked to the sample and it was thoroughly mixed. Packing was made on a 100ml bottle with screw cap containing about 80 mg of the spiked tuna sample. The packed samples were properly labelled and placed in a retort @ 115.6 °C for about 3 hours. After cooling, these also stored at 4°C.

2.2 Reference method

The reference method used to provide the reference value was isotope dilution technique in tandem with liquid chromatography mass spectrometry (ID-LCMSMS) and with gravimetric sample preparation. Benzoic acid quantitation [1] by this technique was calculated using Equation (1) where,

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

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

The same equation was applied for histamine quantitation. This was also crosschecked with HPLC technique with gravimetric sample preparation. The HPLC technique for benzoic acid [2] and histamine analysis published elsewhere with o-phthaldialdehyde (OPA) [3], [4] were applied. The ID-LCMSMS method for benzoic acid [1] and histamine [5] with slight modification were used.

3. RESULTS AND DISCUSSION

3.1. Method Validation

The HPLC technique for benzoic acid and histamine with gravimetric sample preparation were validated and used for the characterization of the homogeneity and stability of the RMs produced. The acceptable validation results by HPLC for benzoic acid and histamine in each matrix are tabulated in Table 1.

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
Accuracy, % 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

The reference method was also developed for benzoic acid and histamine analysis using a candidate higher order method: ID-LCMSMS. Certified reference materials were also analyzed using the reference method developed. The reference value obtained by this method established the traceability of the measurement to international system (SI) of units. The reference value is expressed with an estimate of measurement of uncertainty. Acceptable % recovery (n=10) was obtained for the CRM (HRM-1001A Benzoic acid in Orange Juice) at two levels of benzoic acid concentration using ID-LCMSMS with gravimetric sample preparation. LOD and LOQ for benzoic acid using ID-LCMSMS are 0.0292 mg/kg and 0.0974 mg/kg respectively

On the other hand, acceptable % rsd = 1.35 (n=10) and acceptable % recoveries were also obtained for control materials used using ID-LCMSMS with gravimetric sample preparation. LOD

and LOQ for histamine by this technique are 0.0014 mg/kg and 0.0046 mg/kg respectively.

3.2. Reference material characterization

The RMs prepared are required to be homogeneous and stable. Result of statistical evaluation (Tables 2 and 3) for the homogeneity test are good. Stability of RM for benzoic acid was about 6 months while 12 months was achieved for the stability of RM for histamine.

Trend analysis [6] was used for short-term and long-term stability evaluation. No significant instability of the material for both RMs was observed at about 4°C, 30°C, and 40°C for a period of three weeks exposure and up to at least 6 months. The RM for histamine in canned tuna reached stability of up to 12 months as shown in Fig. 1.

Table 1. Summary of statistical analysis for homogeneity of benzoic acid in mango juice

Criteria	Critical Value	Obtained Result	Remarks
Mean, X (mg/kg)		791	
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 2. Summary of statistical analysis for homogeneity of histamine in canned tuna

Criteria	Critical Value	Obtained Result	Remarks
Mean, X (mg/kg)		136	
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

3.3 Reference value assignment

The reference value assigned for benzoic acid in mango juice and histamine in canned tuna (Table 3) is the mean of duplicate measurements from three bottles obtained from the batch and analyzed by the ID-LCMSMS method. Acceptable % recovery of

the CRM HRM-1001A and control materials was achieved for both RMs.

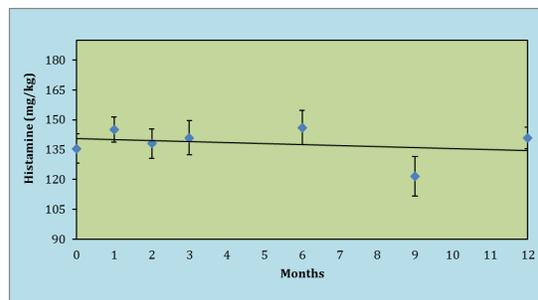


Fig. 1. Long-term stability for histamine in canned tuna

The Mass spectrometer (MS) was operated in electrospray ionization in negative mode. Benzoic acid and its isotope analogue were detected by selectively monitoring the collision-induced dissociation channels of $[M-H]^- \rightarrow [M-CO_2H]^-$ at m/z 121.1 \rightarrow 77.2 and m/z 126.1 \rightarrow 82.1, respectively.

The internal and calibration standards used for histamine analysis were Histamine dihydrochloride (A,A,B,B-D4) from Cambridge Isotope Laboratory and histamine dihydrochloride from National Institute for Metrology (NIM) China, respectively. The Mass spectrometer (MS) was operated in electrospray ionization in positive mode. Histamine and its isotope analogue were detected by selectively monitoring the collision-induced dissociation channels of $[M+H]^+ \rightarrow [M+H-NH_3]^+ \rightarrow [M+H-CH_2CH_2NH_2]^+$ at m/z 112.1 \rightarrow 95.1 \rightarrow 68.2 and m/z 116.1 \rightarrow 99.1 \rightarrow 85.2, respectively.

Table 3. 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

3.4. Measurement of uncertainty

The reported expanded uncertainties of the reference values of the RMs produced were estimated based on the ISO GUM approach and ISO Guide 35. Highest contribution (94.6%) to the expanded uncertainty of the reference values of RM benzoic acid 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 rest of the uncertainty sources include masses of samples, standards and solutions and purity of the calibration standard.

3.5. Conduct of Proficiency Test (PT) Scheme

The RMs developed were also used for PT schemes organized to assess the performance of local testing laboratories. The PT schemes were participated by government and private testing laboratories, research institutes and the academe using routine method of analysis. In the PT round for benzoic acid in mango juice (MiCPT-14-01) most of the participant used HPLC. Nine (9) laboratories (82 %) out of 11 participating laboratories obtained satisfactory performance and two laboratories (18 %) obtained unsatisfactory results. The unsatisfactory results were observed to be due to computation error and overestimation of result because of matrix effects.

Two different analytical techniques based on fluorometry and ELISA were used by the twelve participants for the PT round for histamine (MiCPT-14-04). However only 50% satisfactory performance was achieved by the participating laboratories. Under estimation of results from four laboratories used the method AOAC 977.13. The relative standard deviation of the participating laboratories with satisfactory performance is approximately 9%. Different parameters for the extraction techniques and need for validation of the method were identified problems in this PT.

4. CONCLUSION

In this work, the capability to develop 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 (ID-LCMSMS) and the conduct of PT schemes were demonstrated. The RMs were homogeneous, stable and were made available for the first time to the local laboratories. Through the PT exercises conducted using these RMs, an assessment of better performance of local laboratories for benzoic acid than for histamine analysis was observed. Likewise this was also a way for our Institute to disseminate the traceability of measurement to SI units. Continuous development of RMs and conduct of PT schemes by the Institute, and use of RMs, conduct of method validation, participation in PT schemes of local testing laboratories are deemed

essential to further improve testing laboratory performance in the country and facilitate fair global trade in food.

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