

Certificate of Analysis

CERTIFIED REFERENCE MATERIAL HRM – 1021A

Preservatives in Soy Sauce

Batch Number

STY-0096-001

Description

The certified reference material (CRM) consists of 50 mL of soy sauce fortified with benzoic acid, methyl paraben and n-propyl paraben. The material was bottled in an amber glass bottle and screw-capped under nitrogen.

The CRM was produced with reference to the requirements set out in ISO/IEC 17025:2017 [1], ISO 17034:2016 [2] and ISO Guide 35:2017 [3].

Certified Mass Fraction Values

A certified value is a value for which a laboratory has the highest confidence in its accuracy, in that all known or suspected sources of biases have been investigated and accounted for. The certified mass fraction values for benzoic acid, methyl paraben and n-propyl paraben listed in the Table below were determined by gas chromatography-isotope dilution mass spectrometry (GC-IDMS).

Analyte	Certified Mass Fraction	Coverage Factor (at approximately 95% confidence)	Unit
Benzoic acid	759 ± 74	3.18	mg/kg
Methyl paraben	220 ± 14	2	mg/kg
n-Propyl paraben	117 ± 13	2	mg/kg

The mass fraction value is expressed as the certified value ± the expanded uncertainty.

The uncertainty listed with the certified value is an expanded uncertainty about the mean, calculated at approximately 95% confidence. The certified value has an associated measurement uncertainty attributed to uncertainty contribution from characterisation of the material (U_{char}), uncertainty in the homogeneity of the material (U_{bb}) and uncertainty in the stability of the material (U_{stab}). The U_{char} was evaluated by combining uncertainties from method precision, weighing, the concentration of calibration solution (which included the purity of the standard), bias using different ion pairs, bias using different instruments [i.e. liquid chromatography-diode array detection (LC-DAD) vs GC-MS]), dilution factor and method recovery, in accordance with ISO/IEC Guide 98-3:2008 [4].

Homogeneity

Homogeneity testing on benzoic acid and methyl paraben in the soy sauce was evaluated based on prior experience from previous batches of soy sauce materials. The materials were demonstrated to be sufficiently homogeneous.

Homogeneity testing on n-propyl paraben in the soy sauce was performed on 11 bottles with two sub-samples taken from each bottle. GC-MS was employed for the determination of n-propyl paraben. The sample size taken for homogeneity testing was about 1 g. No significant differences in the between- and within-bottle variances were found using one-way ANOVA at 95 % confidence level [3]. Thus, n-propyl paraben in the material was regarded to be sufficiently homogeneous. The u_{bb} was evaluated from the uncertainty due to between-bottle inhomogeneity.

Stability

The short-term stability of benzoic acid and methyl paraben in the soy sauce was evaluated based on prior experience from previous batches of soy sauce materials, where the materials were demonstrated to be stable at 40 °C (maximum allowable transportation temperature) for up to 10 days.

The short-term stability of n-propyl paraben in the soy sauce was studied. The material was stored at 40 °C for at least 10 days. The results showed that n-propyl paraben showed sign of instability. In view of possible instability of n-propyl paraben at an extreme temperature (40 °C) that might occur during transportation, the CRM is transported in cold condition (in ice packs).

The long-term stability of the analytes in the material at storage temperature (18 °C to 25 °C) was evaluated on three occasions over a period of up to 6 months after preparation. The results showed that all analytes were stable over the study period with repeated sampling. The u_{stab} was evaluated from the standard error of the slope.

Validity of Certified Mass Fraction Values

The certified mass fraction values are valid within their respective measurement uncertainties until **23 Mar 2028**, provided that the CRM is subjected to the same handling and storage conditions as stated in this Certificate of Analysis (COA).

The CRM will be continuously monitored during the validity period to determine if any substantive change to the certified values has occurred. If necessary, its user will be advised if the property value of the CRM is found to have changed or an updated COA may be issued.

Analytical Methods

The certified mass fractions of benzoic acid, methyl paraben and n-propyl paraben in the material were determined by exact-matching GC-IDMS. CRMs [benzoic acid (HRM-1002A), methyl paraben (HRM-1003A) and n-propyl paraben (HRM-1022A)] from HSA were used as calibration standards. Isotope-labelled $^{13}\text{C}_6$ -benzoic acid and $^{13}\text{C}_6$ -n-propyl paraben from Cambridge Isotope Laboratories Inc., $^{13}\text{C}_6$ -methyl paraben from Sigma Aldrich were used as the internal standards.

The calibration blends were prepared gravimetrically by mixing appropriate amount of calibration standard solutions and internal standard solutions. The sample blends were gravimetrically prepared by mixing of 1 g of diluted soy sauce and internal standard solutions. Quality control blends were also prepared and analysed concurrently. All the blends were left to equilibrate before being extracted with acetonitrile. Each sample and quality control blends were bracketed with a calibration blend and analysed at least three times.

Metrological Traceability

The certified mass fraction values for benzoic acid, methyl paraben and n-propyl paraben are traceable to the International System of Units (SI) through the use of CRMs from HSA.

Intended Use

For the validation of methods or as quality controls used in the determination of the mass fraction of benzoic acid, methyl paraben and n-propyl paraben in soy sauce and materials of similar matrix.

Instructions for Use

Prior to use, the material should be mixed by vortexing the contents **thoroughly** for at least 1 min. **After use, the bottle should be purged with nitrogen gas, re-capped, sealed with Parafilm and stored at about 4 °C.** The minimum sample size for the analysis should be about 1 g. If results differ from certified value in subsequent sampling, customers are advised to purchase a new CRM.

Transport and Storage

HRM-1021A is transported under cold condition (in ice packs). Upon receipt, the CRM should be stored at room temperature (18 °C – 25 °C) in its original bottle prior to opening. After opening, the CRM should be stored at about 4 °C after purging with nitrogen gas. Exposure to direct intense light, ultraviolet radiation and other temperatures should be avoided. Storage of the material at room temperature after opening may result in changes in the concentrations of the analytes.

Safety Precautions for Users

Treat the material as hazardous substance. Use appropriate work practices when handling the material, in order to avoid skin or eye contact or ingestion.

Further Information

Please direct all enquiries regarding this CRM to the contact provided in this COA.

References

1. ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories.
2. ISO 17034:2016 General requirements for the competence of reference material producers.
3. ISO Guide 35:2017 Reference materials – Guidance for characterisation and assessment for homogeneity and stability.
4. ISO/IEC Guide 98-3:2008 Uncertainty of measurement – Part 3: Guide to the expression of uncertainty in measurement (GUM:1995).

Certificate Revision Records

Certificate Ref. No.	Date of issue	Reason for issuance
CML-HRM-1021A/01	23 Mar 2020	Issuance of first certificate
CML-HRM-1021A/02	18 Mar 2021	Extension of expiry date
CML-HRM-1021A/03	28 May 2021	Revision on certified values
CML-HRM-1021A/04	25 Feb 2022	Extension of expiry date
CML-HRM-1021A/05	1 Mar 2023	Extension of expiry date Removal of sorbic acid due to instability
CML-HRM-1021A/06	14 Mar 2025	Extension of expiry date. Removal of n-butyl paraben due to instability

Note

HSA does not assume any liability with respect to any loss caused by improper use and/or storage of the reference material by the customer.



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