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Ref. No.: CML-HRM-1025A/03
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Certificate of Analysis

CERTIFIED REFERENCE MATERIAL
HRM – 1025A

Artificial Sweeteners in Soft Drink

Batch Number
STY-0101-001

Description
The certified reference material (CRM) consists of 50 mL of soft drink fortified with acesulfame potassium, sodium cyclamate and sucralose. 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 acesulfame potassium, sodium cyclamate and sucralose listed in the Table below were determined by liquid chromatography-isotope dilution tandem mass spectrometry (LC-IDMS/MS):

Analyte	Certified Mass Fraction	Unit
Acesulfame potassium	340 ± 12	mg/kg
Sodium cyclamate	241 ± 14	mg/kg
Sucralose	291 ± 15	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, with coverage factor 2 (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}), uncertainty in the short-term stability of the material (u_{sts}) and uncertainty in the long-term stability of the material (u_{lts}). The u_{char} was evaluated by combining uncertainties from method precision, concentration of calibration solution (which included the purity of the standard), weighing, bias using different ion pairs (for acesulfame potassium, and sucralose), bias using different LC columns, bias using different filtration membranes for clean-up, bias from analysis by different analysts and method recovery, in accordance with ISO/IEC Guide 98-3:2008 [4].

Homogeneity

Homogeneity testing on the analytes in the material was performed on ten bottles with two sub-samples taken from each bottle. LC-MS/MS was employed for the determination of all the analytes. The sample size taken for homogeneity testing was about 0.5 g (approximately 0.5 mL). No significant differences in the between- and within-bottle variances were found for sodium cyclamate using one-way ANOVA at 95 % confidence level [3]. The between-bottle standard deviation for acesulfame potassium, and sucralose was sufficiently small compared to the standard uncertainty of the certified mass fraction value [3]. Thus, 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 testing of the analytes in the material was studied. The material was stored at 40 °C (maximum allowable transportation temperature) for up to 14 days. The results showed that no significant trend was found for sodium cyclamate using Student's *t*-test at 95 % confidence level [3]. The standard uncertainty of short-term stability for sucralose was sufficiently small compared to the standard uncertainty of the certified mass fraction value [3]. Thus, sodium cyclamate and sucralose in the material were regarded to be sufficiently stable at 40 °C over the study period. The results of acesulfame potassium in the material exposed to 40 °C for up to 7 days were within the expanded uncertainty range of the certified mass fraction value. In view of possible instability of acesulfame potassium in the material at an extreme temperature (40 °C) that might occur during transportation, the CRM is transported under cool condition (e.g., in ice gel packs). The u_{sts} was evaluated from the standard error of the slope from the short-term stability study.

The long-term stability of the analytes in the material at 4 °C (storage temperature) was evaluated on three occasions over a period of up to 3 months after preparation. The results showed that all analytes were stable when stored at 4 °C over the study period. The u_{lts} was evaluated from the standard error of the slope from the long-term stability study.

Validity of Certified Mass Fraction Values

The certified mass fraction values are valid within their respective specified measurement uncertainties until **14 Sep 2026**, 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 mass fraction values has occurred. If necessary, its user will be advised or an updated COA may be issued when the property value of the CRM is found to have changed.

Analytical Methods

The certified mass fractions of acesulfame potassium, sodium cyclamate and sucralose in the material were determined by exact-matching LC-IDMS/MS. CRMs [acesulfame potassium (HRM-1012A), sodium cyclamate (HRM-1009A) and sucralose (HRM-1015A)] from HSA were used as calibration standards. Isotope-labelled D_4-acesulfame potassium and D_6-sucralose from Toronto Research Chemicals Inc. and D_{11}-sodium cyclamate from C/D/N Isotopes Inc. were used as 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 about 0.5 g of the material and internal standard solutions. Quality control blends were also prepared and analysed concurrently. All the blends were left to equilibrate before subjected to clean-up procedures. Each sample and quality control blends were bracketed with a calibration blend and analysed five times.

Metrological Traceability

The certified mass fraction values for acesulfame potassium, sodium cyclamate and sucralose 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 to determine the mass fraction of acesulfame (e.g., acesulfame and acesulfame potassium), cyclamates (e.g., cyclamic acid and its salt forms) and sucralose in aqueous-based beverages and materials of similar matrix.

Instruction for Use

Prior to use, the material should be equilibrated to room temperature and thoroughly mixed by inverting the bottle. After use, the bottle should be re-capped, sealed with parafilm and stored at 4 ^{o}C. The minimum sample size for the analysis should be about 0.5 g. If results differ from certified value in subsequent sampling, customers are advised to purchase a new CRM.

Transport and Storage

HRM-1025A is transported under cool condition (e.g., in ice gel packs). Upon receipt, the CRM should be stored at a temperature of 4 °C in its original bottle. Exposure to direct intense light, ultraviolet radiation and other temperatures should be avoided.

Safety Precautions for Users

Treat the material as hazardous substance. Use appropriate work practices when handling 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 Record

Certificate Ref. No.	Date of issue	Reason for issuance
CML-HRM-1025A/01	14 Sep 2022	Issuance of first certificate
CML-HRM-1025A/02	29 Aug 2023	Extension of expiry date
CML-HRM-1025A/03	26 Aug 2024	Extension of expiry date; revision to exclude saccharin from the CoA due to observed 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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