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Certificate of Analysis

CERTIFIED REFERENCE MATERIAL
HRM – 1030A

Fipronil and Fipronil Sulfone in Chicken Egg Powder

Batch Number
STY-0162-001

Description
The certified reference material (CRM) consists of 5 g of lyophilised chicken egg fortified with fipronil and fipronil sulfone. The lyophilised chicken egg was powdered by grinding and sieving. The material was bottled in an amber glass bottle, crimp-sealed with rubber septum and packaged under argon.

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 fipronil and fipronil sulfone in reconstituted egg listed in the Table below were determined by gas chromatography-isotope dilution tandem mass spectrometry (GC-IDMS/MS).

Analyte	Certified Mass Fraction in the Reconstituted Material	Unit
Fipronil	27.5 ± 4.0	µg/kg
Fipronil sulfone	29.4 ± 4.4	µg/kg

The mass fraction value is expressed as the certified value ± the expanded uncertainty. The mass fraction values apply to the material when it is reconstituted in accordance with the procedure outlined in the “Instruction for Use”.

The uncertainty listed with the certified value is an expanded uncertainty about the mean, with coverage factor, k = 2, corresponding to 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, the concentration of calibration solution (which included the purity of the standard), weighing, bias using different ion pairs, bias using different instruments [i.e. liquid chromatography-tandem mass spectrometry (LC-MS/MS) vs GC-MS/MS], bias using different IDMS approaches 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 seven bottles with two sub-samples taken from each bottle. GC-MS/MS was employed for the determination of all the analytes. The sample size taken for homogeneity testing was about 0.5 g. No significant differences in the between- and within-bottle variances were found using one-way ANOVA at 95 % confidence level [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 30 days. The results showed that no significant trend was found for fipronil sulfone using Student's *t*-test at 95 % confidence level [3]. Thus, fipronil sulfone in the material was regarded to be sufficiently stable at 40 °C over the study period. The results of fipronil 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 fipronil in the material at an extreme temperature (40 °C) that might occur during transportation, the CRM is transported in frozen state (in dry ice).

The long-term stability of the analytes in the material at -20 °C (storage temperature) was evaluated on three occasions over a period of up to 5 months. The results showed that all analytes were stable when stored at -20 °C during the study period. The u_{stab} 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 **28 Mar 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 fipronil and fipronil sulfone in the material were determined by exact-matching GC-IDMS/MS. CRMs [fipronil (P1668) and fipronil sulfone (P1731)] from the National Measurement Institute (NMI, Australia) were used as calibration standards. Isotope-labelled $^{13}C_4$,$^{15}N_2$-fipronil and $^{13}C_4$, $^{15}N_2$-fipronil sulfone from Cambridge Isotope Laboratories Inc. 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 reconstituting about 0.5 g of the material with 1.5 g of water. The mixtures were then spiked with appropriate amounts of internal standard solutions. Quality control blends were also prepared and analysed concurrently. All the blends were left to equilibrate before subjected to extraction and clean-up procedures. Each sample and quality control blends were bracketed with a calibration blend and analysed for at least three times.

Metrological Traceability

The certified mass fraction values for fipronil and fipronil sulfone are traceable to the International System of Units (SI) through the use of CRMs from NMI, Australia.

Intended Use

For the validation of methods or as quality controls used to determine the mass fraction of fipronil and fipronil sulfone in chicken egg and materials of similar matrix.

Instruction for Use

1) Prior to use, the powder material should be equilibrated to room temperature and mixed thoroughly by stirring the contents with a clean spatula.
2) The material must be reconstituted before the measurement. The certified values are expressed based on reconstituted egg.
3) Weigh accurately a sample size of 0.5 g ± 0.05 g. The weighing should be conducted promptly upon opening the bottle to minimise the absorption of water by the lyophilised powder.
4) Add accurately 1.5 g ± 0.03 g of purified water to the powder for reconstitution.
5) After use, the bottle should be purged gently with an inert gas (e.g. argon or nitrogen), re-sealed with a crimp cap and stored at -20 °C.
6) If the laboratory's method working instruction specifies a sample intake higher than 2 g of reconstituted material, it is imperative to uphold the 1:3 mass ratio of powder to purified water.
7) If results differ from certified value in subsequent sampling, customers are advised to purchase a new CRM.

(Note: Freeze-thaw stability study was evaluated for up to two cycles. The certified values may not be valid after two cycles of freeze-thaw as the stability of the analytes subjected to more than two freeze-thaw cycles has not been investigated.)

Transport and Storage

HRM-1030A is transported in frozen state (in dry ice). Upon receipt, the CRM should be stored at a temperature of -20 °C in its original bottle. Exposure to direct intense light, ultraviolet radiation should be avoided. Storage of the material at room temperature or in the refrigerator may result in the changes in the mass fraction of the analytes.

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-1030A/01	28 Mar 2024	Issuance of first certificate
CML-HRM-1030A/02	30 Jul 2024	Revision on certified values
CML-HRM-1030A/03	26 Mar 2025	Extension of expiry date

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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