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Certificate of Analysis

Certified Reference Material

BOTS-1

Bovine Muscle Certified Reference Material for Veterinary Drug Residues

BOTS-1 is a certified reference material (CRM) consisting of bovine muscle tissue that has been naturally contaminated with veterinary drugs. This CRM is designed for use in method development, validation, and quality control for the analysis of veterinary drug residues in bovine muscle or related matrices. The certified values for BOTS-1 shown in Table 1 represent the mass fraction of veterinary drug residues as supplied and after reconstitution of one part of BOTS-1 material with two parts of aqueous solution [1].

Table 1: Certified values and expanded uncertainties (*k* = 2) for BOTS-1

Compound	Mass fraction µg/kg	Mass fraction reconstituted* µg/kg
chlorpromazine (a,b,c,d)	490 ± 100	160 ± 33
ciprofloxacin (a,b,c,d)	44 ± 4.4	15 ± 1.5
clenbuterol (a,b,c,d)	3.3 ± 1.4	1.1 ± 0.5
dexamethasone (a,b,c,d)	9.5 ± 0.8	3.2 ± 0.3
enrofloxacin (a,b,c,d)	57 ± 4.8	19 ± 1.6
meloxicam (a,b,c,d)	3.0 ± 0.4	1.0 ± 0.1
ractopamine (a,b,c,d)	12.4 ± 1.2	4.1 ± 0.4
sulfadiazine (a,b,c,d)	2290 ± 120	763 ± 40

*mass fractions after reconstitution with 2 parts aqueous solution
Refer to the sections below for additional explanations

Period of validity: until August 2033
Storage conditions: –20 °C or below

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Table 2: Information value for BOTS-1

Compound	Mass fraction g/kg
water (e)	5.1

Intended use

This certified reference material is primarily intended for use in method development, validation, and quality control for the analysis of chlorpromazine, ciprofloxacin, clenbuterol, dexamethasone, enrofloxacin, meloxicam, ractopamine, and sulfadiazine in bovine muscle or similar matrices.

Preparation of material

BOTS-1 was produced via dosage of a healthy, 2-year old, veterinary-inspected heifer with chloramphenicol (antibiotic), chlorpromazine (tranquilizer), clenbuterol (β-agonist), dexamethasone (anti-inflammatory), enrofloxacin (antibiotic), meloxicam (anti-inflammatory), nitrofurantoin (antibiotic), phenylbutazone (anti-inflammatory), ractopamine (β-agonist), sulfadiazine (antibiotic), trenbolone (growth promoter), and tulathromycin (antibiotic). The heifer was euthanized at the appropriate time and the muscle tissue was harvested and ground. The ground muscle tissue was then wet homogenized, freeze-dried, milled to a fine powder, homogenized, and bottled under argon in ≥10 g aliquots.

Characterization of material

The explanatory list of letters next to each compound (Tables 1 and 2) refers to the instrumental method used for measurements:

a) Double isotope dilution liquid chromatography tandem mass spectrometry (ID^2−LC−MS/MS)
b) Standard addition double isotope dilution liquid chromatography tandem mass spectrometry (SA−ID^2−LC−MS/MS)
c) Standard addition liquid chromatography tandem mass spectrometry (SA−LC−MS/MS)
d) External calibration liquid chromatography tandem mass spectrometry (LC−MS/MS)
e) Mass loss after vacuum desiccator drying

Certified values are based on results from data generated from the National Research Council of Canada (NRC), the Canadian Food Inspection Agency (CFIA), the United States Department of Agriculture (USDA), and the German Federal Office of Consumer Protection and Food Safety (BVL) using LC−MS/MS. Additional results for enrofloxacin and sulfadiazine were obtained from the international comparison exercise CCQM-K141/P178 performed within the Consultative Committee for Amount of Substance, of the International Committee of Weights and Measures [2].

Metrological traceability

Results presented in this certificate are traceable to the International System of Units (SI) through gravimetrically prepared standards of NIST SRM 350b (benzoic acid) employed as an internal standard for proton quantitative nuclear magnetic resonance spectroscopy (^{1}H-qNMR).

Homogeneity

The material was tested for within-unit homogeneity at the NRC via ID^2−LC−MS/MS and for between-unit homogeneity via ID^2−LC−MS/MS and SA-ID^2−LC−MS/MS. Results from subsamples (0.5 g) from randomly selected bottles were evaluated using the DSL random effects model [3, 4]. These uncertainties were combined to assign an associated uncertainty component.

Stability

The transportation, freeze-thaw (F/T), and long-term stability of the veterinary drug residues in BOTS-1 were assessed at the NRC via ID^2−LC−MS/MS. The transportation stability was carried out using an isochronous approach. Replicate bottles of BOTS-1 were stored at −80, −20, +4, +20, and +37 °C. All samples were removed from storage conditions after two weeks and analyzed simultaneously using the −80 °C samples as a reference. Freeze-thaw stability was assessed over twenty F/T cycles from −80 to +20 °C using a single bottle of BOTS-1. The bottle was removed from the freezer, allowed to equilibrate at room temperature for 1 hour, opened, mixed, and resealed without argon purging. Sub samples were removed at cycles 1, 5, 10, 15, and 20 and stored at -80 °C until the last cycle when all samples were analyzed simultaneously. Long-term stability for ciprofloxacin, clenbuterol, enrofloxacin, ractopamine, and sulfadiazine was assessed at −80 °C after 10 years and for chlorpromazine, dexamethasone and meloxicam at −80 °C after 9 years.

All stability results were evaluated using the DerSimonian-Laird (DSL) random effects model [3]. No significant differences in the measured mass fractions were observed for the transportation stability samples for ciprofloxacin, clenbuterol, dexamethasone, enrofloxacin, meloxicam, ractopamine, or sulfadiazine and the uncertainty components were thus considered negligible and assigned to zero. Small differences in the measured mass fractions were observed for the transportation stability samples for chlorpromazine and an uncertainty component was therefore included. No significant differences in the measured mass fractions were observed for the F/T stability samples for chlorpromazine, ciprofloxacin, clenbuterol, dexamethasone, enrofloxacin, meloxicam, ractopamine, or sulfadiazine and the uncertainty components were thus considered negligible and assigned to zero. No significant differences in the measured mass fractions were observed for the long-term stability samples for clenbuterol, dexamethasone, meloxicam, or ractopamine and the uncertainty components were thus considered negligible and assigned to zero. Small differences in the measured mass fractions were observed for the long-term stability samples for chlorpromazine, ciprofloxacin, enrofloxacin, and sulfadiazine and an uncertainty component was therefore included.

Uncertainty

The expanded uncertainty (U) for all values is equal to $U = ku_c$, where u_c is the combined standard uncertainty calculated according to the Joint Committee for Guides in Metrology (JCGM) [5] and k is the coverage factor. A coverage factor of $k = 2$ was applied which corresponds to a level of confidence of approximately 95 %.

All reasonable sources of uncertainty related to the certified values in Table 1 were considered. Included in the combined uncertainty estimate are uncertainties in the batch characterization, uncertainties related to possible between-unit and within-unit variation, and uncertainties related to stability. The characterization uncertainty includes contributions from measurement, primary standards, and an additional dark uncertainty component which was revealed from the analysis of results from the CCQM-K141/P178 international comparison study [2].

Storage

The material shall be stored at –20 °C or below.

Instructions for handling and use

Prior to opening, each bottle should be allowed to warm to room temperature and the contents should be thoroughly mixed. After use, bottles should be tightly sealed and immediately returned to the freezer. Repeated sampling is permitted, although care must be taken not to introduce contamination. A minimum sample mass of 0.5 g is recommended. It is recommended that the

material be rehydrated immediately prior to use. To each 0.5 g of BOTS-1, add 1.0 g of water or appropriate buffer, mix well and allow to equilibrate for a minimum of 10 minutes.

Health and safety information

Only qualified personnel should handle the material and appropriate disposal methods should be used. A Safety Data Sheet (SDS) is available at doi.org/10.4224/crm.2018.bots-1. For laboratory use only; not for human consumption, therapeutic, drug, household, or any other uses.

Period of validity

The certified values are valid until August 2033, provided the storage and instructions for handling and use specified in this certificate are followed.

Quality Management System

The NRC is Canada's national metrology institute (NMI) and is a signatory of the International Committee for Weights and Measures Mutual Recognition Arrangement (CIPM MRA). The CIPM MRA was developed in a response to a growing need for an open, transparent, and comprehensive scheme to give users reliable quantitative information on the comparability of national metrology services and to provide the technical basis for wider agreements negotiated for international trade, commerce, and regulatory affairs. Our Quality Management System for measurement services and certified reference materials conforms to the requirements of ISO/IEC 17025 and ISO 17034.

Description of terms

Certified values are those for which the NRC has the highest confidence and that all known and suspected sources of bias have been considered by the NRC and are reflected in the stated expanded uncertainties.

Information values are those that may be of interest to users, but for which the NRC has not established sufficient information to provide an estimate of uncertainty, or that reflect a lack of agreement between different methods of analysis.

Supplemental information

Bibliographic information and any additional technical supplemental information is available at doi.org/10.4224/crm.2018.bots-1.

References

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3. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials. 1986; 7: 177-188. https://doi.org/10.1016/0197-2456(86)90046-2

4. ISO Guide 35. Guidance for characterization and assessment of homogeneity and stability. Geneva (Switzerland): International Organization for Standardization (ISO); 2017. https://www.iso.org/standard/60281.html
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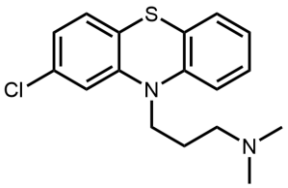
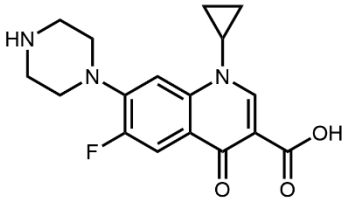
Appendix



chlorpromazine
CAS registry number: 50-53-3
InChI Key: ZPEIMTDSQAKGNT-UHFFFAOYSA-N
Molecular formula: $C_{17}H_{19}ClN_2S$
Molar mass: 318.87 g/mol



ciprofloxacin
CAS registry number: 85721-33-1
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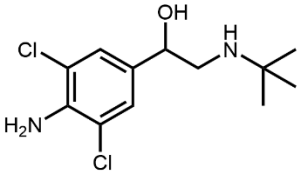
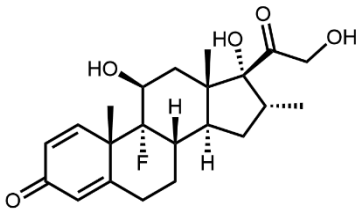
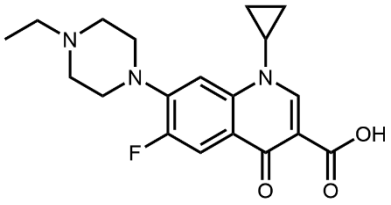
Molecular formula: $C_{17}H_{18}FN_3O_3$
Molar mass: 331.34 g/mol



clenbuterol
CAS registry number: 37148-27-9
InChI Key: STJMRWALKKWQGH-UHFFFAOYSA-N
Molecular formula: $C_{12}H_{18}Cl_2N_2O$
Molar mass: 277.19 g/mol



dexamethasone
CAS registry number: 50-02-2
InChI Key: UREBDLICKHMUKA-CXSFZGCWSA-N
Molecular formula: $C_{22}H_{29}FO_5$
Molar mass: 392.47 g/mol



enrofloxacin
CAS registry number: 93106-60-6
InChI Key: SPFYMRJSYKOXGV-UHFFFAOYSA-N
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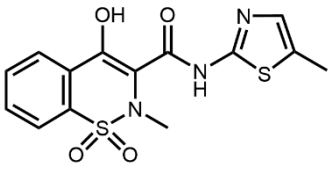
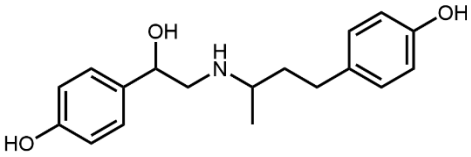
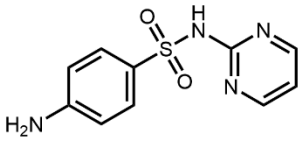
Molar mass: 359.40 g/mol



meloxicam
CAS registry number: 71125-38-7
InChI Key: ZRVUJXDFFKFLMG-UHFFFAOYSA-N
Molecular formula: $C_{14}H_{13}N_3O_4S_2$
Molar mass: 351.41 g/mol



ractopamine
CAS registry number: 97825-25-7
InChI Key: YJQZYXCXBBCEAQ-UHFFFAOYSA-N
Molecular formula: $C_{18}H_{23}NO_3$
Molar mass: 301.38 g/mol



sulfadiazine
CAS registry number: 68-35-9
InChI Key: SEEPANYCNGTZFQ-UHFFFAOYSA-N
Molecular formula: $C_{10}H_{10}N_4O_2S$
Molar mass: 250.28 g/mol

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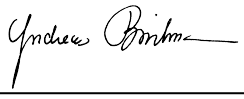
Approved by: 

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