DRAFT TANZANIA STANDARD

Honeybee venom (apitoxin) — Specification

TANZANIA BUREAU OF STANDARDS

First Edition 2026

Introduction

Honeybee venom, also called apitoxin, is a complex mixture of proteins, peptides, and enzymes secreted by worker honeybees for defence. It is a transparent liquid which dries up easily even at room temperature, odourless, ornamental pungent smell, a bitter taste, hydrolytic blend of proteins with basic pH (4.5 to 5.5) that is used by bees for defense. When coming into contact with mucous membranes or eyes, it causes considerable burning and irritation. Honeybee venom is soluble in water and insoluble in alcohol and ammonium sulfate. When it comes in contact with air it forms grayish-white crystals.

Dried Honeybee venom takes on a light yellow colour and some commercial preparations are brown, thought to be due to oxidation of some of the venom proteins. Honeybee venom contains a number of very volatile compounds which are easily lost during collection, it is considered a rich source of enzymes, peptides and biogenic amines.

The therapeutic value of Honeybee venom was already known to many ancient civilizations, due toits anticoagulant and anti-inflammatory properties. Honeybee venom was mainly used to treat many inflammatory disorders such as arthritis, bursitis, tendinitis, dissolving scar tissue (e.g. keloids), Herpes zoster, joint disease, and rheumatoid arthritis. Today, Honeybee venom is used as medicine in human and veterinary.

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Foreword

This Draft Tanzania Standard was developed by the Pharmaceuticals Technical Committee under supervision of the Chemicals Divisional Standards Committee and it is in accordance with the procedures of the Bureau.

This Draft Tanzania Standard has been prepared with assistance drawn from:

Newzeeland specifications

In reporting the test results of a test or analysis made in accordance with this standard, if final value, calculated or observed is to be rounded off, it shall be done in accordance with TZS 4 *Rounding off numerical values*.

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Honeybee venom - Specification

1. Scope

This Draft Tanzania Standard specifies requirements, sampling and test methods of Honeybee venom to be used as a pharmaceutical product.

2. Normative references

The following documents are referred in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

TZS 4 *Rounding off numerical values*
TZS 773 *Labeling and marking of pharmaceutical products – Specifications*
TZS 59 Water for analytical laboratory use - Specification and test method

3. Terms and definitions

Honeybee venom

cytotoxic and hemotoxic bitter colourless bees excrete liquid containing proteins, peptides, and enzymes which may produce immune response.
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4. Requirements

4.1 General requirements

4.1.1The honeybee venom shall be transparent liquid which dries up easily even at room temperature.

4.1.2 Air Dried honeybee venom shall form white to yellow colour crystals.

4.1.3The honeybee venom shall be free from foreign matters like dust, pollen, wax, or other bee-derived residues.

4.1.4 The honeybee venom shall have ornamental pungent smell.

4.2 Specific requirements

The honeybee venom shall comply with the specific requirements in Table 1 when tested as per methods specified.

Table 1: Requirements for Honeybee venom

S/N	Characteristics	Requirements	Method of Test
1.	pH	4.4-5.5	Annex A
2.	Density, g/cm^3	1.13- 1.30	Annex B
3.	Moisture content, % m/m, max	10.8	Annex C
4.	Melittin, %	40 - 50	Annex D
5.	Histamine, %	0.7 - 1.6	Annex D
6.	Apamin, %	2 - 3	Annex D
7.	Phospholipase A2 %	10 - 12	Annex D

5. Packing and labelling

5.1 Packaging

The honeybee venom shall be supplied in clean, dry and air tight containers which does not allow light.

5.2 Labelling

In addition to TZS 773 each container shall be labelled with the following information given in prominent, legible and durable labelling:

a) Physical address and name of the manufacturer
b) Recognized trade mark, if any.
c) Name of the product as “Honeybee venom”
d) Nominal weight
e) Batch number.
f) Manufacturing date
g) Expiry date

6. Storage

7.1 The honeybee venom shall be stored in suitable container which shall not affect integrity of the product in terms of quality.

7.2 The product may be refrigerated to keep it safe. Proper storage at low temperatures (ideally -20°C or lower) and protection from light and humidity are vital to maintain quality.

ANNEX A
(Normative)
Determination of pH

A.1 Materials

A.1.1 Honeybee venom sample
A.1.2 Distilled water
A 1.3 Standard pH buffers

A.2 Apparatus
A.2.1 pH meter equipped with a glass electrode
A.2.2 Beakers
A.2.3 Stirrer

A.3 Procedures

A.3.1 Calibration of the pH Meter:

Calibrate the pH meter using standard pH buffer solutions. Rinse the electrode with distilled water after each calibration point to prevent contamination. Or use instrument user manual for calibration

A.3.2 Preparation of Honeybee Venom Sample:

If the venom is in powder form, dissolve a small, accurately measured amount (e.g., 1 mg) in a known volume of distilled water (e.g., 10 mL).Use a ratio appropriate for the pH meter's sensitivity.

ANNEX B
(Normative)
Determination of density

B.1 Materials
Honeybee venom sample:
Distilled water

B.2 Apparatus
Analytical balance
Volumetric flask or graduated cylinder
Pipette or dropper
Temperature control device

B.3 Procedure
B.1 Prepare the Venom Solution:
Weigh 1 g (or appropriate weight) of the venom in a beaker. Add 10 mL of distilled water and stir to complete dissolution.

Weigh the Empty Container:

Use the analytical balance to weigh the container you will use to measure the venom solution's mass. Record this weight.

Measure the Volume:

Transfer a specific volume of the venom solution into the volumetric flask or graduated cylinder. For accuracy, ensure no air bubbles are present.

Weigh the Filled Container:

Weigh the container with the venom solution. Record this weight.

Calculate the Mass of the Venom Solution:

Subtract the weight of the empty container from the weight of the filled container to determine the solution's mass.

Calculate the Density:

Use the formula: Density(ρ)=$\frac{Mass(g)}{Volume(cm3)}$
Where;

Mass is the mass of the solution
Volume is the volume of the solution

Temperature Consideration:

Measure and record the temperature of the venom solution, as density can vary with temperature. Ideally, conduct the test at a standard temperature (e.g., 20°C) for consistency.

ANNEX C
(Normative)
Determination of moisture content

C.1 Materials
Honeybee venom sample

C.2 Apparatus
C.2.1 Moisture analyzer
C.2.2 Aluminium dish
C.2.3 Analytical balance
C.2.4 Dessicator

C.3 **Principle**: The sample is heated at a controlled temperature, and the loss in weight represents the moisture content.

C.4 **Procedure**:

1. Weigh a sample (about 1 – 2 g) in an aluminum dish.
2. Place in a moisture analyzer or drying oven at 105°C for 3 hours**.**
3. Weigh the sample again after cooling in a desiccator.
4. Calculate moisture content using:

$$Moisture\ content(\%) = \frac{Initial\ weight - Final\ weight}{Initial\ weight} * 100$$

Annex D
(Normative)
Determination of Melittin, Histamine, Apamin,
and Phospholipase A2

D.1 Materials
D.1.1 Honeybee venom sample
D.1.2 Standard solutions of Histamine, Apamin, Phospholipase A2 and Mellitin

D.2 Equipments
D.2.1 HPLC
D.2.2 Beaker

D.3 Procedure
D.3.1 Prepare 25 µg/ml cytochrome c solution (internal standard) in deionized water and use it as diluent of all samples.

D.3.2 Prepare the honey Honeybee venom solutions by diluting 3 mg of the product in 10 ml of internal standard solution (see step 1).

D.4.4 Prepare the standard solutions of apamine, mast cell degranulating peptide, PLA2 and melittin by dissolving them in internal standard solution. The solutions will be used for calibration curves construction. The recommended concentrations of standard solutions are as follows (at least six dilutions): apamine – from 2 to 20 µg, mast cell degranulating peptide – from 5 to 30 µg, PLA2 – from 10 to 100 µg, melittin – from 30 to 300 µg. Sonicate all prepared solutions for 5 min and then filter through 0.45-µm membrane filters.

D.3.5 Perform HPLC analysis of prepared standard solutions and honey Honeybee venom solutions.Use the SynChropack C8 6.5 µm, 4.6 x 100 mm column or the column with the same parameters.Separation conditions: linear gradient 5% B – 80% B at 30 min (eluent A – 0.1% TFA in water, eluent B – 0.1% TFA in acetonitrile: water (80:20)); flow rate = 1 ml/min, injection volume = 40 µl, separation temperature = 25 °C, λ = 220 nm.

D.3.6 Identify apamine, mast cell degranulating peptide, PLA2 and melittin using their retention times.

D.3.7 Construct the calibration curves for apamine, mast cell degranulating peptide, PLA2 and melittin using corresponding relative peak areas (peak area of an analyte divided by peak area of internal standard).

D.3.8 Calculate the concentrations of analyzed honey Honeybee venom from the standard calibration curves equations.

Bibliography

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