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भारतीय मानक मसौदा

क्षारसूत्र – परीक्षण की पद्धतियाँ

Draft Indian Standard

Ksharasutra – Methods of test

ICS 11.120.10

Ayurveda Sectional Committee, AYD 01 **Last Date of Comments:** 04 September 2024

FOREWORD

(*Formal Clause would be added later*)

Ksharasutra therapy (parasurgical procedure using a thread treated by alkalies) is being practiced in Indian system of medicine since ancient time for management of ano-rectal disorders. It is considered as a safe and cost-effective method of treatment for fistula-in-ano, Haemorrhoids and other sinus diseases. The preparation of *Ksharsutra* has undergone many changes and has passed through various stages before it reached the present standard of manufacturing. Several clinical trials have been carried out for evaluation and establishment of its action in India as well as in other countries.

Standardization in *Ksharasutra* production and application is crucial for ensuring efficacy, safety, quality control, and patient confidence. With this objective the Bureau of Indian Standards (BIS) has taken up the task of formulating Indian Standards for method of preparation and test methods of *Ksharasutra*.

In the formulation of this standard, significant assistance has been derived from the Ayurvedic Pharmacopoeia of India, Part II, Vol. II, 2008 published by the Ministry of Ayush, Government ofIndia. Inputs have also been derived from the information available in the public domain in print and electronic media including authoritative books.

In the formulation of this standard due consideration has been given to the provisions of the *Drugs and Cosmetics Act*, 1940 and Rules framed thereunder. However, this standard is subject to the restrictions imposed under these Rules and Regulations, wherever applicable.

For the purpose of deciding whether a particular requirement of this standard is complied with, thefinal value, observed or calculated expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 2022 ‘Rules for rounding off numerical values (second revision)’. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

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Ksharasutra – Methods of test

1 SCOPE

This standard prescribes the methods of microscopical, physical and chemical tests for *Ksharasutra*.

2 REFERENCES

The standards listed below contain provisions which, through reference in this text, constitute provision of this standard. All standards are subject to revision, and parties to agreement based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated:

IS No.	*Title*
IS 1070 : 1992	Reagent grade water – Specification (*third revision*)
AYD 01 (24024) (Under preparation)	*Ksharasutra* - Specification

3 MICROSCOPY

3.1 Take a thread, wash thoroughly with chloroform 2 or 3 times followed by hot water also 3 times to remove the coated materials. Cut the washed thread into small pieces and digest it by boiling with a 10% aqueous solution of sodium carbonate. Wash to remove sodium carbonate and take small amount of the material on a micro slide and crush it with a glass rod. Mount and observe the characteristics.

3.2 Take a small portion of the washed material, mount in Cuoxam (0.5 g of copper carbonate triturated with 10 ml of distilled water, gradually adding strong solution of ammonia, specific gravity 0.88, with continued stirring) and observe the characteristics.

4. PHYSICAL TESTS

4.1 Length

4.1.1 *Apparatus*

Meter Scale (marked in mm)

4.1.2 *Procedure*

Fix a standard meter scale on a table. Place the thread with one cut end exactly coinciding with a division on the scale. Applying just enough tension to keep the thread straight, place the other cut end on the scale, and note the division on the scale with which it coincides. Read the length and record it in mm on the meter scale. Repeat the test on four more threads belonging to the same batch. The average is taken as the length of the thread.

4.2 Weight

4.2.1 *Apparatus*

Weighing Balance

4.2.2 *Procedure*

Record the weight of each thread used in the test 5.1 on a balance of sensitivity 0.1 mg (0.0001 gm) and the average shall be taken as weight of thread.

4.3 Diameter

4.3.1 *Apparatus*

Dial Gauge (Sensitivity of 0.0025 mm)

NOTE - Table of the dial gauge should be about 5 cm in diameter, with a pressor foot of about 12.5 mm. The total load applied by the foot when in use shall be 200 g ± 15 g.

4.3.2 *Procedure*

Take the thread to be measured from its tube and expose it to room temperature for about half an hour. Hold the thread across the gauge table with just the tension required to keep it straight, and allow the pressor foot to touch it. Record the reading on the dial gauge as the thickness of the thread at that point. Three readings shall be taken for each thread, one at mid-point, and two at equidistance on either side of the midpoint. No point shall be within 3 cm of either end of thethread.

The test is repeated with four more threads of the same batch. The average is taken as the diameter of the thread.

4.4 Tensile strength

4.4.1 *Apparatus:*

Tensiometer

4.4.2 *Procedure*

The thread is tied to a hook suspended from a stand. A weighing pan of 250 g is attached to the other end of the thread, and a weight of 2 kg is placed on the pan. Weights are added to the pan inincrements of 50 g, allowing five seconds between such additions. At the time the thread breaks, the total weights in the pan and weight of the pan itself is recorded as the breaking load of the thread. If the breakage occurs within 1 cm from either end, the test shall be repeated on

a fresh thread. The average of five tests is recorded as the breaking load of one batch.

5 CHEMICAL TESTS (repeated for 3 times)

5.1 Loss on drying

5.1.1 *Apparatus*

5.1.1.1 *Tared Petri Dish*

5.1.1.2 *Oven*

5.1.1.3 *Desiccator*

5.1.1.4 *Weighing Balance*

5.1.2 *Procedure*

Take 5 *Ksharasutra* and weigh accurately, place in the form of a coil in a tared petri dish and keepat 105 °C in an oven for 3 hours. Then cool in a desiccator and, weigh to constant weight and calculate loss on drying using following formula:

Percentage of Loss on Drying = Weight Loss/Weight of Sample × 100

5.2 Water soluble extractive

5.2.1 *Apparatus*

5.2.1.1 *Weighing Balance*

5.2.1.2 *Reflux Apparatus*

5.2.1.3 *Graduated Tube*

5.2.2 *Reagents*

Water

5.2.3 *Procedure*

Take 5 *Ksharasutra* and weigh accurately. Macerate the test material with water (1: 40 w/v) for 5 min at room temperature. Reflux for 5 min on steam bath then cool to room temperature and filter into a graduated tube. Make up the original volume with water, then evaporate a known volume and dry to a constant weight at 100 °C to 105 °C.

5.3 n- Hexane soluble extractive

5.3.1 Carry out the procedure same as given above in 6.2 using n-hexane instead of water.

5.4 pH (Alkalinity)

5.4.1 *Apparatus*

5.4.1.1 *Vortex Mixer*

5.4.1.2 *Digital pH Meter*

5.4.2 *Reagents*

Carbon dioxide free water

5.4.3 *Procedure*

Take about 0.1 gm. of coated material of *Ksharasutra* and add 10 ml of carbon dioxide free water. Vortex the mixture for 1 min and set aside for 15 mins. Vortex again for 1 min and filter the mixture. Determine the pH of clear supernatant using digital pH meter.

5.5 Sodium and Potassium

5.5.1 *Apparatus*

5.5.1.1 *Flame Photometer*

5.5.1.2 *Volumetric Flask*

5.5.2 *Reagents*

5.5.2.1 *Sodium Chloride*

5.5.2.2 *Potassium Chloride*

5.5.2.3 *Triple Distilled Water*

5.5.3 *Procedure*

Prepare separate stock solution of sodium / potassium (500 mEq) by dissolving 2.9230 g sodium chloride / 3.7280 g potassium chloride in 100 ml triple distilled water. Prepare separate working standard solutions containing 0.5, 1.0, 2.0, 4.0 and 5.0 mEq of sodium/potassium from the respective standard stock solutions.
Using flame photometer with appropriate filters, calibrate the standard solutions and prepare separate calibration plots respectively for sodium/potassium. Take 0.1 gm coated material of *Ksharasutra* and add 15 ml of triple distilled water in 50 ml of volumetric flask and shake vigorously and make the volume up to the mark. Filter the solution and choosing sodium and potassium filter, calculate the content of the sodium/potassium respectively in the coated material of *Ksharasutra* by interpolation from the calibration plot.

5.6 Total alkalies

5.6.1 *Apparatus*

pH Meter

5.6.2 *Reagents*

N/25 hydrochloric acid

5.6.3 *Procedure*

Estimate the total alkalies as carbonate in the coated material of *Ksharasutra* by titrating a known volume of the aqueous solution prepared for determination of pH, with N/25 hydrochloric acid using pH meter to an end point pH of 3.6. Calculate percentage of total alkali as carbonate using the titer value.

5.7 Turmeric

5.7.1 *Apparatus*

Vortex mixer

5.7.2 *Reagents*

5.7.2.1 *Turmeric*

5.7.2.2 *Hydrochloric acid*

5.7.2.3 *Acetone*

5.7.3 *Procedure*

Moisten 0.2 g of coated material of *Ksharasutra* and 0.05 g Turmeric, each separately, with 0.5 ml % v /v hydrochloric acid for 5 minutes. Extract each separately with 4 x 5 ml acetone by vortexing for 30 seconds, at 0, 5th and 10th minutes. Pool the respective extracts, filter and make up the volume to 25 ml using acetone. Read the absorbance of each extract after suitable dilution, at 418 nm against acetone Blank. Calculate the percentage of Turmeric in the coated material of *Ksharasutra* using the absorbance of Reference Turmeric.

5.8 Curcumin

5.8.1 *Apparatus*

Vortex mixer

5.8.2 *Reagents*

5.8.2.1 *Curcumin*

5.8.2.2 *Chloroform*

5.8.2.3 *Methanol*

5.8.2.4 *Hydrochloric acid*

5.8.2.5 *Acetone*

5.8.3 *Procedure*

Moisten 0.2 g of coated material of *Ksharasutra* with 0.5 ml % v/v hydrochloric acid for 5 min. Extract the mixture with 4 x 5 ml acetone by vortexing for 30 seconds, each at 0, 5th and 10th min. Pool the extracts, filter and make up the volume to 25 ml using acetone. Take 10 ml of the solution, evaporate at room temperature to about 0.1 ml. Apply quantitatively 0.1 ml of sample solution, 15 µl (1 mg/ml) solution of Reference Curcumin in acetone and 50 µl of acetone as Blank on a chromatoplate. Develop the Plate in chloroform: methanol (49:1). Mark the yellow coloured Curcumin zone in reference, test sample and blank. Separate the spots and extract each with 5 x 4 ml methanol and make up the volume to 25 ml in each case. Read the absorbance of methanol solution of coated material of *Ksharasutra* and Curcumin after suitable dilution against blank at 418 nm. Calculate the percentage of Curcumin in the sample with respect to the Reference Curcumin.

5.9 Sulphated Ash

5.9.1 *Apparatus*

5.9.1.1 *Silica Crucible*

5.9.1.1 *Desiccator*

5.9.1.1 *Weighing Balance*

5.9.2 *Reagents*

Sulphuric acid

5.9.3 *Procedure*

Heat silica crucible to redness for 10 minutes, allow it to cool in a desiccator and weigh. Take 3 *Ksharasutra*, in the crucible and weigh accurately. Ignite gently at first, until the substance is thoroughly charred. Cool, moisten the residue with 1 ml of concentrated sulphuric acid, heat gently until white fumes are no longer evolved and ignite at 800ºC until all black particles have disappeared (conduct the ignition in a place protected from air currents). Allow the crucible to cool, add a few drops of concentrated sulphuric acid and heat. Ignite as before, allow to cool and weigh to constant weight. Calculate the percentage of Sulphated ash.

5.10 Euphol

5.10.1 *Apparatus*

5.10.1.1 *Vortex mixer*

5.10.1.2 *Chromatogram Plate*

5.10.1.3 *Oven*

5.10.1.4 *Water Bath*

5.10.1.5 *Test Tube*

5.10.1.6 *Ice Bath*

5.10.2 *Reagents*

5.10.2.1 *n-Hexane*

5.10.2.2 *Chloroform*

5.10.2.3 *Methanol*

5.10.2.4 *Euphol (Reference Standard)*

5.10.2.5 *Acetic Anhydride*

5.10.2.6 *Sulphuric Acid*

5.10.3 *Procedure*

Extract 0.2 g of coated material of *Ksharasutra* with 5 x 5 ml n-hexane by vortexing for 30 seconds, each at 0, 5th, 10th, 15th and 20th minute. Pool the extracts, filter and recover the solvent under reduced pressure and re-dissolve the residue in 1 ml chloroform: methanol (3:2). Apply quantitatively 100 µl of the above solution, 100 µl (5 mg /ml) solution of Reference Euphol in n-hexane and 100 µl of n-hexane as Blank on a chromatogram plate. Develop the plate in chloroform: n-hexane (4:1). Mark the Euphol zones in sample, Reference Euphol and Blank by visualizing in iodine chamber. Remove the iodine by vaporizing in an oven at 50^{o}C for 20 minutes. Separate the zones individually, extract each with 5 x 4 ml n-hexane and make up the volume to 25 ml in each case. Take 2 ml from each extract separately in a test tube and dry on a boiling water bath. Cool the residue to the room temperature and add 4 ml of acetic anhydride to each and cool further in an ice bath for 15 minutes. Add 0.05 ml of cold conc. sulphuric acid carefully to each tube and mix thoroughly and set aside in a dark cupboard for exactly 1.5 hours and read the absorbance at 281 nm against Blank. Calculate the percentage of Euphol in the coated material of *Ksharasutra* with respect to the Reference Euphol.

6 QUALITY OF REAGENTS

6.1 Reagents including pure chemicals used shall be of analytical grade.

6.2 Reagent grade water for laboratory use shall be as per IS 1070.

NOTE — ‘Pure chemicals’ shall mean chemicals that do not contain impurities which effect the results of analysis.