भारतीय मानक
Indian Standard

IS 11142 : 2023

हिना (मेहंदी) पाउडर — विशिष्टि

(दूसरा पुनरीक्षण)

Henna (*Mehendi*) Powder — Specification

(Second Revision)

ICS 71.100.70

भारतीय मानक ब्यूरो
BUREAU OF INDIAN STANDARDS
मानक भवन, 9 बहादुर शाह ज़फर मार्ग, नई दिल्ली - 110002
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI - 110002
www.bis.gov.in www.standardsbis.in

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Cosmetics Sectional Committee PCD 19

FOREWORD

This Indian Standard (Second Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Cosmetics Sectional Committee had been approved by the Petroleum, Coal and Related Products Division Council.

Henna (*mehendi*) is the leaf of a small shrub. Botanically the plant is known as *Lawsonia Inermis* Linn. syn. L. *alba*, *fam. Lythraceae*. The leaves of the plant, dried and powdered, are used to dye the hair and for colouring the finger nails, palms and soles of the feet. It is also exported in considerable quantities.

Microscopic examination of the powdered *Henna* leaves shows the following histological structures:

Olive green or brownish green numerous fragments of cuticle and leaf parenchyma rosette aggregates and monoclinic prisms of calcium oxalate frequently up to 15 microns and occasionally up to 40 microns in diameter, globular mucilage cells, numerous fragments of intra-vascular tissues, long narrow and shorter fusiform sclerenchyma fibres with thick walls, some of the latter being wavy toothed, fragments of epidermis with stomata and striated cuticle, the stomata being surrounded by ordinary epidermal cells; occasional papillae or non-glandular hair fragments.

Henna powder quality is generally determined by its colour, purity, dyeing property and fineness. The principal colouring matter is lawsone. At times *Henna* powder may be adulterated with sand, stems, fruit of *Henna* plant, husk of paddy, leaves and twigs of other shrubs, etc. Certain requirements and tests, as in the case of powdered spices have been included to restrict malpractices.

Earlier, two separate Indian Standards were available for *Henna* powder and methods of test for *Henna* powder, namely IS 11142 : 1984 and IS 7159 : 1984. In the first revision, the committee felt that two separate standards are not necessary, and a comprehensive standard needs to be formulated for *Henna* powder including specifications as well as the methods for test for *Henna* powder. Accordingly, IS 11142 : 1984 was revised by amalgamating it with IS 7159 : 1984.

Nowadays, *Henna* powder cosmetic products enriched with natural ingredients such as *amla*, *Shikakai*, *Neem*, *Bringaraj*, *Arishtaka*, *Haritaki*, *Jatamansi*, *Baheda*, *Brahmi* and aloe vera powder etc are available in the Indian market. However, the first revision of this standard (2019 version) was not applicable for such products as it was limited to pure *Henna* powder only. Hence, a need was felt to revise it to incorporate requirements for two new types of '*Henna* powder with natural ingredients'. In this revision, following major change has been made:

Type 2 —'*Henna* powder with natural ingredients without preservatives' and its requirements have been incorporated.

Type 3 —'*Henna* powder with natural ingredients with preservatives' and its requirements have been incorporated.

The composition of the Committee responsible for the formulation of this standard is given in Annex L.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test of analysis, shall be rounded of 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 valued in this standard.

Indian Standard

HENNA (*MEHENDI*) POWDER — SPECIFICATION

(Second Revision)

1 SCOPE

This standard prescribes the requirements and methods of sampling and test for *Henna* (*mehendi*) powder.

2 REFERENCES

The standards listed in Annex A contain provisions, which through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of these standards.

3 TYPES

There shall be three types of the *Henna* powder, namely.

3.1 Type 1

Pure *Henna* powder (natural without any other ingredients/adjuncts/preservatives).

3.2 Type 2

Henna powder with natural ingredients/adjuncts without preservatives.

3.3 Type 3

Henna powder with natural ingredients/adjuncts with preservatives.

4 REQUIRMENTS

4.1 Description

The material shall be in the form of fine powder obtained from dried fresh leaves of *Henna* plant. A minimum of 95 percent of *Henna* powder shall pass through 250 µ IS sieve [*see* IS 460 (Part 1)]. It shall be free from extraneous adulterants like sand, stems, fruit of *henna* plant, husk of paddy, leaves and twigs of other shrubs, etc.

4.2 All the natural ingredients/adjuncts of Type 2 *Henna* powder shall be chosen appropriately in accordance with the natural ingredients described in the authoritative books of *Ayurvedi*c and Siddha as specified in the first schedule of the latest edition of *The Drugs and Cosmetics Act*, 1940, or ingredient which is being anywhere in the world or is recognised for use in cosmetics in any national or international literature. It shall comply to the requirements of [*see* IS 17316 (Part 1) and (Part 2)] for the claims like natural, organic etc as applicable.

4.3 All ingredients of *Henna* powder shall also comply with the provisions of IS 4707 (Part 1) and IS 4707 (Part 2) subject to the provisions of *The Drugs and Cosmetics Act* and rules framed thereunder.

4.4 The total percent by mass of natural ingredients incorporated shall be decided to comply with the lawsone pigment requirement and other requirements given in Table 1 when tested as prescribed in col (6) of Table 1.

5 PACKING AND MARKING

5.1 Packing

5.1.1 The material shall be packed in polythene lined hessian bags or in suitable containers as agreed to between the purchaser and the supplier.

5.1.2 All containers container or closures in which the material is packed shall be dry, clean and tight so that extraneous impurities are not introduced.

5.2 Marking

Each container (pouch/jar/bottle/bag etc) and package containing this in a carton/box shall be marked with following information:

a) Name of the material;
b) Name and address of the manufacturer;
c) Net content;
d) Month and year of manufacture (MM/YY);
e) Use before or expiry date as per statutory requirements;
f) Batch number;
g) **WARNING** — Do not use for colouring eyelashes or eyebrows. Do not use on cut, irritated or abraded skin;
h) Any other information required by statutory authorities;
j) List of ingredients as per statutory requirements [1), 2)]; and
k) Instructions for use.

[1)] The list can be given at either outer or inner label depending upon on the specific packing under which it will be sold to the consumer, such listing could also be done by way of leaflets and/or neck tag securely tied with the package/container as the case may be.
[2)] Provided that this statement need not appear for packs of less than 60 ml of liquids and 30 g of solid and semi solids.

Table 1 Requirements for *Henna* (*Mehendi*) Powder

(*Clause* 4.4)

Sl No.	Characteristic	Requirement			Method of Test, Ref to	
		Type 1	Type 2	Type 3	Annex	IS No.
(1)	(2)	(3)	(4)	(5)	(6)	(7)
i)	Moisture and volatile matter, percent by mass, *Max*	10	10	12	B	—
ii)	Cold water extract, percent by mass, *Min*	25	25	25	C	—
iii)	Crude fibre, percent by mass, *Max*	15	15	15	D	—
iv)	Mineral matter, percent by mass, *Max*	12	15	15	E	—
v)	Acid insoluble ash, percent by mass, *Max*	6	6	6	F	—
vi)	Absence of extraneous/ synthetic dyes	To pass the test	To pass the test	To pass the test	G and H	—
vii)	Lawsone pigment, percent by mass, *Min*	1.0	0.9	0.85	J/K	—

NOTE — In case of any dispute with respect to lawsone content, method of test prescribed at Annex H shall be the reference method.

5.2.1 *BIS Certification Marking*

The product(s) conforming to the requirements of this standard may be certified as per the conformity assessment schemes under the provisions of the *Bureau of Indian Standards Act*, 2016 and the Rules and Regulations framed thereunder, and the products may be marked with the Standard Mark.

6 SAMPLING OF *HENNA* POWDER

6.1 Representative samples of *Henna* (*mehendi*) powder shall be drawn as prescribed in IS 3958.

6.2 Tests for all the requirements shall be carried out on a composite sample.

6.3 The *Henna* (*mehendi*) powder shall be taken to have conformed to this standard if the composite sample passes all the tests.

7 QUALITY OF REAGENTS

Unless otherwise specified, pure chemicals and distilled water (*see* IS 1070) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

8 PREPARATION OF SAMPLE FOR ANALYSIS

8.1 Procedure

Mix the sample thoroughly, divide and keep at least 100 g portion in a non-corrodible, clean and dry air-tight container for analysis.

ANNEX A

(*Clause* 2)

LIST OF REFERRED STANDARDS

IS No.	*Title*	*IS No.*	*Title*
IS 460 (Part 1) : 2020	Test sieves — Specification: Part 1 Wire cloth test sieves (*fourth revision*)	(Part 2) : 2017	List of raw materials generally not recognized as safe for use in cosmetics (*fourth revision*)
IS 1070 : 2023	Reagent grade water — Specification (*fourth revision*)	IS 17316	Guidelines on technical definitions and criteria for natural and organic cosmetic ingredients and products:
IS 3958 : 2021	Methods of sampling cosmetics (*second revision*)		
IS 4011 : 2018	Methods of test for safety evaluation of cosmetics (*third revision*)	(Part 1) : 2019/ ISO 16128-1 : 2016	Definitions for ingredients
IS 4707	Classification of cosmetic raw materials and adjuncts:	(Part 2) : 2019/ ISO 16128-2 : 2017	Criteria for ingredients and products
(Part 1) : 2020	Colourants (*fourth revision*)		

ANNEX B

[*Table* 1, *Sl No.* (i)]

DETERMINATION OF MOISTURE AND VOLATILE MATTER

B-1 PROCEDURE

Weigh accurately about 5 g of the prepared sample material in a moisture dish, about 6 cm to 8 cm in diameter and about 2 cm to 4 cm in depth. Dry in an air oven at a temperature of 105 °C ± 2 °C to constant mass (within ± 5 mg).

B-2 CALCULATION

Moisture and volatile matter, percentage by mass =

$$\frac{100 \times M_1}{M}$$

where

M_1 = loss in mass, in g, on drying; and
M = mass, in g, of the material taken for the test

ANNEX C

[*Table* 1, *Sl No.* (ii)]

DETERMINATION OF COLD WATER EXTRACT

C-1 PROCEDURE

Weigh to the nearest 0.001 g, about 2 g of the prepared sample. Transfer the material quantitatively with water to a 100 ml volumetric flask and fill to the mark with cold water. Stopper the flask and shake at approximately 30 min intervals for 8 h and allow to settle for another 16 h without shaking. Filter the extract through a dry grade 40 filter paper. Reject first few milliliters, then evaporate a 25 ml aliquot to dryness in a tarred dish on the water-bath and heat in the oven at 100 °C ± 2 °C to constant mass. Record the final mass.

C-2 CALCULATION

Cold water soluble extract, percent by mass =

$$4 \times \frac{M_2}{M_1} \times 100$$

where

M_1 = mass, in g, of the test sample; and
M_2 = mass, in g, of the residue obtained.

ANNEX D

[*Table* 1, *Sl No.* (iii)]

DETERMINATION OF CRUDE FIBRE

D-1 REAGENTS

D-1.1 Petroleum Ether — boiling range 40 °C to 60 °C

D-1.2 Dilute Sulphuric Acid — 1.25 percent (*m/v*), accurately prepared

D-1.3 Sodium Hydroxide Solution — 1.25 percent (*m/v*), accurately prepared

D-1.4 Ethyl Alcohol — 95 percent (*v/v*)

D-2 PROCEDURE

Weigh accurately about 2.5 g of the prepared sample. Transfer the material into a one litre flask. Take 200 ml of the dilute sulphuric acid in a beaker and bring to boil. Transfer the whole of the boiling acid to the flask containing material and immediately connect the flask with a water-cooled reflux condenser and heat, so that the content of the flask begins to boil within 1 min. Rotate the flask frequently, taking care to keep the material from remaining on the sides of the flask and out of contact with the acid. Continue boiling for exactly 30 min. Remove the flask and filter through fine linen (about 18 threads to the centimeter) or through a coarse acid-washed, hardened filter paper, held in a funnel, and wash with boiling water until the washings are no longer acid to litmus. Bring some quantity of sodium hydroxide solution to boil under a reflux condenser. Wash the residue on the filter into the flask with 200 ml of boiling sodium hydroxide solution. Immediately connect the flask with the reflux condenser and boil for exactly 30 min. Remove the flask and immediately filter through the linen or through filter paper. Thoroughly wash the residue with boiling water and transfer to a Gooch crucible G4, prepared with a thin but compact layer of ignited asbestos. Wash the residue thoroughly first with hot water and then with about 15 ml of ethyl alcohol and with three successive washings of 15 ml of petroleum ether each. Dry the Gooch crucible and contents at 105 °C ± 1 °C in an air-oven for 3 h, cool and weigh. Repeat the process of drying for 30 min, cooling and weighing until the difference between two consecutive weighing is less than 1 mg. Incinerate the contents of the Gooch crucible in the muffle furnace at 550 °C ± 20 °C until all the carbonaceous matter is burnt. Cool the Gooch crucible containing the ash in a desiccators and weigh.

D-3 CALCULATION

$$\text{Crude fibre, percent by mass} = \frac{(M_1 - M_2)}{M} \times 100$$

where

M_1 = mass in g, of Gooch crucible and contents before ashing;
M_2 = mass in g, of Gooch crucible and contents after ashing; and
M = mass in g, of the material taken for the test.

ANNEX E

[*Table* 1, *Sl No.* (iv) *and Annex* F]

DETERMINATION OF MINERAL MATTER

E-1 PROCEDURE

Weigh accurately about 5 g of the prepared sample in a silica dish. Heat the dish at first on a low flame and then in a muffle furnace maintained at about 600 °C for 2 h. Cool in a desiccator and weigh. Repeat the process of heating, cooling and weighing until constant mass is obtained. Preserve the ash for test under Annex F.

E-2 CALCULATION

Mineral matter, percent by mass = $\frac{M_2 \times 100}{M_1}$

where

M_2 = mass in g, of the ash; and
M_1 = mass in g, of the material taken for the test.

ANNEX F

[*Table* 1, *Sl No.* (v) *and Annex* E]

DETERMINATION OF ACID INSOLUBLE ASH

F-1 REAGENT

F-1.1 Dilute Hydrochloric Acid — approximately 5 N

F-2 PROCEDURE

To the ash preserved in Annex E add 25 ml of dilute hydrochloric acid (5 N), heat on water-bath for 10 min, allow to cool and filter the contents of the dish through Whatman filter paper No. 42, wash the filter with distilled water till the washing is free from acid. Return the filter and residues to the dish. Keep it in oven to dry and ignite to free from carbon (in muffle furnace to 600 °C) for 2 h. Cool the dish in desiccators and weigh. Repeat the process of igniting, cooling and weighing, until the difference between two successive weighing is less than one milligram. note the lowest mass.

F-3 CALCULATION

Acid insoluble ash, percent by mass = $\frac{M_2}{M_1} \times 100$

where

M_2 = mass, in g, of the residue; and
M_1 = mass, in g, of the material taken for the test.

ANNEX G

[*Table* 1, *Sl No.* (vi)]

DETECTION OF EXTRANEOUS/SYNTHETIC DYES BY TLC

G-0 OUTLINE OF METHOD

To detect contamination of chemical dyes or synthetic pigments if any, the thin layer chromatography (TLC) method shall be used.

G-1 APPARATUS

G-1.1 Weighing Balance

G-1.2 TLC Plates — pre-coated silica gel 60 F_{254} 0.2 mm thickness

G-1.3 TLC Apparatus/Beakers — 250 ml (narrow)

G-1.4 Iodine Chamber

G-1.5 Test Tubes —with stoppers

G-1.6 Volumetric Flask — 10 ml

G-1.7 Micro Syringe — 10 µl

G-1.8 Silicone Wax

G-2 REAGENTS AND STANDARDS

G-2.1 Chloroform

G-2.2 Methanol

G-2.3 Glacial Acetic Acid

G-2.4 Ethyl Acetate

G-2.5 Iodine Granules

G-2.6 Standards — pure *Henna*, lawsone pigment, chemical/synthetic dye ingredients like *p*-phenylenediamine, *p*-aminophenol, *m*-aminophenol, 2-nitro-*p*-phenylenediamine, etc.

G-2.7 Natural Ingredients Standards — (for Type 2 and Type 3 *Henna* powder only) reference sample of *Amla*, *Shikakai*, *Neem*, *Bringaraj*, *Arishtaka*, *Haritaki*, *Jatamansi*, *Baheda*, *Brahmi*, aloe vera powder etc.

G-2.8 Test Sample — one gram of *Henna* powder sample being tested.

NOTE — to be used if unknown spots are seen on the sample plates.

G-3 PREPARATION OF SOLUTION

G-3.1 Mobile Phase

Chloroform : ethyl acetate : methanol : glacial acetic acid (8 : 2 : 1 : 1 *v/v*). Take the solvents chloroform, ethyl acetate, methanol and glacial acetic acid in 8 : 2 : 1 : 1 *v/v*, mix well and keep stoppered.

G-3.2 Sample Solutions

Disperse about 3 g of test sample in about 7 ml water and make a smooth paste, allow to stand for 4 h. Mix 1 g of this paste with chloroform into the 10 ml flask and make up volume to 10 ml with chloroform then centrifuge at 4 000 rpm for about 10 min and use the supernatant liquid (filter it if suspended particles are observed) for spotting and apply one drop of the extract on the base line of the TLC plate.

G-3.3 *Henna* Powder Reference Solutions

Disperse about 3 g of *Henna* powder prepared from authentic *Henna* leaves in about 7 ml water and make a smooth paste, allow to stand for 4 h. Mix 1 g of this paste with chloroform into the 10 ml flask and make up volume to 10 ml with chloroform, then centrifuge at 4 000 rpm for about 10 min and use the supernatant liquid (filter it if suspended particles are observed) for spotting and apply one drop of the extract on the base line of the TLC plate.

G-3.4 Reference Solutions — A

Weigh individually about 50 mg ± 1 mg of reference substance like lawsone, *p*-phenylenediamine, *p*-aminophenol, *m*-aminophenol and 2-nitro-*p*-phenylenediamine in 10 ml flask. Dissolve and dilute to 10 ml with chloroform. Additionally, if required, any other suspected dyes may also be prepared separately.

G-3.5 Reference Solutions — B (To be used only for Type 2 and Type 3 Sample)

Extract separately about 1 g of reference sample of natural ingredients like *Amla*, *Shikakai*, *Neem*, *Bringaraj*, *Arishtaka*, *Haritaki*, *Jatamansi*, *Baheda*, *Brahmi*, aloe vera powder etc in 10 ml of chloroform. Centrifuge the resulting mixture to obtain supernatant liquid and use the supernatant liquid for spotting.

NOTE — Preservatives to be used as per IS 4707 (Part 2). Any interference need to be checked by *Henna* manufacturer as per preservative used in formulation.

G-4 PROCEDURE

G-4.1 Pour 15 ml to 20 ml of mobile phase into the 250 ml narrow beaker (developing chamber) and

cover with a suitable petri dish using silicone wax as a sealant.

G-4.2 Apply to a chromatography plate, about 5 µl of each of above described reference solutions and sample solution along a line approximately 1.5 cm from the edge of the plate.

G-4.3 Place the plate in a tank previously saturated with the development solvents and allow to develop at room temperature in the tank until the solvent front moved about 80 percent of plate height.

G-4.4 Remove the TLC plate allow the plate to dry completely and place a plate in iodine chamber or UV chamber to develop the chromatogram.

G-5 IDENTIFICATION

G-5.1 Compare the R_f value and the colour obtained from the sample with those of the reference substances chromatographed and identify spots. Compare the TLC pattern of the sample with that of the lawsone reference sample and standard *Henna* powder reference sample which is prepare d from authentic *Henna* leaves.

G-5.2 Any other spots[1)] on the chromatogram of sample other than lawsone, chlorophyll and spots due to standard *Henna* and added natural ingredients obtained on chromatogram of reference sample indicates the possible presence of extraneous dyes which needs to be confirm. In case, unknown spots from suspected chemical dyes are observed, further testing be done by applying the standards of suspected synthetic dyes and check for matching R_f values and spot characteristics.[2)]

Representative elution order of above samples with some commonly used synthetic adulterants (lowest R_f to highest R_f): *P*-Phenylenediamine, *p*-aminophenol, *m*-aminophenol, 2-nitro-*p*-phenylenediamine, lawsone[3)], chlorophyll.

G-5.3 Confirmation of doubtful identification may sometimes be achieved by a spiking method, adding the corresponding reference substance solution to the sample extract or if sample shows additional spots, confirmation is to be done by scraping the spot and conducting the UV or IR spectrum.

[1)] Since the *Henna* (*mehendi*) is a natural product, TLC pattern may slightly vary depending upon the change in weather conditions, age of plant, season and different agro-climatic locations. Hence, if other spots are observed in addition to the spots due to chlorophyll and lawsone this needs to be reconfirmed with TLC pattern of *mehendi*. Confirmatory may be necessary to conclude the presence of extraneous dyes. This may be done by scraping out the respective spot and solubilizing and checking using other analytical methods like Spectrophotometric, HPLC etc.

[2)] If the concentration of one or more of the substances found in the sample is excessive, dilute the sample extract and repeat the test.

[3)] A reddish orange spot due to lawsone will elute, and the greenish spot due to chlorophyll will be coincident with liquid front. Other spots due to the addition of natural ingredients may also be seen. These spots may interfere with the chemical/synthetic dye ingredients/pigments present in the sample if any. In such case alternative validated chromatographic conditions are acceptable in order to improve/achieve clear separation, or reproducibility. Responsibility of changes in chromatographic conditions lies with manufacturer.

ANNEX H

[*Table* 1, *Sl No.* (vi)]

DETECTION OF PICRAMIC ACID/SODIUM PICRAMATE ACID BY TLC

H-1 APPARATUS

H-1.1 Volumetric Flasks — 10 ml

H-1.2 Analytical Balance

H-1.3 Development Chamber or Beaker — spotless

H-1.4 Glass Rods

H-1.5 Pipette — (1 ml × 10 ml)

H-1.6 TLC Plates — (pre-coated silica gel 60 F_{254}, 0.2 mm thickness)

H-1.7 Silicone Wax

H-1.8 Syringe — 5 µl or 10 µl capacity

H-2 REAGENTS AND CHEMICALS

H-2.1 Acetonitrile

H-2.2 Methanol

H-2.3 Glacial Acetic Acid

H-2.4 Lawsone — reference standard

H-2.5 Picramic Acid — reference standard

H-3 PREPARATION OF PLATES

Mix 10 g of the silica gel (of TLC grade, particle size 10 µm to 40 µm) with 20 ml of distilled water to make a slurry and spread over glass plates to a depth of 250 microns. Activate the plates for 30 min, by keeping in an oven maintained at 105 °C.

NOTE — Precoated silica gel 60 F_{254} plate may also be used.

H-4 PREPARATION OF LAWSONE REFERENCE SAMPLE

Dissolve about 10 mg of lawsone reference sample with 10 ml of chloroform and apply about 5 µl of the extract on the base line of the plate.

H-5 PREPARATION OF PICRAMIC ACID REFERENCE SAMPLE

Dissolve about 10 mg of picramic acid reference sample with 10 ml of chloroform and apply about 5 µl of the extract on the base line of the plate.

H-6 PREPARATION OF *HENNA* POWDER REFERENCE SAMPLE

Disperse about 3 g of *Henna* powder prepared from authentic *Henna* leaves in about 7 ml water and make a smooth paste, allow to stand for 4 h. Mix 1 g of this paste with chloroform into the 10 ml flask and make up volume to 10 ml with chloroform, then centrifuge at 4 000 rpm for about 10 min and use the supernatant liquid (filter it if suspended particles are observed) for spotting and apply one drop of the extract on the base line of the TLC plate.

NOTE — More time may be required to ensure appropriate extraction of lawsone from the leaf powder.

H-7 PREPARATION OF SAMPLE

Disperse about 3 g of test sample in water and make a smooth paste, allow to stand for 4 h. Mix 1 g of this paste with chloroform into the 10 ml flask and make up volume to 10 ml with chloroform. Then centrifuge at 4 000 rpm for about 10 min and use the supernatant liquid for spotting and apply about 5 µl of the extract on the base line of the plate.

NOTE — More time may be required to ensure appropriate extraction of lawsone from the leaf powder.

H-8 DEVELOPMENT OF CHROMATOGRAM

Keep the prepared plates in a jar containing a mixture of acetonitrile : methanol : glacial acetic acid (9 : 1 : 0.05). Observe the spots obtained as such and under ultra violet light.

Elution order: (lower R_f to higher) lawsone, picramic acid, chlorophyll

H-9 OBSERVATION

Compare the TLC pattern due to sample solution with that of lawsone reference sample, picramic acid and *Henna* powder reference sample which is prepared from authentic *Henna* leaves.

Check for spot corresponding to picramic acid in colour and characteristics for the sample on the plate. This indicates the presence of picramic acid/sodium picramate.

NOTES

1 Picramic acid and sodium picramate gives their spots at the same R_f on the chromatograph.

2 Confirmatory test may be necessary to conclude the presence of picramic acid/sodium picramate. This may be done by scraping out the respective spot and solubilizing and checking using other analytical methods like spectrophotometric, HPLC etc.

ANNEX J

[*Table* 1, *Sl No*. (vii)]

DETERMINATION OF LAWSONE PIGMENT CONTENT

J-1 OUTLINE OF METHOD

The pigment is extracted and the lawsone content is determined by comparing the observed optical density (measured colorimetrically) with a calibration curve, relating optical density to various concentrations of 2-hydroxy-1,4-naphthoquinone.

J-2 APPARATUS

J-2.1 Spectrophotometer or Photoelectric Colorimeter — with a filter of 490 nm

J-3 REAGENTS

J-3.1 Sodium Bicarbonate Solution — 5 percent (*m/v*)

J-3.2 2-Hydroxy-1, 4-Naphthoquinone

J-4 PROCEDURE

J-4.1 Preparation of Standard Calibration Curve

Prepare 0.1 percent standard stock solution of 2-hydroxy-1,4-naphthoquinone by dissolving it in 5 percent (*m/v*) sodium bicarbonate solution. Pipette 0 ml (blank), 1 ml, 2 ml, 3 ml, 4 ml and 5 ml of 0.1 percent standard stock solution in 100 ml volumetric flasks and dilute with 5 percent (*m/v*) sodium bicarbonate solution up to the mark. Measure the optical density of these solutions having varying concentration from 0.0 ppm, 10 ppm, 20 ppm to 50 ppm of 2-hydroxy-1,4-naphthoquinone, at 490 nm with a spectrophotometer and construct a calibration curve. The stock solution should not be stored for more than one month.

J-4.2 Sample Preparation

Weigh 2.0 g of the prepared sample. Transfer it to a 100 ml volumetric flask. Add 5 percent (*m/v*) sodium bicarbonate solution and make up the volume to mark. Shake the contents of the flask every half an hour or so for about 8 h. Allow to settle overnight. Thereafter filter the solution through a filter paper and reject the first few millilitres. Take 10 ml of the filtrate in a 25 ml volumetric flask and dilute with 5 percent (*m/v*) sodium bicarbonate solution up to the mark. Further take 10 ml of this solution in a 100 ml volumetric flask and dilute with 5 percent (*m/v*) sodium bicarbonate solution up to the mark. Measure the optical density of this solution with a spectrophotometer at 490 nm.

J-5 CALCULATION

Refer to the calibration curve and determine the percent lawsone content of the sample from the curve.

ANNEX K

[*Table* 1, *Sl No.*(vii)]

DETERMINATION OF LAWSONE PIGMENT BY HPLC

K-1 CHROMATOGRAPHIC CONDITIONS

K-1.1 Column — C_{18} 250 × 4.6 mm

K-1.2 Mobile Phase : Methanol — water with 1 percent ortho-phosphoric acid (50 : 50)

K-1.3 Wavelength — 286 nm

K-1.4 Flow Rate — 1 ml/min

K-1.5 Temperature — ambient

K-1.6 Run Time — about 15 min

K-2 SAMPLE PREPARATION

Weigh 2.0 g of sample. Transfer it to a 100 ml volumetric flask. Add 5 percent (*m/v*) sodium bicarbonate solution and make up the volume to mark. Shake the contents of the flask every half an hour or so for about 8 h. Allow to settle overnight, there-after filter the solution through a filter paper and reject the first few millilitres. Take 10 ml of the filtrate in a 25 ml volumetric flask and dilute with distilled water up to the mark. Use this solution for HPLC.

K-3 STANDARD PREPARATION

Take weight 0.02 g/100 ml of standard 2-hydroxy-1,4-naphthoquinone and prepared same as described under sample preparation.

K-4 STABILITY AND STORAGE PREPARATION

The standard and sample are stable when stored in glass volumetric flask and are refrigerated.

K-5 CALCULATIONS

Lawsone pigment, percent by mass =

$$\frac{Area\ of\ sample\ \times concentration\ of\ stanard\ \times purity\ (actual)}{Area\ of\ standard\ \times concentration\ of\ sample}$$

ANNEX L

(*Foreword*)

COMMITTEE COMPOSITION

Cosmetics Sectional Committee, PCD 19

Organization	*Representative (s)*
Central Drugs Standard Control Organization, New Delhi	DR RAJEEV SINGH RAGHUVANSHI **(*Chairperson*)**
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Central Drugs Testing Laboratory, Mumbai	SHRIMATI S. U. WARDE SHRIMATI SUJATA S. KAISARE (*Alternate*)
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Directorate of Food and Drugs Administration, Bambolim	SHRIMATI JYOTI J. SARDESSAI
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In Personal Capacity (*1098, 10th Cross,7th Block, HMT Layout, Vidyaranyapura, Bangluru - 560097*)	DR SUNDARAM RAMACHANDRAN
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Headquarters:

Manak Bhavan, 9 Bahadur Shah Zafar Marg, New Delhi 110002
Telephones: 2323 0131, 2323 3375, 2323 9402 *Website*: www.bis.gov.in

Regional Offices: *Telephones*

Central : 601/A, Konnectus Tower -1, 6th Floor, DMRC Building, Bhavbhuti Marg, New Delhi 110002 — 2323 7617

Eastern : 8th Floor, Plot No 7/7 & 7/8, CP Block, Sector V, Salt Lake, Kolkata, West Bengal 700091 — 2367 0012, 2320 9474

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