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PAKISTAN STANDARD

FOR

SKIN CREAMS

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PAKISTAN STANDARDS AND QUALITY CONTROL AUTHORITY,
STANDARDS DEVELOPMENT CENTRE,
PSQCA Complex Plot # ST-7/A, Block-3,
Scheme No: 36, Gulistan-e- Johar,
Karachi.

PAKISTAN STANDARDS AND QUALITY CONTROL AUTHORITY
STANDARDS DEVELOPMENT CENTRE
(CHEMICAL DIVISION)

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PAKISTAN STANDARD SPECIFICATION
FOR
SKIN CREAMS

0. FOREWORD

This Pakistan Standard was adopted by the Pakistan Standards and Quality Control Authority on 28, February, 2017 after the draft finalized by the Cosmetics and Toilet Goods Technical Committee had been approved by the National Standards Committee for Chemical.

0.1 This standard was originally issued in 1972, first revised in 1978 and 2nd revision in 2016. The Technical Committee responsible for its formulation has decided to revise it in the light of experience gained since its publication. In order to allow new innovations in skin creams, requirement limit of total fatty substance content has been lowered in this revision, since the cosmetic industry has successfully produced acceptable creams with lesser content of fatty matter making it less sticky and oily on skin. Skin creams should not be the cause of bacteriological and fungal contamination. In this revision, a requirement limit for microbial content has been specified, while requirement of *pH* has been modified. Important marking requirements for best use before, list of key ingredients on containers and ECO Mark certification have also been incorporated in this revision.

0.2 Recently, skin whitening creams are being manufactured and marketed in local markets. A large segment of population uses these creams with the belief that they looked fairer. It is reported that these creams contain mercury, corticosteroid, hydroquinone and other harmful substances to a level that are hazardous to health. Skin specialists and the concerned have expressed their concern over the hydroquinone and mercury containing creams. In view of this health impact, the technical committee decided to revise the standard. In revising the standard the committee gave due attention to views of the consumers, manufacturers, trade bodies and other stakeholders.

0.3 In this Pakistan Standard, heavy metals and / or organic compounds such as lead, arsenic, mercury, corticosteroid and hydroquinone shall not be added as ingredients in cosmetic products. On the other hand, it is deemed that despite being manufactured from pure raw materials, cosmetic products might get contaminated by heavy metals coming from water, machine and environment which create confusion to the mind of stakeholders. To remove this contradiction it is felt necessary to revise the standard.

0.4 Specialized skin creams, which have an effect on the physiological functions of the body or for which therapeutic claims are generally made, are not included in this standard

0.5 This standard is intended chiefly to cover the technical provisions relating to the supply of material & it does not include all the necessary provisions of a contract.

0.6 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 or analysis shall be rounded off in accordance with PS: 103. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

1. SCOPE

This standard prescribes the basic requirements, test methods and sampling for physical and chemical properties and for the marking and labelling of skin cream for use by public. The creams with the intended use other than general purpose as defined under this standard shall be subject to Drug Act. This standard also does not apply to the creams for which therapeutic claims are made.

2. DEFINITIONS

For the purpose of this standard, the following definitions shall apply:

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2.1A COSMETICS PRODUCT:-

Any substance or preparation intended for applying on various external parts of the human body such as skin, hair, nails, lips, external genital organs, teeth and the mucous membranes of the oral cavity or products intended to come into contact with the mucous membranes include those used in the vicinity of the eyes, on the lips, in the oral cavity or on the external genital organs, with a view exclusively or principally to cleaning them, perfuming them or protecting them in order to keep them in good conditions, change their appearance or correct body odours. This standard does not cover the products that come into brief contact with the skin such as shampoos and shower gel. Cosmetic products do not have any effect on the physiological functions of the body and do not provide any therapeutic claims.

2.2 CREAMS – Cosmetics preparations which are semi-solid at ambient temperatures. creams are made by mixing oil with water. They tend to be moisturizing in action and may or may not contain active ingredients such as antioxidants or skin lighteners.

2.3 Cosmetic Whitening (Fairness) / Beauty Cream: Creams that are applied to the skin in an attempt to lighten skin tone or provide an even skin complexion . These should not include Mercury, Corticosteroids and Hydroquinone as an ingredient.

2.4 **GERNERAL SKIN TPYES** –

a) Oily types of skin – A greasy skin, due to large number of oil-secreting glands per given area of the skin or due to hyper-activity of such glands.

b) Dry type of skin – A skin with fine pores characterized by flaking and tautness due to few oil-secreting glands or due to under-activity of such glands.

c) Normal or combination type of skin – A skin which is dry only at some areas oily at others.

3. CLASSIFICATION

For specially made creams, for dry or oily skin, in clause 2.13, it is recommended that they be labelled as follow:

i) 'For dry skin'.
ii) 'For oily skin'.
iii) 'For normal skin'.

4. **GENERAL REQUIREMENTS**

4.1 The skin cream shall be in the form of an emulsion. It shall be white, or pigmented, or of uniform colour, and free from visible impurities.

4.2 There shall be no hard particles formed from the rubbing of the creams on to a freshly cleaned skin.

4.3 INGREDIENTS

4.3.1 All ingredients shall be of Halaal origin and in case of imported items an authentic certificate to that effect will be mandatory. Clause 2.7.1 of PS: 5319-2014 for "GENERAL GUIDELINES FOR HALAAL COSMETICS AND PERSONAL CARE PRODUCTS|" shall be applied.

4.4 Unless specified otherwise, all the raw materials used in the manufacture of skin creams shall conform to the requirements prescribed in the relevant Pakistan Standards where such standards exist.

4.5 The cream shall contain reasonable amount of the permissible ingredients necessary to affect the intended use as stipulated in the definitions in clause 2.

4.6 The manufacturers shall certify that all the ingredients used, e.g. fatty matter, extracts from natural herbs, dyes, perfumes etc shall be in pure form and shall not be harmful or irritating to the skin.

4.7 Product shall be safe for its intended application.

5. Skin creams shall also comply with the requirements given in Table 1 when tested

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according to the methods described therein.

TABLE 1.
REQURIEMENTS FOR GENERAL PURPOSE CREAMS

SL #	CHARACTERISTIC	REQUIREMENT	METHOD OF TEST TO (REF. TO APPENDIX) *
	Thermal stability	To pass the test	A
i	pH range	4.0 - 9.0	B
iii	Total fatty substance content, percent by mass, min.	5.0	C
iv	Microbial content / limit (a) Total viable count cfu/ g (b) Gram negative pathogens	Not more than 1000 Less than 10	D
v	Heavy metals ppm (as Lead) Max.	20	E
vi	Arsenic (as As_2O_3), ppm, Max.	2	F
vii	Mercury (as Hg), ppm, Max.	1	G
viii	Hydroquinone	Shall be absent	H
ix	Corticosteroid substance	Shall be absent	-

*The relevant testing method of PS, ISO, ASTM, FAO, WHO, International recognized standard method or self developed validated methods, may be taken into account for analysis purpose. (For ref. microbiological limits ISO 17516: 2014).

6. LABELLING AND MARKING

6.1 The labelling for general purpose creams shall be clear and truthful in all respects and shall state the intended uses, e.g. moisturizing cream vanishing cream, cleaning cream, etc.

6.2 It is recommended that the labelling should contain the instructions of use.

6.3 MARKING – The package shall be legibly marked with the following information:

i) Name of cream / Type of cream

ii) Manufacture's name and/or registered trade mark.

iii) Net amount in gm or ml.

iv) Batch number/code number.

v) Date of manufacture/expiry.

vi) List of key ingredients; and

vii) Any other information required by statutory authorities.

viii) The containers may also be marked with the Standard Mark. The use of the Standard Mark is governed by the provisions of the PSQCA Act VI, of 1996 and the Rules and Regulations made there under.

7. PACKING

The products shall be packed in suitable containers to ensure stability during storage and protection from damage during transportation.

8. METHODS OF TEST

8.1 QUALITY OF REAGENTS – Unless specified otherwise, Analytical Grade (ARG) chemicals (chemicals that do not contain impurities which affect the result of analysis) and distilled water (an electrical conductivity of not more than 11 µS/cm and total dissolved solids of less than 10 mg/litre) shall be used in the tests/analysis.

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8.2 The tests shall be carried out as prescribed in the appendices below, or accordance to any other suitable standard methods.

APPENDIX A

DETERMINATION OF THERMAL STABILITY

A-1 **APPARATUS**

A thermostatically controlled oven, capable of maintaining a temperature of 45 $\pm$ 1°C.

A-2. **PROCEDURE**

Place a fresh, un-opened sample of the cream, in its original container, into the thermostatically controlled oven at 45 $\pm$ 1°C for 48 hours, making sure that the sample is securely sealed.

If the product is packed in a opaque container (e.g. tube) remove 50 g of the sample and place it into an affectively sealed tube or vial, and test as above.

A-3 **RESULTS**

The product shall be taken to have passed the test if, on removal form the oven, the following indications of instability are not observed:

(i) Change of colours.

(ii) Change in smell or odour.

(iii) Phase separation.

(iv) Formation of granules or crystal growth.

(v) Shrinkage due to evaporation of water.

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APPENDIX B

DETERMINATION OF pH

B-1 **APPARATUS**

A pH meter, preferably equipped with a glass electrode.

B-2. **PROCEDURE**

B-2.1 FOR OIL – IN – WATER EMUSLTION CREAMS – Weigh accurately 5 $\pm$0.01 g of the cream in a 100 ml beaker. Add 45 ml of water and disperse the cream in it. Determine the pH of the suspension at 25°C using the pH meter.

B-2.2 FOR WATER – IN – OIL EMUSLTION CREAMS – Weigh 10 g of the cream to the nearest 0.1 g. Add 90 ml of rectified spirit previously adjusted to pH 6.5 to 7.0 Warm, if necessary, to 45°C and stir thoroughly for 15 minutes. Filter the alcoholic layer through a filter paper and measure the pH of the filtrate at 25 °C using the pH meter.

NOTE: - Determine the type of cream: a small amount of water soluble dye, such as methylene blue is added to the emulsion, now if water is the continuous phase (O/W emulsion), dye will dissolve uniformly throughout the system. If oil is the continuous phase (W/O emulsion), dye will remain as cluster on

the surface of the system. In case of doubt, matter is confirmed by electricity whether the product is capable of conducting electricity: if so the cream is deemed to be water-continuous phase.

APPENDIX C

DETERMINATION OF TOTAL FATTY SUBSTANCE CONTENT

C-1. PRINCIPLE OF THE METHOD

The emulsion is broken with dilute mineral acid and the fatty matter is extracted with petroleum ether. It is weighed after removal of the solvent.

C-2. REGAGENTS

C-2.1 DILUTE HYDROCHLORIC ACID – **1:1 (v / v).**

C-2.2 PETROLEUM ETHER – B.P 40 °C to 60 °C**.**

C-2.3 METHLY ORANGE INDICATOR SOLUTION – Dissolve 0.1 g of methyl orange in 100 ml of water.

C-2.4 SODIUM SULPHATE – Desiccated**.**

C-3. PROCEDURE

Weigh accurately about 2 g of the material into a conical flask, add 25 ml of dilute hydrochloric acid, fit a reflux condenser into the flask and boil the contents until the solution is perfectly clear. Pour the contents of the flask into a 300-ml separating funnel and allow it to cool to 20 °C. Rinse the conical flask with 50 ml of petroleum ether in portions of 10 ml. Pour the Petroleum ether rinsing into the separating funnel, shake the separation funnel well and leave until the layers separate. Separate out the aqueous phase and shake it out with 50 ml portions of Petroleum ether twice. Combine all the Petroleum ether extracts and wash them with water until free of acid (when tested with methyl 1 orange indicator solution). Filter the Petroleum ether extracts through a filter paper containing sodium sulphate into a conical flask which has been previously dried at a temperature of 60 ± 2 °C and then weighed. Wash the sodium sulphate on the filter with Petroleum ether and combine the washing with the filtrate. Distil off the Petroleum ether and dry the material remaining in the flask at a temperature of 60 ± 2°C to constant mass.

C-4. CALCULATION

Total fatty substance, percent by mass = $100(M_1/M_2)$

Where,

M_1 = mass in g of the residue, and

M_2 = mass in g of the material taken for the test.

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APPENDIX D

MICROBIOLOGICAL EXMINATION OF SKIN CREASM AND LOTIONS

D-1. **OUTLINE OF THE METHOD**

The test consists of plating a known dilution of the sample or any digest agar medium (soya bean casein is recommended) suitable for the total count of aerobic bacteria and fungi after incubating them for a specified period to permit the development of visual colonies.

IMPORTANT: Take precaution in ascertaining that only fresh samples, from carefully sealed containers that had not been opened before, are used for this test. This is very necessary for getting accurate results.

D-2. **APPARTUS**

D-2.1 TUBES – Of resistant glass, provided with closely fitting metal caps.

D-2.2 AUTOCLAVES – Of sufficient size. They shall keep uniform temperature within the chamber up to and including the sterilizing temperature of 122. They all be equipped with an accurate thermometer, located so as to register the minimum temperature within the sterilizing chamber, a pressure gauge and properly adjusted safety values.

D-2.3 PETRI DISHES – Of 100 ml diameter and 15 mm depth. The bottom of the dishes shall be free form bubbles and scratches and shall be flat. Disposable, single use plastic petri dishes (90 mm diameter and 15 mm depth) or reusable glass petri dishes may be used.

D-3.4 COLONY COUNTER – An approved counting aid, such as Quebec colony counter. If such a counter is not available, counting may be done with a lens giving a magnification of 1.5 diameter. In order to ensure uniformity of conditions during counting, illumination equivalent to that provided by the Quebec colony counter shall be employed.

D-3. **MEDIA AND BUFFER**

D-3.1 SOYA BEAN CASEIN DIGEST AGAR MEDIUM – Dissolve 15.0 g pancreatic digest of casein, 5.0 g of Papaic digest of soybean meal, 5.0 g of Sodium chloride, 15.0 g of Agar or the dehydrated complete medium in 1000 ml water by mixing while heating. Dispense the medium into suitable containers. Sterilize in the autoclave at 121°C for 15 min. After sterilization and cooling down, the pH shall be equivalent to 7.3 ± 0.2 when measured at room temperature.

D-3.2 STOCK SOLUTION PH 7.2 PHOSPHATE BUFFER – Dissolve 34 g of monobasic potassium phosphate in about 500 ml water contained in 1000 ml volumetric flask. Adjust the pH to 7.2 ± 0.1 by the addition of sodium hydroxide solution (4 %). Make up the volume with water and mix. Sterilize at 122 °C for 20 minutes, and store under refrigeration.

D-3.3 DILUTE PHOSPHATE BUFFER SOULTION PH 7.2 Dilute 1 ml of stock solution with distilled water in the ratio of 1:800. Fill 50 ml in each of the conical flasks of 100 ml capacity. Plug the flasks with cotton and sterilize at 122 °C for 20 minutes.

D4. **STERILIZING OF APPARATUS**

D4.1 TUBES – These shall be sterilized in the autoclave at 122 °C temperature and 1.05 Kg/cm^2 pressure for 20 minutes or in a hot air oven at 160 °C for one hour.

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D4.2 PETERI DISHES – These shall be packed in drums and autoclaved at 122 °C temperature and 1.85 kg/cm^2 pressure for 20 minutes or individually wrapped in Kraft paper and sterilized in a hot air oven at 160 °C for one hour.

D4.3 PIPETTES – These shall be placed in pipette cones (copper, stainless steel or aluminium) after plugging the broader end with cotton and sterilized in the autoclave at 122 °C temperature and 1.05 kg/cm2 pressure for 20 minutes, or at 160 °C for one hours in the air oven.

D5. **PROCEDURE**

D5.1 Melt a sufficient number of soya bean casein digest agar medium tubes in a hot water bath and transfer while hot into a constant temperature water bath maintained at 48 $\pm$ 2 °C.

D5.2 Weigh and transfer aseptically 1 g of the sample to a conical flask containing sterile 50 ml or any suitable dilution factors of dilute phosphate buffer at pH 7.2. Shake well. Pipette out in 1 ml portions into three sterile Petri dishes. Pour melted and cooled (at 45 °C) soya bean casein digest agar medium over it, and rotate the plates to mix thoroughly. Incubate at 32 °C for 72 hours in an inverted position.

D6. **EXPRESSION OF RESULT**

Get the average number of colonies on soya bean casein digest agar medium plates and determine the number of micro-organisms per gram of the sample. If no colony is recovered from any of the plates micro-organisms can be stated as being less than 50 per gram.

APPENDIX - E

TEST FOR HEAVY METLAS

E-1 OUTLINE OF THE METHOD The colour produced with hydrogen sulphide solution is matched against that obtained with standard lead solution.

E-2 APPARATUS

E-2.1 Nessler Cylinders — 50-ml capacity.

E-3 REAGENTS

E-3. I Dilute Hydrochloric Acid — Approximately 5 N.

E-3.2 Dilute Acetic Acid — Approximately 1 N.

E-3.3 Dilute Ammonium Hydroxide —Approximately 5N

E-3.4 Hydrogen Sulphide Solution — Standard.

E-3.5 Standard Lead Solution — Dissolve 1.600 g of lead nitrate in water and make up the solution to I 000 ml. Pipette out 10 ml of the solution and dilute again to 1000 ml with water. One millilitre of this solution contain 0.01 mg of lead (as Pb).

E-4 PROCEDURE Weigh about 2,000 g of material in a crucible and heat on a hot plate and then in a muffle furnace to ignite it at 600°C to constant mass. Add 3 ml of dilute hydrochloric acid, warm (wait till no more dissolution occurs) and make up the volume to 100 ml. Filter the solution. Transfer 25 ml of the filtrate into a Nessler's cylinder. In the second Nessler's cylinder, add 2 ml of dilute acetic acid, 1.0 ml of standard lead solution and make up the volume with water to 25 ml. Add 10 ml of hydrogen sulphide solution to each Nessler cylinder and make up the volume with water to 50 ml. Mix and allow to stand for 10 min. Compare the colour produced in the two Nessler's cylinders. Blank determination without samples are recommended to avoid errors arising out of reagents.

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E-5 RESULTS The sample may be taken to have passed the test, if the colour developed in the sample solution is less than that of standard solution.

NOTE: Any suitable method of analysis for heavy metals, e .g. by spectroscopic instrumentation, may also be used.

APPENDIX-F

DETERMINATION OF ARSENIC

F-1 OUTLINE OF THE METHOD

Arsenic present in a solution of the material is reduced to arsine, which is made to react with mercuric bromide paper. The stain produced is compared with a standard stain.

F-2 REAGENTS

F-2.1 Mixed Acid - Dilute one volume of concentrated sulphuric acid with four volumes of water. Add 10g of sodium chloride for each 100 ml of the solution.

F-2.2 Ferric Ammonium Sulphate Solution
Dissolve 64 g of ferric ammonium sulphate in water containing 10 ml of mixed acid and make up to one litre.

F-2.3 Concentrated Hydrochloric Acid - See PS: 37

F-2.4 Stannous Chloride Solution - Dissolve 80 g of stannous chloride ($SnCl_2$, $2H_2O$) in 100 ml of water containing 5 ml of concentrated hydrochloric acid.

F-3 PROCEDURE
Carry out the test adding into the Gutzeit bottle, 2 ml of Ferric ammonium sulphate solution, 0.5 ml of stannous chloride solution and 25ml of sample solution as prepared in E-4.

For comparison, prepare a stain using 0.001 mg of arsenic trioxide.

APPENDIX G

DETERMINATION OF MERCURY

BY ATOMIC ABSORPTION SPECTREPHOTOMETRIC METHOD

(MERCURY VAPORIZER UNIT)

G-1 Principle —Digested mercury react with reducing agent to form elemental mercury.

G-1.1 Nitric acid—See PS: 34

G-1.2 Distilled water —See PS: 593.

G-1.3 Potassium permanganate solution.

G-1.4 Sulphuric acid —see PS: 38

G-1.5 Hydrocloric acid —see PS: 37.

G-1.6 Reductant—10% $SnCl_2$ stabilised in 22 mL HCl.

G-2 Quality of Reagents

G-2.1 Unless specified otherwise, AR grade chemicals and distilled waters (See PS: 593) shall be used in tests.

G-2.3 Apparatus/Equipments

G-3.1 Volumetric flasks : 50 mL, 100 mL.

G-3.2 Beaker-400 mL

G-3.3 Filter paper- No-41.

G-3.4 Centrifuge rotary machine

G-3.3 Method of sample preparation: Weigh approximately about 2.0 g of the sample into a 400 mL beaker and then add 5-10 mL of distilled water. Digest with 2.5 mL nitric acid by heating on a water bath at 70-80^oC for about 1 hour, evaporate to dryness. Mix a small amount of distilled

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water and cool the sample to room temperature. Centrifuge at 400-500 rpm for10-15 minutes and filter the solution through a filter paper.

Transfer the filtrate into a volumetric flask and make up the volume upto the mark (50 mL or 100 mL) with distiled water.

G-4 Blank preparation : 2.5 mL concentrated nitric acid and make up the volume upto mark (50 mL or 100 mL) with distilled water.

G-4.1 Instrumental parameters / Conditions

Method name- Cold Vapour Atomic Absorption Spectroscopy (CVAAS).

G-4.2 Spectrometer Parameters

G-4.3 Wavelength-253.7 nm

G-4.4 Background correction-D2

Calculation

$$\text{Mercury content (ppm)} = \frac{\text{Absorbance} \times \text{Total volume}}{\text{Weight of sample} \times 1000}$$

APPENDIX H

DETERMINATION OF HYDROQUINONE BY H.P.L.C METHOD

Method-1

1.0 Application —Applicable to skin creams

2.0 Principle—Extraction of hydroquinone using methanol and analysed by reverse phase HPLC method.

3.0 **Reagents/Chemicals**:

3.1 Water HPLC grade.

3.2 Methanol HPLC grade

4.0 **Apparatus/Equipments**

4.1 Volumetric flasks- 100 mL

4.2 Beaker -100 mL

4.3 Funnel

4.4 Whatman filter paper.

4.5 Pipette- 5 mL, 10 mL

4.6 HPLC system equipped with UV-VIS detector and 20 microlitre loop and data processor.

4.7 C-18 reverse phase coloumn (25 cm x 4.6 mm) with particle size of 5 micron.

5.0 **Procedure**

5.1 Preparation of calibration standard.

5.1.1 Weight accurately 100 mg ± 0.01mg of hydroquinone in a 100 mL Volumetric flask and dissolve it in methanol and dilute to 100mL with same solvent.

5.1.2 Dilute 0.5, 1, 1.5, 2 and 2.5mL from step 5.1.1 to 100 mL with methanol (corresponds to 5, 10, 15, 20 and 25 mg/mL of hydroquinone).

5.2 Preparation of sample

5.2.1 Weight accurately about 1g cream in to a 100 mL beaker and dissolve it in 50 mL methanol and quantitatively transfer to a 100 mL volumetric flask with methanol. Make volume to 100 mL with methanol and shake well. Filter the solution.

5.3 Preparation of mobile phase and HPLC conditions.

5.3.1 Mobile phase—Water:Methanol (90:10).

5.3.2 Set the HPLC flow rate to 1.3 mL/min and UV detection at 225 nm.

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5.3.3 Inject the standards and draw a calibration curve of concentration vs peak area of the hydroquinone.

5.3.4 Inject the sample under same conditions as standard and calculate the hydroquinone content from the linear regression.

5.4 Calculation

1 Hydroquinone content from linear regression = x ppm (mcg/mL).

% Hydroquinone = v

APPENDIX H
DETERMINATION OF HYDROQUINONE BY H.P.L.C METHOD
Method-2

1.0 Application —Applicable to creams.

2.0 REAGENTS AND MATERIALS:

2.1 Methanol HPLC Grade

2.2 Water HPLC Grade

2.3 Hydroquinone Reference Standard

2.4 Diluent: 50:50 water/methanol(v/v)

Mix 500mL of methanol with 500mL of water in a glass bottle with screw lid.

3.0 **APPARATUS AND EQUIPMENT:**

4.1 HPLC with UV-Vis detector

4.2 C18 Reverse Phase column: 250mm × 4.6 mm, 5µm

4.3 0.45 µm Nylon syringe filter

3.4 Standard laboratory glassware

3.5 Water bath maintained at 60°C

3.6 A four decimal place Analytical balance

4.0 Sampling technique:

4.1 If the sample is packed in a tube, discard initial few centimetres of product and weigh directly into the flask

4.2 If the sample is not packed in a tube, transfer with spatula and weigh.

5.0 Procedure

5.1 Preparation of calibration standard.

A. Preparation of 1000 ppm Hydroquinone standard.

5.1.1 Weigh accurately 100 mg ± 0.1mg of hydroquinone in a 100mL volumetric flask. Add 50 mL of diluent (2.4), dissolve and make up the volume with same

B. Preparation of 100ppm and 10 ppm Hydroquinone standards.

5.1.2 Pipette out 10 mL of above solution (5.1.1) to a 100 mL volumetric flask and dilute up to the mark with diluent (2.4). Resulting solution will be equivalent to 100 ppm of hydroquinone.

5.1.3 Pipette out 10 mL of above solution (5.1.2) to a 100 mL volumetric flask and dilute up to the mark with diluent (2.4). Resulting solution will be equivalent to 10 ppm of hydroquinone.
C. Preparation of calibration solutions

5.1.4 Pipette out 0.5 mL, 1 mL, 5 mL, 10 mL and 15 mL of the above solution (5.1.3) to 5 different 100mL volumetric flasks and dilute up to the mark with diluent (2.4). This will yield 0.05.0.1, 0.5, 1.0 and 1.5 ppm of hydroquinone calibration standards respectively.

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5.2 Sample preparation

5.2.1 Weigh accurately 1.0±0.1g (nearest to 0.001 gram) of skin cream sample directly into a 50 mL volumetric flask (refer section number-4.1 and 4.2). Add 25 mL diluent (2.4), close the flask and shake vigorously until a homogenous suspension is obtained. Immerse the flask in a water bath maintained at 60±2°C for 5 minutes to enhance the extraction. Cool the solution to room temperature and make up to the volume (V_1) with diluent (2.4) and shake well. Filter the above solution through a 0.45 µm Nylon syringe filter (3.3). Discard initial few mL and collect the filtrate in a 2 mL vial. Perform the HPLC determination within 24 hours of preparing the extract.

5.3 HPLC Analysis
Analyse the blank, calibration standards and sample solutions using the below mentioned HPLC conditions.

5.3.1 HPLC conditions

Mobile phase	Water: Methanol 95:5 (v/v) (Mix 950 mL of water with 50 mL of Methanol.
Flow rate	1.5 mL/min
Wavelength	295 nm
Column temperature	36°C
HPLC Column	Zorbax ODS,250 mm× 4.6 mm,5 µm or Equivalent
Injection volume	10µL
Run time	35 minutes

5.3.2 Inject blank (2.4), calibration solutions (5.1.4) and note down the peak areas.

5.3.3 Draw the calibration curve and calculate the slope.

6.0 Calculation and expression of results:

6.1 Calculate the Hydroquinone content from calibration curve
Y= mX+C
where, m= Slope of calibration curve
C= Intercept
Y= Sample area
X= Concentration of Hydroquinone (ppm)

$$\text{Hydroquinone(ppm)} = \frac{\text{(Sample Area)} \times \text{Volume prepared } (V_1) \text{ in mL}}{\text{Slope of calibration} \times \text{Weight of Sample(g)}}$$

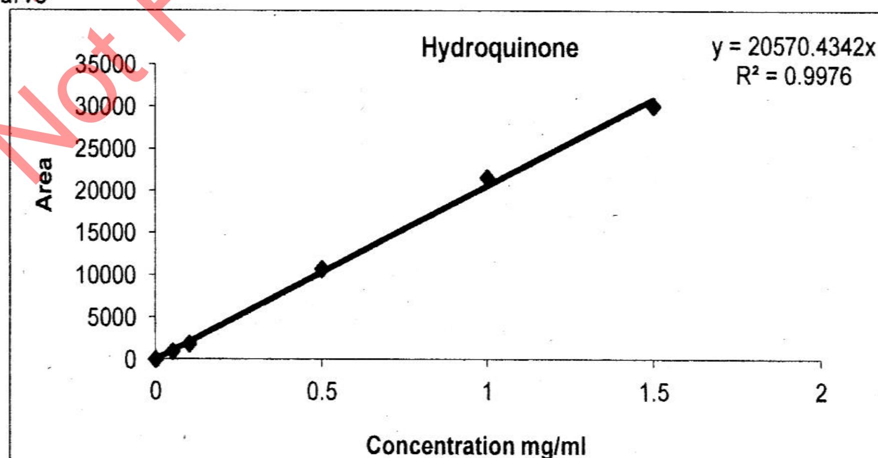

Fig – 1
For Determination of Hydroquinone

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