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ICS 71.100.70

# DRAFT EAST AFRICAN STANDARD

## Skincare special purpose product— Specification — Part 1: anti-aging

DRAFT EAST AFRICAN STANDARD FOR PUBLIC REVIEW

## EAST AFRICAN COMMUNITY

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# Contents

Page



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# Foreword

Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in the East African Community. It is envisaged that through harmonized standardization, trade barriers that are encountered when goods and services are exchanged within the Community will be removed.

The Community has established an East African Standards Committee (EASC) mandated to develop and issue East African Standards (EAS). The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the public and private sector organizations in the community.

East African Standards are developed through Technical Committees that are representative of key stakeholders including government, academia, consumer groups, private sector and other interested parties. Draft East African Standards are circulated to stakeholders through the National Standards Bodies in the Partner States. The comments received are discussed and incorporated before finalization of standards, in accordance with the Principles and procedures for development of East African Standards.

East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

The committee responsible for this document is Technical Committee EASC/TC 071, *Cosmetics and related products.*

Attention is drawn to the possibility that some of the elements of this document may be subject of patent rights. EAC shall not be held responsible for identifying any or all such patent rights.

EAS 1205 consists of the following parts, under the general title *Skincare special purpose product— Specification:*

— *Part 1: Anti-aging*

— *Part 2: Sunscreen*

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# Skincare special purpose product— Specification — Part 1: anti-aging

## 1 Scope

This Draft East African Standard specifies requirements, sampling and test methods for anti-aging/ anti-wrinkle products.

This standard does not apply to skincare products covered by EAS 786, aromatherapy substances, sun protection products and hair creams, lotions and gels.

## 2 Normative references

The following documents are referred to 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.

EAS 346, *Labelling of cosmetics — General requirements*

EAS 377 (all parts), *Cosmetics and cosmetic products*

EAS 846, *Glossary of terms relating to the cosmetic industry*

EAS 847-1, *Cosmetics — Analytical methods — Part 1: Glossary of terms*

EAS 847-16, *Cosmetics — Analytical methods — Part 16: Determination of lead, mercury and arsenic content*

EAS 847-17, *Cosmetics — Analytical methods — Part 17: Determination of pH*

EAS 847-18, *Cosmetics — Analytical methods — Part 18: Determination of thermal stability*

ISO 18416, *Cosmetics — Microbiology — Detection of Candida albicans*

ISO 21149, *Cosmetics — Microbiology — Enumeration and detection of aerobic mesophilic bacteria*

ISO 21150, *Cosmetics — Microbiology — Detection of Escherichia coli*

ISO 22717, *Cosmetics — Microbiology — Detection of Pseudomonas aeruginosa*

ISO 22718, *Cosmetics — Microbiology —Detection of Staphylococcus aureus*

ISO 24153, *Random sampling and randomization procedures*

## 3 Terms and definitions

For the purposes of this document, the terms and definitions given in EAS 846 and EAS 847-1 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

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— ISO Online browsing platform: available at http://www.iso.org/obp

**3.1**
**cream**
semi-solid emulsion which contain mixtures of oil and water as a cosmetic preparation applied to the skin. Examples include vanishing creams, foundation creams, cold creams, night creams, moisturizer creams, hand creams, face creams, body creams, toning creams, emollient creams, purifier creams, nourisher creams, facial scrub creams, facial mask creams, facial wash creams and such products among others.

**3.2**
**lotion**
thick, smooth liquid preparation designed to be applied to the skin for cosmetic purposes. Examples include vanishing lotions, foundation lotions, cold lotions, night lotions, moisturizers, cleanser lotions, hand lotions, face lotions, body lotions, toner lotions, emollients, purifiers, and nourishers and such products among others.

**3.3**
**gel**
semi-solid colloidal suspension of a solid dispersed in a liquid to be applied to the skin for cosmetic purposes. Examples include serums and such products among others".

**3.4**
**anti-aging skincare product**
cosmetic formulation designed to help reduce or mitigate the signs of aging on the skin such as but not limited to; fine lines, hyperpigmentation, age spots, dark eye circle and crow feet

**3.5**
**anti-wrinkle skincare product**
cosmetic formulation intended to reduce the appearance of wrinkles in the skin

**3.6**
**gel-based serum**
water or oil based preparation containing plant extracts, carrier oils, essential oils, and sometimes hydrosols or other materials with anti ageing properties

# 4 Requirements

## 4.1 Ingredients

**4.1.1** Skincare special purpose products shall contain antioxidants, exfoliants, moisturizers and cell regulators such as retinols, peptides and growth factors. It may contain sunscreens.

**4.1.2** All ingredients used including dyes, pigments and colours shall comply with all parts of EAS 377.

## 4.2 General requirements

Skincare special purpose products shall be:

a) clear or homogeneous and

b) free from visible impurities.

## 4.3 Specific requirements

**4.3.1** Anti-aging products shall comply with the specific requirements given in Table 1 when tested in accordance with the test methods specified therein.

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**Table 1 — Specific requirements for anti-aging products**

| S/N | Characteristic | Requirement | Test method |
|---|---|---|---|
| i. | Thermal stability | To pass test | EAS 847-18 |
| ii. | pH range | 3.5 – 8.5 | EAS 847-17 |
| iii. | Total fatty substance content[a], % by mass, min. | 5 | Annex A |
| iv. | Hydroquinone content | Not detected | Annex B |
| v. | Antioxidant activity,% Radical scavenging activity, min | 10 | Annex C |
| a applicable to products in form of creams and lotions | | | |

**4.3.2** Non-emulsified and gels anti-aging product shall comply with the specific requirements given in Table 2 when tested in accordance with the test methods specified therein.

**Table 2 — Specific requirements for Non-emulsified and gels anti-aging product**

| S/N | Characteristic | Requirement | | Test method |
|---|---|---|---|---|
| i. | pH range | Non skin lightening products | 4.5 - 8.5 | EAS 847-17 |
| | | Skin lightening products | 3.5 - 8.5 | |
| ii. | Hydroquinone content | Not detected | | Annex B |

## 4.4 Microbiological limits

Anti-aging products shall comply with the microbiological limits given in Table 3 when tested in accordance with the test methods specified therein.

**Table 3 — Microbiological limits for Anti-aging products**

| S/N | Micro-organism | Limit | Test method |
|---|---|---|---|
| i. | Total viable count for aerobic mesophyllic microorganisms CFU/g or CFU/ml, max. | 100 | ISO 21149 |
| ii. | *Pseudomonas aeruginosa* | Not detected in 1 ml or 1 g of cosmetic product | ISO 22717 |
| iii. | *Staphylococcus aureus* | | ISO 22718 |
| iv. | *Candida albicans* | | ISO 18416 |
| v. | *Escherichia coli* | Not detected in 1 g of cosmetic product | ISO 21150 |

## 4.5 Heavy metal contaminants

Anti-aging products shall comply with the heavy metal limits given in Table 4 when tested in accordance with the test methods specified therein.

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**Table 4 — Heavy metal limits for skincare special purpose creams, lotions and gels**

| S/N | Heavy metal | Maximum limit[a] mg/kg | Test method |
|---|---|---|---|
| i. | Lead | 10 | EAS 847-16 |
| ii. | Arsenic | 2 | |
| iii. | Mercury | 2 | |
| [a] The total amount of heavy metals as lead, mercury and arsenic, in combination in the finished product, shall not exceed 10 mg/kg. | | | |

## 5 Packaging

The product shall be packaged in suitable well-sealed containers that shall protect the contents and shall not cause any contamination or react with the product.

## 6 Labelling

In addition to the labelling requirements given in EAS 346, each package shall be legibly and indelibly labelled with the following:

a) product name as “Cream”, “Oil” and “Jelly”, “Lotion”, “Serum” or “Gel”, with an indication of where the product is to be applied; and;

b) an indication on whether the product is “anti-aging” or “anti-wrinkle”.

## 7 Sampling

Sampling shall be carried out in accordance with ISO 24153.

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# Annex A
(normative)

# Determination of total fatty substance content

## A.1 Outline 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.

## A.2 Reagents

**A.2.1** **Dilute hydrochloric acid,** 1:1 (v/v)

**A.2.2** **Petroleum ether,** B.P. 40 °C to 60 °C

**A.2.3** **Methyl orange indicator solution,** dissolve 0.1 g of methyl orange in 100 ml of water.

**A.2.4** **Sodium sulphate,** dehydrated

## A.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 separation 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 ether rinsings into the separation 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 ether twice. Combine all the ether extracts and wash them with water until free of acid (when tested with methyl orange indicator solution). Filter the ether extracts through a filter paper containing sodium sulphate into a conical flask which has been previously dried at a temperature of 60 °C ± 2 °C and then weighed. Wash the sodium sulphate on the filter with ether and combine the washings with the filtrate. Distil off the ether and dry the material remaining in the flask at a temperature of 60 °C ± 2 °C to constant mass.

## A.4 Calculation

The total fatty substance, expressed as percent by mass, shall be calculated as follows:

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

where

$M_1$ is the mass, in grams, of the residue; and

$M_2$ is the mass, in grams, of the material taken for the test.

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# Annex B
(normative)

# Determination of hydroquinone content

## B.1 Principle

Hydroquinone is extracted from the cosmetic product using a mixture of water and methanol in the ratio of 20:80 in an ultrasonic bath for 20 min. Determination of the analyte in the resulting solution is performed by reverse phase HPLC equipped with UV/VIS detector at a wavelength of 295 nm.

## B.2 Reagents

**B.2.1 HPLC grade water**

**B.2.2 HPLC grade methanol**

**B.2.3 Hydroquinone standard,** purity 99.9+ %

**B.2.4 HPLC grade acetonitrile**

**B.2.5 Mobile phase methanol/water** (80/20)

## B.3 Apparatus

**B.3.1 HPLC** equipped with UV/VIS detector

**B.3.2 Analytical balance** with accuracy of ± 0.000 1 g

**B.3.3 Ultrasonic bath**

**B.3.4 Amber volumetric flasks,** 10-ml, 1 000-ml.

**B.3.5 10-ml and 100-ml beakers**

**B.3.6 250-ml measuring cylinder**

**B.3.7 0.45-µm Teflon syringe filters**

**B.3.8 Aluminium foil paper**

**B.3.9 10-ml glass syringe**

**B.3.10 Stainless steel column,** length 150 mm, internal diameter 4.6 mm, C18, particles size of 5 µm or equivalent.

**B.3.11 Wash-bottle**

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## B.4 Sample preparation

### B.4.1 Preparation of the solvent mixture

Prepare the solvent mixture by measuring about 200 ml of HPLC grade water using 250-ml measuring cylinder and transfer into a 1 000-ml volumetric flask. Fill the 1 000-ml volumetric flask to the mark with HPLC grade methanol using a wash bottle. Cap the flask and shake well to ensure a homogeneous solution is obtained. Label the flask appropriately (methanol/water 80/20) and allow the resultant solution to settle.

### B.4.2 Preparation of calibration standard solutions

Calibration standard solutions are prepared as detailed in B.4.2.1 to B.4.2.8.

#### B.4.2.1 Principle

Calibration standards are gravimetrically prepared by weighing known masses of standards into a known mass of solvent to obtain the desired concentration level of the standards. A stock solution of about 1 000 mg/kg is prepared and is then serially diluted to the desired concentration level for working standards. Calibration standards are directly injected (neat) into the HPLC and their resultant peak areas are used to develop a calibration curve for quantification of the unknown concentration of samples.

#### B.4.2.2 Reagents

All reagents shall be of HPLC grade or better. The purity shall be declared.

#### B.4.2.3 Apparatus

**B.4.2.3.1** **Analytical balance,** with an accuracy of ± 0.000 1 g

**B.4.2.3.2** **Gas-tight syringes,** 1-ml, 2.5-ml, and 5.0-ml

**B.4.2.3.3** **Amber volumetric flask,** 10-ml

**B.4.2.3.4** **Amber autosampler vials,** 1.8-ml

**B.4.2.3.5** **Beaker,** 10-ml

**B.4.2.3.6** **Ultrasonic bath**

**B.4.2.3.7** **Lint-free paper towel**

#### B.4.2.4 Procedure

**B.4.2.4.1 Preparation of stock solution, 1 000 mg/kg**

**B.4.2.4.1.1** Accurately weigh a labelled volumetric flask with cap on and record mass in grams as $M_1$.

**B.4.2.4.1.2** Weigh empty 10-ml beaker and record mass in grams as $M_2$.

**B.4.2.4.1.3** Add approximately 0.01 g of the standard on the pre-weighed beaker and record mass in grams as $M_3$.

**B.4.2.4.1.4** Add about 5 ml of solvent to the beaker and sonicate on an ultrasonic bath.

**B.4.2.4.1.5** Transfer the standard solution into the pre-weighed volumetric flask, and thereafter wash the beaker twice with 2 ml of the solvent adding them to the standard solution in the volumetric flask.

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**B.4.2.4.1.6** Top the volumetric flask to the mark with the solvent, wipe with lint-free paper towel and weigh, record mass in grams as $M_4$.

### B.4.2.5 Preparation of working solutions

**B.4.2.5.1** Label five capped volumetric flasks as Level 1 to Level 5 and weigh them recording the masses in grams as $V_{E1}$ to $V_{E5}$.

**B.4.2.5.2** Aliquot 1 000 µl of the stock solution using a clean 1-ml gas-tight syringe, wipe with lint-free paper towel and weigh on the analytical balance. Record mass in grams as $WS_I$.

**B.4.2.5.3** Transfer 50 µl of the stock solution into the pre-weighed volumetric flasks, and top to the mark with solvent. Cap the flask and weigh and record the mass in grams as $V_F$.

**B.4.2.5.4** Weigh the gas-tight syringe and record mass in grams as $WS_F$.

**B.4.2.5.5** Repeat steps B.4.2.5.3 to B.4.2.5.4 for Level 2 to Level 5, transferring 100 µl, 150 µl, 200 µl and 500 µl respectively, record masses in grams as $WS_{F2}$ to $WS_{F5}$.

**B.4.2.5.6** The target concentrations ($^{mg}/_{kg}$) are 5, 10, 15, 20 and 50.

### B.4.2.6 Documentation and data control

Readings of masses shall be recorded in the analyst's workbook.

### B.4.2.7 Calculation and expression of results

#### B.4.2.7.1 Concentration of calibration standards

**B.4.2.7.1.1** The concentration of stock solution, expressed as milligrams per kilogram, is calculated using the following formula:

$$\frac{([M_3 - M_2] \times 1\,000)}{([M_4 - M_1]\,/1\,000)}$$

where

$M_1$ is the mass, in grams, of the volumetric flask with cap on;

$M_2$ is the mass, in grams, of the empty beaker;

$M_3$ is the mass, in grams, of the standard and the pre-weighed beaker; and

$M_4$ is the mass, in grams, of the volumetric flask topped to the mark with solvent and wiped with lint-free paper towel.

**B.4.2.7.1.2** The concentration of working standards is calculated using the following formula:

$$\frac{[V_F - V_E]}{[WS_I - WS_F]}$$

where

$V_F$ is the mass, in grams, of the pre-weighed volumetric flask and solvent topped to the mark;

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$V_E$ is the mass, in grams, of the empty volumetric flask with the cap

$WS_1$ mass, in grams, of the gas-tight syringe with 1 000 µl of the stock solution and wiped with lint-free paper towel; and

$WS_F$ is the mass, in grams, of the empty gas-tight syringe.

#### B.4.2.8 Expression of results

Levels ≥ 5 mg/kg but < 50 mg/kg to the nearest three significant figures.

Levels ≥ 50 mg/kg to the nearest whole number.

Levels > 1 mg/kg < 5 mg/kg to the nearest two significant figures.

### B.4.3 Sample preparation

Weigh a100-ml beaker and record the mass ($S_1$). Weigh approximately 0.5 g of sample into the pre-weighed beaker and record the mass ($S_2$). Disperse the sample in about 8 ml solvent mixture (B.4.1). Cover the beaker with aluminium foil paper (B.3.8) and place it in an ultrasonic bath (B.3.3) for 10 min. Weigh a 10-ml amber volumetric flask and record the mass ($S_3$). Transfer the sample solution into the volumetric flask and top to the mark with the solvent mixture. Weigh the volumetric flask containing sample mixture and record the mass ($S_4$). Transfer the sample into appropriately labelled amber autosampler vials for HPLC determination. Test samples within 24 h of preparation.

## B.5 Environmental control

The analysis should be carried out in a well-ventilated air-conditioned room maintained at 20 ºC ± 2 ºC since the retention time of hydroquinone fluctuates with temperature changes.

## B.6 Quality control

The stability of the retention time shall be ensured when the Relative Standard Deviation (RSD) of successive injections do not vary one from the other by more than 2 %. If the linearity is below the acceptable limit, repeat the calibration exercise.

The linearity of the calibration curve should be above 0.999XX. If the linearity is below the acceptable limit, repeat the calibration exercise.

## B.7 Procedure: High Performance Liquid Chromatography (HPLC) Setup

**B.7.1** Degas mobile phase solvents in a sonicator for 10 min and then connect solvent reservoirs to the instrument. Turn on the instrument and load the method of test in the software.

**B.7.2** Download the method into the instrument. Allow software to connect to the instrument and then open the pump valve.

**B.7.3** Press the purge button to prime the pump before use. Once purging is complete, close the valve and check if pressure is building up. Check for any air bubbles in the plumbing and purge the system if necessary. Install the appropriate column and check for any leaks. If leaks are detected, tighten the connectors.

**B.7.4** Allow the equipment to run for 20 min to equilibrate, with a different solvent other than the mobile phase flowing through (in this case, the solvent is Acetonitrile). After the equipment has stabilized, switch off the pump and switch to the mobile phase and allow the equipment to run for about 10 min. Ensure that the

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oven temperature of 40°C has been attained and any signals arising from the noise and drift of the equipment has been reduced.

**B.7.5** Adjust the flow rate of the mobile phase to 1.5 ml/min, set runtime at 5 min, the pump mode to isocratic mode, the lamp to D2, and detector wavelength set at 295 nm.

## B.8 HPLC calibration curve

To obtain a calibration curve, inject 1 µl of calibration standard solutions starting with the blank and then standards starting with the lowest concentration. Record the peak areas/heights for each corresponding concentration level. Ensure at least five replicate injections are made for each calibration standard. Prepare the calibration curve by plotting the peak areas/heights against hydroquinone concentration, in milligrams per kilogram (mg/kg). Ensure that the results meet the acceptance criteria stipulated in B.6.

## B.9 HPLC determination

Inject 1 µl of the samples and record the peak area/heights. Using the peak areas/heights of the sample, calculate the concentration of the sample using the Microsoft® Excel spreadsheet calibration curve. Ensure that at least three injections are made and the average is reported as the result if it passes the QC checks stipulated in B.6.

## B.10 Results

### B.10.1 Determination of hydroquinone concentration

Hydroquinone concentration, expressed as milligrams per kilogram, shall be calculated using the following formula:

$$\frac{\text{Peak}_{\text{Area}} - \text{Intercept}}{\text{Scope}} \times \frac{S_4 - S_3}{S_2 - S_1}$$

where

$S_1$ is the mass, in grams, of empty beaker;

$S_2$ is the mass, in grams, of beaker with sample;

$S_3$ is the mass, in grams, of the empty volumetric flask; and

$S_4$ is the mass, in grams, of volumetric flask with sample solution.

### B.10.2 Expression of results

Levels ≥ 10 mg/kg but < 100 mg/kg to the nearest three significant figures

Levels ≥ 100 mg/kg to the nearest whole number

Levels > 0.1 mg/kg < 10 mg/kg to the nearest two significant figures

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# Annex C
(normative)

# Determination of antioxidant activity

## C.1 General

The free RSA is tested using a 1, 1- diphenyl-2-picryl hydrazyl (DPPH) technique.

## C.2 materials

**C.2.1** **Analytical Reagent Methanol.** Absolute ethanol can be used in place of Methanol (AR)

**C.2.2** **UV/Vis spectrophotometer** (able to read absorbance at 517 nm)

**C.2.3** **Amber yellow glass test tubes**

**C.2.4** **Analytical balance** 0.0001g

## Procedure

**C.2.1** Dissolve 24 mg of DPPH in 100 mL of methanol for making the stock solution. Filtration of DPPH stock solution using methanol yields a usable mixture with an absorbance of around 0.973 at 517 nm. If not achieved, dilute the solution with the solvent until absorbance is below one.

**C.2.2** In a test tube, combine 3 mL DPPH workable solutions with 100 µL of sample.

**C.2.3** Three millilitres of solution containing DPPH in 100 µL of methanol kept in complete darkness for 30 minutes shall be used as is often given as a standard.

**C.2.4** keep the tubes in complete darkness for 30 min and determine the absorbance at 517 nm.

## C.3 Calculation

The antioxidant activity expressed as percent, shall be calculated using the formula below:

$$\frac{(A_C - A_S)}{A_C} \times 100$$

Where

*Ac* Control reaction absorbance; and

*As* Testing specimen absorbance.

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