FINAL DRAFT UGANDA STANDARD

FDUS EAS 342

First Edition
2013-mm-dd

Pomades and solid brilliantines — Specification

Reference number
FDUS EAS 342: 2013

Compliance with this standard does not, of itself confer immunity from legal obligations

A Uganda Standard does not purport to include all necessary provisions of a contract. Users are responsible for its correct application

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National foreword

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(a) a member of International Organisation for Standardisation (ISO) and

(b) a contact point for the WHO/FAO Codex Alimentarius Commission on Food Standards, and

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The work of preparing Uganda Standards is carried out through Technical Committees. A Technical Committee is established to deliberate on standards in a given field or area and consists of representatives of consumers, traders, academicians, manufacturers, government and other stakeholders.

Draft Uganda Standards adopted by the Technical Committee are widely circulated to stakeholders and the general public for comments. The committee reviews the comments before recommending the draft standards for approval and declaration as Uganda Standards by the National Standards Council.

This Final Draft Uganda Standard, FDUS EAS 342: 2013*, Pomades and solid brilliantines — Specification,* is identical with and has been reproduced from an East African Standard, EAS 342: 2013*, Pomades and solid brilliantines — Specification,* and is being proposed for adoption as a Uganda Standard.

Wherever the words, “East African Standard" appear, they should be replaced by "Uganda Standard."

EAS 342: 2013

ICS 71.100.70

EAST AFRICAN STANDARD

Pomades and solid brilliantines — Specification

EAST AFRICAN COMMUNITY

First Edition 2013

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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.

In order to achieve this objective, the Community established an East African Standards Committee mandated to develop and issue East African Standards.

The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the private sectors and consumer organizations. 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 procedures of the Community.

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.

EAS 342 was prepared by Technical Committee EAS/TC 071, *Cosmetics and cosmetic products.*

This second edition cancels and replaces the first edition (EAS 342:2004), which has been technically revised.

Pomades and solid brilliantines — Specification

1 Scope

This East African Standard specifies requirements and methods of sampling and test for pomades and solid brilliantines for general use.

It applies to pomades and solid brilliantines which are either vegetable oil or petroleum based but excludes oil emulsions.

This East African standard does not cover liquid brilliantines.

2 Normative references

The following referenced documents are indispensable for the application 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 377-1, *Cosmetics and cosmetic products — Part 1: List of substances prohibited in cosmetic products*

EAS 377-2, *Cosmetics and cosmetic products — Part 2: List of substances which cosmetic products must not contain except subject to the restrictions laid down*

EAS 377-3, *Cosmetics and cosmetic products — Part 3: List of colorants allowed in cosmetic products*

EAS 377-4, *Cosmetics and cosmetic products — Part 4: List of preservatives allowed in cosmetic products*

EAS 377-5, *Cosmetics and cosmetic products — Part 5: Use of UV filters in cosmetic products*

ISO 11930, *Cosmetics — Microbiology — Evaluation of the anti-microbial protection of a cosmetic product*

ISO 24153, *Random sampling and randomization procedures*

3 Definitions

For the purposes of this East African standard, the following terms and definitions shall apply.

3.1
solid brilliantine
preparation based on petroleum jelly used for skin, scalp and hair application. May be coloured and may contain perfumes

3.2
liquid brilliantine
liquid mineral, vegetable or animal oil for body and hair care

3.3
pomade
ointment used for cosmetic purposes may be greasy, soft or hard, perfumed or non-perfumed and may contain mineral oil, vegetable oil, animal fat, or a combination of the three.

4 Requirements

4.1 General requirements

4.1.1 All ingredients used in the manufacture of pomades and solid brilliantines shall comply with all parts of EAS 377.

4.1.2 The pomade and solid brilliantine shall be in the form of a soft, homogenous unctuous mass.

4.2 Specific requirements

4.2.1 The pomades and brilliantines shall comply with the chemical requirements given in Table 1 when tested according to the methods prescribed therein.

Table 1 — Chemical requirements for pomades and solid brilliantines

S/N	Characteristic	Requirements	Method of test
i.	Melting point, °C	45 to 60	Annex A
ii.	Sulphated ash, % by mass, max	0.10	Annex B
iii.	Sulphur and sulphide	To pass the test	Annex C
iv.	Penetration number, 1/10 mm	100 to 275	Annex D
v.	Test for rancidity	Shall be free from rancidity	Annex E
vi.	Bleed number	5 to 15	Annex F
vii.	Stability	To pass the test	Annex G

4.2.2 Pomades and solid brilliantines shall also comply with the microbiological limits given in Table 2 when tested in accordance with the methods indicated therein.

Table 2 — Microbiological limits

Micro-organisms	Limits, max. Cfu/g	Method of test
Total viable count	100 in 0.5 g[1)]	Annex E
	100 in 0.1 g[2)]	
Pseudomonas aeruginosa[3)]	Not detectable	Annex F
Staphylococcus aureus[3)]	Not detectable	
Candida albicans[3)]	Not detectable	

1) For products specifically intended for children under 3 years, eye area and mucous membranes

2) For other products

3) For *Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans*, the limits shall not be detectable in 0.5 g for products specifically intended for children under 3 years, eye area and mucous membranes and in 0.1 g for other products.

4.2.3 The products shall comply with the limits for heavy metal contaminants in accordance with Table 3.

Table 3 — Limits for heavy metal contaminants

S/N	Characteristic	Limitst	Method of test
I.	Lead, ppm, max.	20	Annex C
II.	Arsenic, ppm, max.	2	Annex D
III.	Mercury, ppm, max.	2	Annex E
NOTE The total amount of heavy metals as lead, mercury and arsenic, in combination, in the finished product should not exceed 20 ppm.			

6 Packaging and labelling

6.1 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.

6.2 Labelling

6.2.1 The labelling shall be in English, Kiswahili or French or in combination as agreed between the manufacturer and supplier.

6.2.2 The labelling shall comply with the requirements of EAS 346.

7 Sampling

Random samples of the product shall be drawn for test in accordance with ISO 24153 from the market, factory or anywhere else.

Annex A
(normative)

Determination of melting point

A.1 Heat a quantity of the sample on a water bath while stirring until it reaches a temperature of 90 °C to 92 °C. Cool the molten sample to a temperature of 8 °C to 10 °C above the expected melting point. Chill the bulb of a thermometer (range 1 °C to 100 °C) to 5 °C, wipe it dry and while it is still cold, dip it into the molten sample so that approximately half of the bulb is submerged. Withdraw it immediately and hold it vertically away from heat until the wax surface dulls, then dip it for 5 min into a water bath having a temperature not higher than 16 °C.

A.2 Fix the thermometer prepared in securely in a test tube so that its lowest point is about 15 mm above the bottom of the test tube. Suspend the test tube in a water bath adjusted to 16 °C and raise the temperature of the bath at a rate of 2 °C per minute upto 30 °C, then change the rate of rise to 1 °C per min and note the temperature at which the first drop of the melted sample leaves the thermometer. Repeat the determination twice on a freshly melted portion of the sample. If the variation in three determinations is less than 1 °C take the average of the three as the melting point. If the variation in three determinations is more than 1 °C, make two additional determinations and take the average of five.

Annex B
(normative)

Determination of sulphated ash

B.1 Reagents

Dilute sulphuric acid, approximately 5 N

B.2 Procedure

Heat a porcelain or silica dish of 50 mL to 100 mL capacity to redness; cool in a desiccator and weigh. Place about 20 g of the sample, accurately weighed, in the dish. Heat the dish gently by means of a Bunsen burner until the oil can be ignited at the surface. Remove the burner and allow the oil to burn completely, taking care that all the free carbon on the sides of the dish is completely burnt. Heat the residue with a strong flame or in a muffle furnace until all the carbonaceous matter has disappeared. Cool the dish; add a few drops of dilute sulphuric acid; heat gently to drive off the acid and then heat strongly. Cool the dish again in the desiccator and weigh it. Repeat the heating, cooling and weighing until constant mass is obtained.

B.3 Calculation

The sulphated ash content shall be calculated as follows:

$$\text{Sulphated ash, \% by mass} = \frac{M_2 - M_1}{M} X100$$

where

M_1 is the mass in g of the residue, and

M_2 is the mass in g of the sample taken for the test.

Annex C
(normative)

Test for sulphur and sulphides

C.1 Apparatus

Copper strips, 1 cm in width, and freshly polished

C.2 Procedure

Melt in a beaker about 100 g of the sample and keep in a water bath at a temperature of 95 °C. Then place a strip of copper in the melted sample so that it is partially immersed in it and allow to remain for 10 min.

C.3 Results

The material shall be taken to have passed the test if the copper strip used in the test shows no tarnishing when compared with another freshly polished copper strip.

Annex D
(normative)

Determination of consistency

D.1 Principle

Measuring penetration makes determination of consistency of the material of a standard cone at 25.0 °C ± 0.5 °C.

D.2 Apparatus

D.2.1 Penetrometer

Any suitable penetrometer which permits the specified cone to drop vertically without appreciable friction for at least 40 mm and which indicates accurately the depth of penetration to the nearest 0.1 mm. The instrument shall have a table to carry the test sample that may be adjusted to the horizontal before conducting the test. A mechanism for releasing and clamping the loaded cone shall be provided.

D.2.2 Cone

Consisting of a conical body of brass or corrosion resistant steel with detachable hardened steel up. The total moving mass, namely, that of the cone and its movable attachments shall be 150.0 g ± 0.1 g. The attachments consist of a rigid shaft having a suitable device at its lower end for engaging the cone. The outer surface shall be polished to a very smooth finish.

D.2.3 Constant temperature bath

A water bath capable of regulating the temperature at 25. °C and of suitable design for conveniently brining the sample container to the test temperature. The bath should be provided with a cover to maintain the temperature of the air above the sample at 25 °C.

D.2.4 Timing device

A stopwatch or any other suitable instrument capable of measuring an interval of 5 s to an accuracy of 0.2 s

D.2.5 Sample container

Flat-bottomed; metal or glass cylinders that are 100 mm ± 5 mm in diameter and not less than 60 mm in height.

D.3 Procedure

D.3.1 Melt a quantity of the sample at 82.0 °C ± 2.5 °C, pour into one or more of the sample containers, filling to within 6 mm of the brim. Cool at 25.0 °C ± 0.5 °C over a period of not less than 16 h, protecting from draughts. 2 h before the test; place the containers in a water bath at 25.0 °C ± 0.5 °C. If the room temperature is below 23.5 °C or above 26.5 °C, adjust the temperature of the cone to 25.0 °C ± 0.5 °C by placing it in a water bath.

D.3.2 Without disturbing the surface of the sample, place the container on the penetrometer table, and lower the cone until the tip just touches the top surface of the sample at a spot 25 nm to 39 mm from the edge of the container. Adjust the zero setting and quickly release the plunger, then hold it free for 5 s. Secure the plunger, and read the total penetration from the scale. Make three of more trails each so spaced that there is no overlapping of the areas of penetration. Where the penetration exceeds 20 mm, use a separate container of the sample for each trial. Read the penetration to the nearest 0.1 mm. Calculate the average of three or more readings; and conduct further trials to a total of 10 if the individual results differ from the average by more than ± 3 %.

D.4 Calculation

The consistency shall be calculated as follows:

Consistency = 10*A*

where

A is the mean of all the values of penetration in mm

Annex E
(normative)

Test for rancidity

E.1 General

Test for rancidity is mainly intended for pomades and brilliantines based on vegetable oils. This test detects any onset of incipient rancidity, arising as a result of the product being vegetable oil based.

E.2 Reagents

E.2.1 Concentrated hydrochloric acid, Analytical reagent grade

E.2.2 Phloroglucinol solution, Dissolve 0.1 g of phloroglucinol in 100 mL of diethyl ether.

E.3 Procedure

Shake 10 mL of the material, melt if necessary, with 10 mL of concentrated hydrochloric acid and 10 ml of phloroglucinol solution: Shake for 1 min.

E.4 Results

The material shall be taken to have passed the test if no pink colour develops.

Annex F
(normative)

Bleed number

Procedure

Heat the sample to 95 °C. Then allow to cool to 100 °C above its melting point. Dip a glass tube (or internal diameter 4 mm and wall thickness 1 mm) into the sample so that when it is removed with the upper end closed with a finger, it contains approximately 25 mm column of molten sample. From approximately 12 mm above the filter paper (Whatman No. 1 or equivalent), allow 5 evenly spaced drops of the sample to fall separately on the paper. The droplets should have a diameter of 6 mm - 8 mm. When the droplets solidify, place the paper on a watch glass and insert in an oven kept at 30 °C for 24 h. After 24 h, determine the diameter of each droplet plus the oil ring which surrounds it. Subtract the diameter of the droplet from the oil ring and record the result in mm. Calculate the average of these result in milimetres.

Annex G
(normative)

Test for stability

G.1 Apparatus

Ultra violet lamp , with emission at 360 mm

G.2 Procedure

Place 50 mL of the material in a 100- mL glass beaker. Turn on the ultra violet lamp and expose the samples at a distance of 12 cm - 14 cm below the lamp for 6 h. After the specified time, remove the sample, cool to room temperature and compare for any change in odour or colour. The same volume of material shall be employed for all tests so that comparison is ensured on a reproducible basis.

NOTE The output of the ultra violet lamp diminishes with time in service. A log of number of hours of the lamp in use should be maintained. The lamp is to be replaced after the specified hours of service, as recommended by the lamp manufacturer.

G.3 Evaluation

Evaluation is done by comparing the test material against an unexposed specimen from the same sample.

Annex H
(normative)

Determination of *Pseodomonas aeruginosa, Staphylococcus aureus* and *Candida albicans* in cosmetic products

H.1 Introduction

A general aseptic and safety procedures should be followed. Table H.1 gives the results of the interlaboratory study supporting the acceptance of the method.

Table H.1 — Inter-laboratory study results for determination of preservation of non-eye area water-miscible cosmetic and toiletry formulations

Incidence of false- negatives among total positive samples[a)]				Incidence of false-positives among total negative samples[b)]		
Product name	**Number**	**Percentage**	**Sensitivity rate**	**Number**	**Percentage**	**Sensitivity rate**
Shampoo	2/49	4	96	0/53	0	100
Conditioner	3/48	6	94	0/54	0	100
Water-in-oil emulsion	0/52	0	100	1/50	2	98
Oil-in-water emulsion	0/51	0	100	0/51	0	100
All combined	5/200	2	98	1/208	0.5	99.5

a) False-negative analysis indicates a sample is adequately preserved.

b) False-positive analysis indicates a sample is not adequately preserved.

H.2 Principle of the method

Bacteria yeast and mould grown on laboratory media, harvested, calibrated, and inoculated into test products. Using serial dilutions and plate counts; the numbers of organisms surviving in the test products are determined over time. Products meeting the specified criteria are considered adequately preserved for manufacture and consumer use. Products not meeting criteria are considered inadequately preserved.

H.3 Apparatus

H.3.1 Jars, 2-4 oz wide-mouth, straight-side flint glass ointment jars with linerless metal, polypropylene or teflon lined screw caps

H.3.2 Disposable borosilicate glass culture tubes, 16 mm x 125 mm, with caps

H.3.3 Disposable borosilicate glass culture tubes, 20 mm x 150 mm, with screw caps

H.3.4 Petri plates Z, 100 mm x 15 mm

H.3.5 Sterile 2.2 mL pipettes

H.3.6 Sterile swabs

H.3.7 Glass beads

H.3.8 Sterile gauze

H.3.9 10 µl -20 µl inoculating loops

H.3.10 Vortex mixer

H.4 Reagents

H.4.1 Preparation

For convenience, dehydrated media of any brand equivalent in function may be used. Test each lot of medium for sterility and growth-promotion using suitable organisms.

H.4.2 List of reagents

H.4.2.1 Letheen agar, contains 5.0 g pancreatic digest of casein 1.0 g dextrose, 3.0 g beef extract, 1.0 g lecithin, 7.0 g polysorbate 80 g, and 15.0 g agar per L. Prepare according to manufacturer's directions. Dispense into suitable containers and sterilize by autoclaving at 121 °C for 15 min. Final pH should be 7.0 ± 0.2 at 25 °C . Place in 45 °C water bath until agar is 45 °C ± 2 °C. Use for pour plates.

H.4.2.2 D/E Neutralizing broth (Dey/Engley), contains 5.0 g pancreatic digest of casein, 2.5 g yeast extract, 10 g dextrose, 1.0 g sodium thiogycollate, 6.0g $Na_2 S_2O_3 . 5H_2O$, 2.5 g $NaHSO_3$, 7.0 g lecithin, 5.0 g polysobate 80 g, and 0.02 g bromcresol purple per L.

Prepare according to manufacturer's directions. Dispense 9 mL or 9.9 mL aliquot into tubes and sterilize by autoclaving at 121 °C for 15 min. Final pH should be 7.6 ± 0.2 at 25 °C. Use for aerobic plate count, L, dilutions.

H.4.2.3 Nutrient agar, contains 5.0 g pancreatic digest of gelatin 3.0 g beef extract, and 15.0 g agar per L. Prepare according to manufacturer's directions. Dispense into tubes and sterilize by autoclaving at 121 °C for 15 min. Final pH should be 6.8 ± 0.2 at 25 °C. Cool in inclined position to form a slant. Use for bacterial culture maintenance and inoculum preparation.

H.4.2.4 Y/M agar (yeast/malt extract), contains 3.0 yeast extract, 3.0 g malt extract, 5.0 g peptone.10.0 g dextrose and 20.0 g agar per L. Prepare according to manufacturer's directions. Dispense into tubes and sterilize by autoclaving at 121 °C for 15 min. Final pH should be 6.2 ± 0.2 at 25 °C. Cool in slanted position. Use for yeast culture maintenance and inoculum preparation.

H.4.2.5 Potato dextrose agar (PDA), contains 200 g potato infusion, 20.0 g dextrose, and 15.0 g agar per L. Prepare according to manufacturer's directions. Dispense into tubes and sterile by autoclaving at 121 °C for 15 min. Final pH should be 5.6 ± 0.2 at 25 °C. Cool in slanted position. Use for mould culture maintenance and inoculum preparation.

H.4.2.6 0.85 % Saline, dissolve 8.50 g NaCI in water and dilute to 1 L. Dispense into flasks or bottles and sterilize by autoclaving at 121 °C for 15 min.

H.4.2.7 0.85 % Saline with 0.05 % Polysorbate 80, dissolve 8.5 g NaCI in water, mix in 0.50 g polysorbate 80 g, and dilute to 1 L. Dispense into suitable containers and sterilize by autoclaving at 121 °C for 15 min.

H.4.2.8 Barium sulphate standard No. 2

H.4.2.8.1 Prepare a 1.0 % $BaCl_2$ solution by dissolving 1.0 g $BaCl_2 . 2H_2O$ in 100 mL water. Let this be referred to as solution 1.

H.4.2.8.2 Prepare a 1.0 % H_2SO_4 solution by mixing 1.0 mL H_2SO_4 in 100 mL water. Let this be referred to as solution 2.

H.4.2.8.3 Mix 0.2 mL of solution (1) with9.8 mL solution (2), in screw-capped test tube. Cap tightly and store in the dark at room temperature.

H.4.2.9 Barium sulphate standard No. 7, use solutions from H.4.2.8. Mix 0.7 mL of solution

H.4.2.8.1, with 9.3 mL of solution H.4.2.8.2, in a screw-capped test tube. Cap tightly and store in the dark at room temperature.

H.5 Micro-organisms

H.5.1 *Staphylococcus aureus* — ATCC 6538

H.5.2 *Staphycloccuss epidermidis* — ATCC12228

H.5.3 *Klebsiella pneumoniae* — ATCC10031

H.5.4 *Escherichia coli* — ATCC 8739

H.5.5 *Enterobacter gergoviae* — ATCC 33028

H.5.6 *Pseudomonas aeruginosa* — ATCC 9027

H.5.7 *Burkholderia cepacia* — ATCC25416

H.5.8 *Acinetobacter baumannii* — ATCC 19606

H.5.9 *Candida albicans* — ATCC10231

H.5.10 *Aspergillus niger* — ATCC 16404

NOTE Environmental micro organisms (s) likely to be contaminants of concern during product manufacture or use be included as a separate inoculum. Predominant environmental microbes isolated during manufacturing, equipment cleaning, and sanitizing, or from related deionized water systems are used as supplemental test inocula).

H.6 Product quality check

H.6.1 Weigh 1.0 g product into a screw-capped culture tube containing 9.0 mL sterile neutralizing broth to make a 1:10 dilution. If necessary to disperse product, add ten to twenty 3-mm diameter glass beads to tube. Mix on Vortex mixer until homogeneous.

H.6.2 Pipette 1.0 mL of the 1:10 dilution into each of four sterile petri plates. Pour 15 mL- 20 mL sterile molten Letheen agar (45 °C ± 2 °C) into each plate . Mix by rotating plates to disperse the dilution thoroughly. Let solidify.

H.6.3 Invert and incubate 2 plates at 35 °C ± 2 °C for 48 h and two plates at 25 °C ± 2 °C for five days.

H.6.4 Count the number of colonies on all plates, add, and multiply by 2.5 to determine the number of colony forming units per gram (cfu./g) in the product.

H.6.5 Save plates to be used for the neutralization validation by refrigerating.

H.7 Product preparation

H.7.1 Measure 20 mL sterile saline into four sterile jars, H.3.1. Cap tightly and store at room temperature.

H.7.2 Weigh 20 g product into each of four sterile jars, H.3.1. Cap tightly and store at room temperature.

H.8 Bacterial inoculum preparation

H.8.1 Streak each bacteria culture, H.5.1 – H.5.10, onto a nutrient agar slant, H.4.2.3. Incubate for 48 h at 35 °C ± 2 ° C. Wash each slant with 5.0 mL sterile saline, loosening the culture from the agar surface. Transfer the suspension into a sterile tube. Repeat the wash with second 5.0 mL aliquot of saline. Combine washes and mix on Vortex mixer to disperse evenly.

H.8.2 Adjust each wash with sterile saline to yield a suspension of ca 10^8 cfu/mL using Mc Farland $BaSO_4$ standard No, 2, H.4.2.8, direct microscopic count, turbidimetry, absorbance, or other method correlated to an aerobic plate count (APC), H.13. Perform an APC, H.13, on each suspension to confirm standardization.

H.9 Fungal inoculum preparation

H.9.1 Streak *C. albicans,* H.5.9, on 3 slants of Y/M agar, H.4.2.4. Incubate at 25 °C ± 2 °C for 48 h. Wash each slant sequentially with 5.0 mL aliquot of sterile saline. Repeat with a second 5.0 mL aliquot of sterile saline. Combine washes to produce 10 mL suspension. Mix on Vortex mixer to disperse evenly.

H.9.2 Adjust the wash with sterile saline to yield a suspension of ca 10^7 cfu/ml using a Mc Farland Ba SO_4 standard No. 7, H.4.2.8, direct microscopic count, turbidimetry, absorbance, or other method that has been correlated to an APC, H.13. Perform an APC, H.13, on the suspension to confirm standardization.

H.9.3 Streak *A. Niger*, H.5.10, on 5 slants of potato dextrose agar H.3.5. Incubate at 25 °C ± 2 °C for 10 days. Dislodge mould spores by adding 5.0 mL sterile saline containing 0.05 % polysorbate 80 to each tube and vigorously rubbing the surface of the agar slant with a sterile swab. Repeat with a second 5.0 mL aliquot in each tube. Combine the 10 washes to produce 50 mL suspension. Filter into a sterile container through 3-5 layers of sterile gauze supported in funnel. Perform an APC, H.13, using appropriate dilutions. Adjust mould suspension to ca 10^7 per ml using sterile saline. Use immediately or refrigerate at 2 °C - 5 °C for up to 1 month. Verify mould viability by an APC, H.13, before each use.

H.10 Inoculum pools

H.10.1 Pool equal parts of the S. *aureus* and S . *epidermidis* suspensions, H.8.2 in a sterile container to make incoculum pool 1: Gram-postive cocci.

H.10.2 Pool equal parts of the K *pneumoniae* , E . *coli*, and E . *gergoviae* suspensios, H.8.2, in a sterile container to make inoculum pool 2: Gram- negative fermentors.

H.10.3 Pool equal parts of the P. *aeruginosa*, B .*cepacia* and A. *baumanii* suspensions, H.8.2, in a sterile container to make Inoculum Pool 3: Gram-negative nonfermentors.

H.10.4 Pool equal parts of C. *Albicans,* H.9.2, and A. *Niger,* H.9.3, suspensions in a sterile container to make innoculum pool 4: Fungi.

H.10.5 Use organism pools immediately or refrigerate them at 2 °C - 5 °C for more than 72 h.

H.11 Inoculation

H.11.1 Inoculate each of the four 20.0 mL aliquots of sterile saline, H.7.1, with 0.2 mL of its respective inoculum pool, H.10.1 - H.10.4. Mix thoroughly. Use these suspensions to determine inoculums counts (see Ka).

H.11.2 Inoculate each of the four 20 g products suspensions, with 2.0 mL of its respective inoculums, H.10.1 – H.10.4. Mix thoroughly by shaking, Vortex mixing or stirring, so that each suspension contains 10^6 bacteria or 10^5 fungi per gram, evenly distribute throughout the product. Tightly close inoculated containers and store at ambient temperature (20 °C - 25 °C).

H.12 Sampling intervals

H.12.1 Sample each inoculated saline suspension, H.11.1, for APC, H.13, within 1 h after inoculation to obtain inoculum count.

H.12.2 Test each inoculated product, H.11.2 for APC, H.13, at 7, 14 and 28 days after inoculation to obtain product interval count.

H.13 Aerobic Plate Count (APC)

H.13.1 Mix suspension thoroughly. Weigh 1.0 g product into screw-capped culture tube containing 9.0 mL sterile neutralizing broth for a 1:10 dilution. If necessary to disperse product, add 10 - 20 sterile 3 mm diameter glass beads to the tube. Mix on Vortex mixer until homogeneous.

H.13.2 Aseptically pipette 0.1 mL of the 1: 10 dilution into 9.9 mL tube of neutralizing broth to obtain a 1:1000 dilution. Vortex mix. Pipette 0.1 mL of the 1:1000 dilution into 9.9 mL neutralizing broth to obtain a 1: 100 000 dilution.

The number of dilutions may be decreased if previous counts microbial populations show reduction.

H.13.3 Using a 2.2 mL pipette, aseptically pipette 1.0 mL and 0.1 mL aliquots from the 1:10 dilution into duplicate petri dishes for the 1:10 and 1:100 plates. If necessary, transfer duplicate 1.0 mL and 0.1 mL aliquots from the 1: 1000 dilution for plates 1:1000 and 1:10 000, and from the 1:100 000 dilution for plates 1:100 000 and 1:1000 000. Pour 15 mL - 20 mL sterile Letheen agar H.3.1, (45 °C ± 2 °C into each plate. Mix by rotating the plates to disperse the suspension thoroughly, and let solidify.

H.13.4 Invert bacterial plates and incubate at 35 °C ± 2 °C. Examine bacterial plates after 48 h - 72 h. Count in suitable range (30 - 300 colonies). If no countable plates fall in that range, count the plate(s) nearest that range showing distinct colonies. Average duplicate plates counts and express results as cfu/g of product.

H.13.5 Invert and incubate fungal plates at 25 °C ± 2 °C. Read fungal plates at 2 - 3 days and record results. Count plates in a suitable range (30 - 300 colonies). If no countable plates fall in that range, count the plate(s) nearest that range showing distinct colonies. Re-incubate plates for another 2 - 3 days. Read and record additional colonies. Add to previous results to obtain total counts. Average duplicate plate counts and record as cfu/g of products.

H.14 Neutralization check

Make a 1:10 000 dilution in sterile saline of pools 1, 2 and 3, H.10.1 - H.10.3, and a 1:1000 dilution of pool 4, H.10.4. Streak each dilution for isolation with a 10 μl loop on the plates saved from H.6.5. If plates are not usable due to either desiccation or surface growth, repeat H.6, and streak freshly prepared plates. Incubate as in H.13.4 - H.13.5

H.15 Data analysis

H.15.1 Product quality check, H.6.4, should be found to contain, <100 cfu/g to proceed with the challenge test.

H.15.2 Inoculums counts, H.12.1, should be between 1 to 9.9 x 10^6 cfu /g product for bacteria and 1 to 9.9 x 10^5 cfu/g product for fungi, or the test should be repeated with different dilutions.

H.15.3 Neutralization check, H.14, should show significant growth of all pools to confirm adequate neutralization. A neutralizing broth other than D/E broth can be used. If neutralization does not occur, the test is invalid. Refer to references 4-6 in AOAC official method 998.10 for assistance.

H.15.4 Calculate the percentage reduction:

$$\text{Reduction},\% = \frac{\text{Inoculum count} - \text{product interval count} \times 100}{\text{Inoculum count}}$$

H.15.5 The test product is considered adequately preserved if

a) bacteria show at least 99.9 % (3 log) reduction within one week following challenge and remain at or below that level thereafter, and
b) fungi show at least a 90 % (1 log) reduction within one week following challenge, and remain at or below that level thereafter. This criteria applies to freshly prepared formulations

Annex I
(normative)

Test for lead using atomic absorption spectrophotometer (AAS)

I.1 Scope

This method describes the determination of lead in various cosmetic products. No interference occurs from the high concentrations of bismuth which do interfere in the dithzone calorimetric procedure.

I.2 Reagents

I.2.1 Lead nitrate, $Pb(NO_3)_2$

I.2.2 Dimethylacetamide DMA

I.2.3 Nitric acid HNO_3**,** 3N. Prepare by diluting 195 mL of concentrated HNO_3 (15.4 N) to one litre with deionized water.

I.2.4 Ethanol C_2H_5OH

I.2.5 Bismuth oxychloride BiOCl

I.2.6 Hydrochloric acid HCl, 6 N. Prepare by diluting 516 mL of concentrated HCl (11.6 N) to one litre with deionized water.

I.2.7 Hydrochloric acid HCl, 2 N. Prepare by diluting 172 mL of concentrated HCl (11.6 N) to one litre with deionized water.

I.2.8 Hydrochloric acid HCl, 0.5 N. Prepare by diluting 25 mL of 2 N HCl to 100 mL with deionized water.

I.3 Standard solutions

I.3.1 Lead standard solution, 100 μg/mL. Dissolve 0.1598 g of $Pb(NO_3)_2$ in 10 mL of dilute HNO_3 and dilute to 1000 mL with deionized water.

I.3.2 Lead standard solution, 1000 μg/mL in DMA. Dissolve 0.1598 g of $Pb(NO_3)_2$ in DMA. Dilute to 100 mL.

I.4 Sample preparation

I.4.1 Aerosols

For hair, deodorant spray or similar aerosols, weigh accurately about 5 g of sample and dissolve in 50 mL of ethanol. For shaving cream spray or similar aerosols weigh accurately about 1 g of sample and dissolve in 50 mL of ethanol.

I.4.2 BiOCl or cosmetics containing BIOCl

Dissolve 1 g of sample (5 g if the lead level is expected to be less than 10 μg/g) in 15 mL of 6 N HCl and dilute to 100 mL with 0.5 N HCl. If the sample is coated with an organic material, it is necessary to ignite the sample to 500 °C to ash before analysis.

I.5 Instrument conditions

I.5.1 Standard atomic absorption conditions for lead

I.5.1.1 Recommended flame air-acetylene, oxidizing (lean, blue)

I.5.1.2 Data obtained with a standard nebulizer and flow spoiler. Operation with a High sensitivity nebulizer or impact bead will typically provide a 2-3x sensitivity improvement.

I.5.1.3 Characteristic concentration with a N_2O-C_2H_2 flame at 283.3 nm: 2.7 mg/L.

Table I.1 — HCl data EDL sensitivity values for lead

Wavelength, nm	**Slit** Nm	**Relative noise**	**Characteristic concentration,** mg/L	**Characteristic concentration check,** mg/L	**Linear range,** mg/L
283.3	0.7	0.43	0.45	20.0	20.0
217.0	0.7	1.0	0.19	9.0	20.0
205.3	0.7	1.4	5.4	250.0	-
202.2	0.7	1.8	7.1	350.0	-
261.4	0.7	0.35	11.0	500.0	-
368.3	0.7	0.40	27.0	1200.0	-
364.0	0.7	0.33	67.0	3000.0	-

I.5.2 Standard flame emission conditions for lead

Stock standard solution, lead, 1000 mg/L. Dissolve 1.598 g of lead nitrate, $Pb(NO_3)_2$ in 1 %(v/v) HNO_3 and dilute to 1 litre with 1 % (v/v) HNO_3

Table I.2 — Flame emission conditions for lead

Wavelength (nm)	**Slit (nm)**	**Flame**
405.8	0.2	Nitrous oxide- acetylene

I.5.3 Light sources

Both Electrodeless Discharge Lamps (EDLS) and Hollow Cathode Lamps are available for lead. EDLs provide greater light output and longer life than Hollow Cathode Lamps. For lead, both EDLs and Hollow Cathode Lamps provide approximately the same sensitivity and detection limit. With multi-element lamps containing copper, the Cu 216.5 nm resonance line may interfere with lead determinations at the lead 217.0 nm line. The lead 283.3 nm line should be used instead.

I.5.4 Interferences

Large excesses of other elements (for example, 10 000 mg/L Fe) May interfere with the lead signal.

I.6 Analysis

Determine the concentration of lead in the sample solutions of aerosols and similar products using the standard conditions for lead and standards prepared in ethanol. The standard solutions shall be the following concentrations: 0 ppm, 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm. Determine the concentration of lead in the BiOCI solutions using the standard conditions for lead and the method of additions.

I.7 Calculations

The total amount of lead shall be calculated as follows:

$$\mu g/g\ Pb = \frac{\mu g/mL\ Pb}{g\ of\ sample} \times 50$$

I.8 Interferences

No interference was found from 1000 μg/mL of Na, Mg, K, or Ca, or from 500 μg/mL of Mn, Co or Ni. A background absorption interference was noted with 1000 μg/mL of Al, Fe or Bi, which can be eliminated by correcting the lead absorption at 283 nm by any absorption observed at 280 nm, or by using the Deuterium Background Corrector.

Annex J
(normative)

Test for arsenic using atomic absorption spectrophotometer (AAS)

J.1 Scope

This method describes the determination of arsenic in various cosmetic products.

J.2 Reagents

The reagents used should be of analytical reagent grade. Water shall be distilled or di-ionised.

J.3 Instrument conditions

J.3.1 Standard atomic absorption conditions for arsenic

J.3.1.1 Recommended flame; air-acetylene, reducing (rich, slightly yellow)

J.3.1.2 Data obtained with a standard nebulizer and flow spoiler. Operation with a High Sensitivity nebulizer or impact bead will typically provide a 2-3X sensitivity improvement.

J.3.1.3 Characteristic concentration with a N_2O-C_2H_2 flame at 193.7 M: 1.4mg/L

J.3.1.4 Table contains EDL data. HCL sensitivity values are more than 25 % poorer.

Table J.1 — HCl data EDL sensitivity values for arsenic

Wavelength, nm	**Slit,** Nm	**Relative noise**	**Characteristic concentration,** mg/L	**Characteristic concentration check,** mg/L	**Linear range,** mg/L
193.7	0.7	1.0	1.0	45.0	100.0
189.0	0.7	1.8	0.78	40.0	180.0
197.2	0.7	0.95	2.0	90.0	250.0

J.3.2 Stock standard solution

Arsenic, 1000 mg/L. Dissolve 1.320 g of Arsenious oxide As_2O_3, in 25 mL of 20 % (w/v) KOH solution. Neutralize with 20 % (v/v) H_2SO_4 to a phenolphthalein endpoint. Dilute to one litre with 1 % (v/v) H_2SO_4. The standard solutions shall be of the following concentrations: 0 ppm, 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm.

J.3.3 Flames

The air-acetylene flame absorbs or scatters more than 60 % of the light source radiation at the 193.7 nm arsenic line. Flame absorption is reduced with the use of the nitrous oxide-acetylene flame, although sensitivity is also reduced. Use of background correction is recommended, as it will correct for flame absorption and thus improve the signal to noise ratio.

J.3.4 Light sources

Both HCL and EDL sources are available for arsenic. EDLS, which are more intense, provide better performance and longer life.

J.3.5 Interferences

Sample with high total salt content (greater than 1 %) can produce non-specific absorption at the 193.7 mn arsenic line, even when the metal is absent. It is therefore advisable to set background correction.

J.4 Sample preparation

As in I.4.

J.5 Analysis

As in I.6.

J.6 Calculation

As in I.7

Annex K
(normative)

Test for mercury using the atomic absorption spectrophotometer (AAS)

K.1 Scope

This method describes the determination of mercury in various cosmetic products.

K.2 Reagents

The reagents used should be analytical reagent grade. Water shall be distilled or de-ionised.

K.3 Instrument conditions

K.3.1 Standard atomic absorption conditions for mercury

K.3.1.1 Recommended flame: air-acetylene, oxidizing (lean, blue)

K.3.1.2 Data obtained with a standard nebulizer and flow spoiler. Operation with a High Sensitivity nebulizer or impact bead will typically provide a 2-3X sensitivity improvement.

K.3.1.3 Characteristic Concentration with a N_2O-C_2H_2 flame at 253.7 nm: 12 mg/L

K.3.1.4 Table contains EDL data. HCL sensitivity values more than 25 % poorer

Table K.1 — HCl data EDL sensitivity values for mercury

Wavelength, nm	**Slit,** nm	**Relative noise**	**Characteristic concentration,** mg/L	**Characteristic concentration check,** mg/L	**Linear range,** mg/L
253.7	0.7	1.0	4.2	200.0	300.0

K.3.2 Standard flame emission conditions for mercury

Table K.2 — Flame emission conditions for mercury

Wavelength (nm)	**Slit (nm)**	**Flame**
253.7	0.2	Nitrous oxide- acetylene

K.3.3 Stock standard solution

Mercury, 1000 mg/L — Dissolve 1.080 g of mercury (II) oxide, HgO, in a minimum volume of (1 + 1) HCl. Dilute to one litre with deionized water. The standard solutions shall be of the following concentrations: 0 ppm, 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm.

K.3.4 Light sources

Both Electrodeless Discharge Lamps (EDLS) and Hollow Cathode Lamps are available for Mercury. However, the light output of mercury Hollow Cathode Lamp is significantly poorer than with EDLs, and the sensitivity and detection limit achieved also are much poorer. In addition, the life of Hollow Cathode Lamps is much shorter.

K.3.5 Interferences

Large concentrations of cobalt will absorb at the mercury 253.7 nm resonance line. A 1000 mg/L cobalt solution produces approximately 10 % absorption. Ascorbic acid, stannous chloride, or other reducing agents may reduce the mercury present to Hg (I) or elemental mercury. These give higher sensitivities than Hg (II), and their presence can generate erroneously high results.

K.4 Sample preparation

As in I.4.

K.5 Analysis

As in I.6.

K.6 Calculations

As in I.7.

Annex L
(informative)

List of raw materials conventionally used in the formulation of pomades and brilliantines

L.1 Type 1 - Based on mineral oils and waxes

a) Paraffin wax

b) Microcrystalline wax

c) Petroleum jelly

d) Mineral oil

e) Perfume and colour

L.2 Type 2 - Based on vegetable oils and waxes

a) Castor oil

b) Beeswax

c) Paraffin wax

d) Coconut oil

e) Perfume and colour

L.3 Type 3 - Based on mineral oils and fatty acids

a) Stearic acid

b) Mineral oil

c) Perfume and colour

L.4 Type 4 - Based on mixture of mineral and vegetable oils, animal fats and waxes.

a) Coconut oil

b) Mineral oil

c) Beeswax

d) Petroleum jelly

e) Lanolin

f) Perfume and colour

