KENYA STANDARD

KS 580: 2013
ICS 71.100.70

Skin care creams, lotions and gels for cosmetic use — Specification

Fifth Edition 2013

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In order to keep a breast of progress in industry, Kenya Standards shall be regularly reviewed. Suggestions for improvements to published standards, addressed to the Managing Director, Kenya Bureau of Standards are welcome.

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update

Foreword

This Kenya Standard was prepared by the Technical Committee on Cosmetics and Related Products under the guidance of the Standards Projects Committee, and it is in accordance with the procedures of the Bureau.

Skin creams, lotions and gels are useful not only as skin beautifiers, but also guard the skin against adverse climatic conditions. The performance of any skin cream, lotion or gel depends on the type of skin on which it is applied, and the nature of ingredients added to effect the intended end use.

This Kenya Standard was first issued in 1986. In the second edition, the types of preparations covered by the standard were listed in the preface. All definitions were transferred to KS 03-1509. The requirements for pH and heavy metals were also adjusted.

In the third edition, the application clause was altered to cover baby products as well as skin lightening preparations based on acids and vitamins. A clause restricting the use of hydroquinone in cosmetic preparations was also inserted. Also, the labelling clause was modified to exclude contents covered in KS 1707.

Likewise, the sampling procedures were cross-referred to a separate standard. The methods of test for heavy metals were also replaced with modern methods. A new requirement was inserted in Table 2 to cater for testing of hydroquinone, and the methods of test inserted in Appendix H.

In the fourth edition, new requirements have been included to cater for products containing herbal additives e.g. neem and aloe vera extracts.

In this Fifth Edition, the limit for Hydroquinone has been set in order to distinguish between contamination and deliberate addition of hydroquinone. Also, the Chemical requirements have been separated from the contaminants and microbiological limits.

In the preparation of this standard, reference was made to the following documents:

IS 6608 — 1972 Specification for skin creams.

IS 4011 — 1982 Methods for dermatological tests for cosmetics.

Regulation (EC) No. 1223/2009 of the European Parliament and of the Council of the European Union. (2009): **Cosmetic Products**. Brussels

The assistance derived from these sources is hereby acknowledged with thanks.

Skin care creams, lotions and gels for cosmetic use — Specification

1 Scope

1.1 This Kenya Standard prescribes the requirements and methods of test for skin care creams, lotions and gels for cosmetic use.

1.2 This standard applies to products meant for adult use, as well as baby products.

These include baby creams, baby lotions, baby gels, skin lightening preparations based on vitamins or acids etc., vanishing creams and lotions, foundation creams and lotions, cold creams and lotions, night creams, moisturizers, cleansers, hands creams and lotions, body creams and lotions, sun-protection preparations, toners, emollients, purifiers, nourishers, anti-wrinkle/aging preparations, facial scrubs, facial masks, facial wash, and any other special treatment skin preparations e.g. Alpha Hydroxy Acids (AHA's).

1.3 This standard does not apply to depilatories, body oils, or to shaving creams.

1.4 This standard does not apply to creams lotions and gels for which therapeutic or medicinal claims are made.

2 Normative references

The following Kenya Standards are necessary adjuncts to this standard:

KS -1509, *Glossary of terms relating to the cosmetics industry*

KS -1707, *Labelling of cosmetic products*

KS -1474, *Classification of cosmetic raw materials and adjuncts*

KS -1668, *Methods of sampling cosmetics*

The above-mentioned standards contain provisions, which through reference in this text, constitute provisions of this standard.

3 Requirements

3.1 General requirements

3.1.1 The preparation shall have uniform (homogenous) consistency at the point of use.

3.1.2 The cream, lotion or gel shall be free from visible impurities which are not part of the intrinsic formulation of the product.

3.2 Ingredients

3.2.1 All ingredients used including dyes, pigments and colours shall conform to all parts of KS 1474 and other relevant raw material standards where they exist .

3.2.2 All products claiming antibacterial activity shall pass the test for antibacterial activity outlined in Annex L.

3.2.3 Cosmetic creams, lotions and gels shall not contain hydroquinone, corticosteroids and hydrogen peroxide.

3.2.4 The cream, lotion or gel shall contain acceptable amounts of the ingredients necessary to effect the intended end use performance as stipulated on the label.

3.2.5 All creams, lotions and gels for skin care shall also comply with the requirements given in Table 1 when tested according to the methods described therein.

3.2.6 The products shall comply with the limits for heavy metal contaminants in accordance with Table 2.

3.2.7 Microbiological limits shall comply with the limits specified in Table 3.

Table 1 — Chemical requirements for creams, lotions and gels for skin care

SL No	Category	Requirement	Test method
i.	Hand and body lotions, creams, and gels (including baby products)	4.5 – 8.5	Annex A
	Special purpose products[1]	3.5 – 8.5	
ii.	Thermal stability	To pass test	Annex B
iii.	Total fatty substance content[2] , % by mass, min.	5	Annex C
iv.	Hydroquinone content	Not detectable (Limit of detection 10 mg/kg)	Annex K

Table 2 — Limits for heavy metal contaminants

SL No	Characteristic	Requirement	Test method
i)	Lead, ppm, max.	20	Annex D
ii)	Arsenic, ppm, max.	2.0	Annex E
iii)	Mercury, ppm, max.	2.0	Annex F

Table 3 — Microbiological examination

SL No.	Characteristic	Limits	Test method
i)	*Microbiological examination* Total viable count for aerobic mesophyllic micro-organisms per g, max.	100	Annex G
ii)	*Psedomonas aeruginosa)*	Not detectable in 0.5 g	Annex H

[1] These include cleansers, toners, purifiers, facial washes, nourishers, AHA's, skin lightening preparations based on vitamins and acids, anti-wrinkle/aging preparations, facial scrubs, facial masks, facial wash, non-emulsified lotions and gels, and such products.

[2] This test is not applicable to non-emulsified gels and lotions (solutions).

	Staphylococcus aureus) *Candida albicans)* *E coli)*	of cosmetic product	

4 Packaging and labeling

4.1 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.

4.2 Labelling

4.2.1 The labelling shall be in English and/or Kiswahili Languages, and shall comply with the requirements of KS 1707.

4.2.2 Special purpose products shall in addition bear the following warnings on the label:

a) This product shall not to be used on children below the age of 12 year;

b) This product may cause irritation. If irritation persists, discontinue use;

c) Do patch test before use.

5 Sampling

Representative unopened samples shall be drawn for test from the market or anywhere else following the procedure outlined in KS 1668. The samples shall be declared as conforming to the specification if they satisfy all the specified requirements.

Annex A
(normative)

Determination of pH

A.1 Apparatus

A.1.1 pH meter, preferably equipped with a glass electrode.

A.2 Procedure

A.2.1 For oil-in-water emulsions

Weigh 5 g ± 0.01 g of the sample in a 100 mL beaker. Add 45 mL of water and disperse the sample in it. Determine the pH of the suspension at 25 °C using the pH meter.

A.2.2 For water-in-oil emulsions

Weigh 10 g of the sample 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 min. Filter the alcoholic layer through a filter paper and measure the pH of the filtrate at 25 °C using pH meter.

NOTE: Determine the type of sample by placing some of it on spot tile and sprinkling with a mixed indicator consisting of an intimate mixture of oil soluble dye of one colour, e.g. oil orange, and a water-soluble dye of a different colour e.g. methylene blue. After a few minutes the predominant colour indicates whether the continuous phase is oil or water. In case of doubt matter is confirmed by checking whether the product is capable of conducting electricity: if so the sample is deemed to be water-continuous.

Annex B
(normative)

Determination of thermal stability

B.1 Apparatus

B.1.1 A thermostatically controlled oven, capable of maintaining a temperature of 37 °C ± 1 °C.

B.2 Procedure

Place a fresh, unopened sample of the product in its original container into a thermostatically controlled oven at 37 °C ± 1 °C for 48 h, making sure that the sample is securely sealed. If the product is packed in an opaque container (e.g. a tube), remove 50 g of the sample and place into an effectively sealed glass tube or vial, and test as above.

B.3 Results

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

i) change of colour;

ii) change of smell or odour;

iii) phase separation;

iv) formation of granules or crystal growth;

v) shrinkage.

Annex C
(normative)

Determination of total fatty substance content

C.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.

C.2 Reagents

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

C.2.2 **Petroleum**, b.p. 40 °C to 60 °C.

C.2.3 **Methyl 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 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.

C.4 Calculation

Total fatty substance, % by mass $= \frac{100M_1}{M_2}$

where,

M_1 is the mass, in g of the residue;

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

Annex D
(normative)

Determination of lead content in cosmetics by graphite furnace Atomic Absorption Spectrophotometer (AAS)

D.1 Scope

This test method specifies an electrothermal atomization technique using graphite furnace AAS method for the determination of lead content of cosmetics.

D.2 Warning and safety

The acids used in the test are highly corrosive and should be handled with maximum care and where appropriate, use a fume hood during preparation of standards. Lead is very toxic/carcinogenic and must be handled with maximum care avoiding physical contact.

If spillage occurs, use adequate amounts of water and soap to wash off the spill.

D.3 Principle

Injecting of the prepared solution into a graphite furnace. Spectrometric measurements of the atomic absorption of the 228.8 spectral line emitted by lead hollow cathode lamp.

D.4 Materials

D.4.1 Reagents, chemicals and standards

D.4.1.1 Nitric acid, ρ about 1.4 g/mL.

D.4.1.2 Nitric acid (1+1) v/v , mix 1 volume of conc. HNO_3 with 1 volume of distilled water.

D.4.1.3 Nitric acid (0.1M), place 17 mL of concentrated acid in 100 mL volumetric flask then top to the mark with distilled water and mix.

D.4.1.4 Lead standard solution, 1000 ppm

In 1 litre volumetric flask, dissolve 1.598 g of Pb $(N0_3)_2$ in minimum volume of 1 % v/v HNO_3 and finally top the mark using 1 % HNO_3.

NOTE Commercial grade standards can also be used when available.

D.4.1.5 Lead standard solution, 100 ppb

This shall be prepared freshly by serial dilution of the lead solution (4.1.4)

D.4.1.6 Purge gas, argon

Sufficiently pure, free from water and oil and free from lead.

D.4.2 Apparatus and equipment

D.4.2.1 Atomic Absorption Spectrometer fitted with graphite furnace

The Atomic Absorption Spectrometer used will be satisfactory if after optimization according to the manufacturers instructions and when in reasonable agreement with the values given by the manufacturer and it meets the performance criteria as set out in the manual.

D.4.2.2 Lead hollow cathode lamp

D.4.2.3 Ordinary laboratory apparatus

Note that all glassware shall first be washed in hydrochloric acid (ρ about 1.19 g/mL, diluted.)

D.5 Performance

D.5.1 Sample preparation

Ignite 1 g of sample at 500 °C ± 2 °C to ash. Extract the lead from the ash with 20 mL of 2N HNO_3 , and repeat with 10 ml of 2 N HNO_3. Combine the extracts and dilute to 50 mL with 0.5 N HNO_3.

D.5.2 Calibration

D.5.2.1 Preparation of calibration curve

D.5.2.1.1 Dilute the stock 100 ppb solution with 0.1M HNO_3 to obtain solutions with 10 ppb, 20 ppb, 40 ppbm, 60 ppb, 80 ppb and 90 ppb of lead.

D.5.2.1.2 Inject 20 microlitres each of the six solutions in turns at the same rate starting from the lowest concentrated solution to the highest concentrated solution.

D.5.2.1.3 Record the corresponding absorbance values and plot calibration curve.

D.5.3 Quality control checks

D.5.3.1 Duplicates

D.5.3.1.1 All samples will be analyzed in duplicates and the stated acceptance criteria shall apply: The absolute difference between two independent test results obtained using the same procedure shall not be greater than 10 % of the arithmetic mean of the two results.

D.5.3.1.2 Spike distilled water with 10.0 ppb of lead and obtain the recovery percentage.

D.5.3.1.3 Recovery % ≥ 96.

D.6 Procedure

D.6.1 Test portion

Use sample as prepared in (D.5.1).

D.6.2 Blank test

D.6.2.1 Run a parallel reagent blank determination replacing the test solution with distilled water.

D.6.2.2 Reagent blank should be ≤ 0.0001 ppb of lead.

D.6.3 Instrumentation

D.6.3.1 Follow the manufacturer's instructions for preparing the instrument for use.

D.6.3.2 Install the appropriate lamp and adjust the current to the recommended value.

D.6.3.3 Ensure that the autosampler pipette is correctly aligned and that the drain is available.

D.6.3.4 Select the sample tray type installed.

D.6.3.5 Ensure that the graphite tube is in good condition and correctly aligned.

D.6.3.6 Switch on the cooling system, turn on the purge gas and finally start the instrument software.

D.6.3.7 Select the relevant method and then condition tube.

D.6.4 Instrument conditions

The following conditions shall be used for the furnace during analysis of lead:

D.6.4.1 Wavelength: 283.3

D.6.4.2 Slit: 0.7

D.6.4.3 Atomization site: pyro/platform

Table D.1 — Furnace conditions for lead

Step	Temperature ^{0}C	Ramp time (seconds)	Hold time (seconds)	Internal gas flow (L/min)	Gas type
1 (drying)	120	10	60	250	Normal
2 (pretreatment)	700	1	30	250	Normal
3 (cooling)	20	1	15	250	Normal
4 (atomization)	1800	0	5	0	Normal
5 (cleanout)	2600	1	5	250	Normal

D.6.5 Spectrometric measurements

D.6.5.1 Inject into the flame the calibration standards, the blank solution and the test solution.

D.6.5.2 Record the absorbance reading.

D.6.5.3 If the absorbance of the sample in greater than the highest calibration standard, dilute the test solution appropriately using 0.1 M HNO_3 for lead then measure the absorbance.

D.6.5.4 Inject the calibration solutions in ascending order.

NOTE: The calibration curve shall only be acceptable for analysis when the correlation coefficient (r) ≥ 0.99.

D.7 Expression of results

D.7.1 Method of calculation

The lead content of the sample expressed in mg/L of product is equal to:

$$[(C_1 - C_2) * V] / M_0$$

where ,

C_1 is the lead content of test solution expressed in mg/L read from calibration curve;

C_2 is the lead content of blank solution expressed in mg/L read from calibration curve;

M_0 is the grams of sample taken for analysis (5 g);

V is the Volume of sample taken for analysis (100 mL).

NOTE If the test solution was diluted, then the dilution factor shall be taken into consideration in calculation.

D.7.2 Expression of results

D7.2.1 Report results of manganese content in mg/L as Pb in the sample into two decimal points.

D.8 Method validation

D.8.1 Method validation data

Table D.2 — Method validation data

Element	Linearity	LOQ ppb	LOD ppb
Pb	$r \geq 0.99$	32.356	3.804

D.8.2 Precision: Repeatability

The absolute difference between two independent tests results obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time should not be greater than 10 % of the arithmetic mean of the two results.

D.8.3 Working range

Pb 10 -100 ppb.

D.8.4 Reporting limits

Pb 30 ppb.

Annex E
(normative)

Test for arsenic using Atomic Absorption Spectrophotometer (AAS)

E.1 Scope

This method describes the determination of arsenic in foam baths and shower gels.

E.2 Reagents

E.2.1 0.15 mol/L (] 1.5 % v/v) hydrochloric acid, carefully add 15 mL conc. HCl to deionized water and make up to 1 L.

E.2.2 0.25 mol/L (] 1 % w/v) NaOH solution, carefully dissolve 10 g sodium hydroxide flakes in deionized water and make up to 1 L.

E.2.3 0.8 mol/L (] 3 % w/v) $NaBH_4$ solution, dissolve 3 g sodium tetrahydroborate in 1 % NaOH solution and make up to 100 mL with 1 % NaOH solution.

E.3 Stock solution

E.3.1 The stock solution contains 1000 mg/L As. The use of commercially available concentrated calibration solutions for AAS is recommended.

WARNING Arsenic solutions are toxic.

E.4 Calibration solution

1 mg As/L (in 1.5 % HCl)

E.4.1 Aliquots for calibration: 10, 25, 50 µL

E.4.2 Corresponding to: 10, 25, 50 ng As

E.4.3 Diluent: 1.5 % (v/v) hydrochloric acid

E.4.4 Calibration volume: 10 mL

E.5 Reductant solution

3 % $NaBH_4$ in 1 % NaOH solution.

E.6 Oxidation state

The hydride is generated much more slowly from As (V) than from As (III). To prevent interferences, As (V) must be pre-reduced to As (III) prior to the determination.

Pre-reduction can be performed with KI in semi-concentrated (5 mol/L) HCl solution or, preferably, with L-cysteine.

E.7 Pre-reduction

E.7.1 KI solution, dissolve 3 g KI and 5 g L (+)- ascorbic acid in 100 mL water. Prepare fresh daily. Add 1 mL of the KI solution per 10 mL of the sample solution in 5 mol/L HCl and stand for 30 min.

or,

E.7.2 L-cysteine solution, dissolve 5 g L-cysteine in 100 mL 0.5 mo1/L HCl. This solution is stable for at least a month. Add 2 mL of the L-cysteine solution per 10 mL of the sample solution and stand for 30 min.

E.8 Instrument conditions

E.8.1 Analytical wavelength: 193.7 nm

E.8.2 Slit width and height: 0.7 nm Low

E.8.3 Radiation source: Electrodeless discharge lamp for As.

E.8.4 QTA heating: Heat the QTA in a lean, blue air-acetylene flame.

E.8.5 Prepared measurement volume: 10 mL minimum to 50 mL maximum.

E.8.6 Pre-reaction purge time: Approx. 50 s

E.8.7 Post-reaction purge time: Approx. 40 s

E.8.8 Characteristic mass: 0.95 ng As for 1 % absorption (A = 0.0044)

E.8.9 Characteristic concentration: 0.095 μg/L 1 % absorption for 10 mL calibration volume.

E.8.10 Characteristic concentration check: 50 μL of the 1000 mg/L As stock solution (50 ng) give an absorbance of approx. A = 0.2

Alternate analytical wavelengths

Wavelength nm	Slit width nm	Sensitivity relative to main analytical wavelength
189.0	0.7	0.8
197.2	0.7	2.0

E.9 Notes

Condition the QTA in cold hydrofluoric acid if there is a decrease in sensitivity (and other causes are excluded).

Annex F
(normative)

Test for mercury using Atomic Absorption Spectrophotometer (AAS)

F.1 Method 1: Using sodium tetrahydroborate ($NaBH_4$) as reductant

F.1.1 Scope

This method describes the determination of mercury in pomades and solid brilliantines using sodium tetrahydroborate ($NaBH_4$) as reductant.

F.1.2 Reagents

F.1.2.1 0.15 mol/L (] 1.5 % v/v) hydrochloric acid, carefully add 15 mL conc. HCl to deionized water and make up to 1 L.

F.1.2.2 0.22 mol/L (] 1.5 % v/v) nitric acid, carefully add 15 mL conc. $HN0_3$ to deionized water and make up to 1L.

F.1.2.3 5% (w/v) $KMnO_4$ solution, dissolve 5 g potassium permanganate in deionized water and make up to 100 mL.

F.1.2.4 0.25 mol/L (] 1 % w/v) NaOH solution, carefully dissolve 10 g sodium hydroxide flakes in deionized water and make up to 1 L.

F.1.2.5 0.8 mol/L (] 3 % w/v) $NaBH_4$ solution, dissolve 3 g sodium tetrahydroborate in 1 % Na0H solution and make up to 100 mL with 1 % Na0H solution.

F.1.3 Stock solution

The stock solution contains 1 000 mg/L Hg. The use of commercially available concentrated calibration solutions for AAS is recommended.

WARNING Mercury solutions are toxic.

F.1.4 Calibration solution

1 mg Hg/L (in 1.5 % HNO_3 stabilized by the addition of a few drops of 5 % $KMn0_4$ solution).

F.1.4.1 Aliquots for calibration:	100, 200, 500 μL
F.1.4.2 Corresponding to:	100, 200, 500 ng Hg
F.1.4.3 Diluent:	1.5 % (v/v) nitric acid or 1.5 % (v/v) hydrochloric acid
F.1.4.4 Calibration volume:	10 mL

F.1.5 Reductant solution:

3 % $NaBH_4$ in 1 % Na0H solution

F.1.6 Instrument conditions

F.1.6.1 Analytical wavelength:	253.6 nm
F.1.6.2 Slit width and height:	0.7 nm Low
F.1.6.3 Radiation source:	Electrodeless discharge lamp or hollow cathode lamp for Hg.

F.1.6.4 QTA heating: No flame required. If condensation in the QTA is a problem, heat the QTA gently by mounting an infrared lamp above it.

F.1.6.5 Prepared measurement volume: 10 mL minimum to 50 mL maximum.

F.1.6.6 Pre-reaction purge time: Approx. 5 s

F.1.6.7 Post-reaction purge time: Approx. 50 s

F.1.6.8 Characteristic mass: 4.68 ng Hg for 1 % absorption (A=0.0044).

F.1.6.9 Characteristic concentration: 0.468 μg/L 1 % absorption for 10 mL calibration volume.

F.1.6.10 Characteristic concentration check: 250 μL of the 1000 mg/L Hg stock solution (250 ng) give an absorbance of approx. A = 0.2.

F.1.7 Notes

F.1.7.1 Stabilize stock and calibration solutions by adding $KMnO_4$ or KI solution.

F.1.7.2 Stabilize all solutions in the reaction flask by adding 1 drop of 5 % (w/v) $KMnO_4$ solution before starting the determination.

F.2 Using tin (II) chloride ($SnCl_2$) as reductant

F.2.1 Scope

This method describes the determination of mercury in pomades and solid brilliantines using tin (II) chloride ($SnCl_2$) as reductant.

F.2.2 Reagents

F.2.1.1 **0.15 mol/L (⎣ 1.5 % v/v) hydrochloric acid**, carefully add 15 mL conc. HCl to deionized water and make up to 1 L.

F.2.1.2 **1 mol/L (⎣ 10 % v/v) hydrochloric acid**, carefully add 100 mL conc. HCl to deionized water and make up to 1L.

F.2.1.3 **0.22 mol/L (⎣ 1.5 % v/v) nitric acid**, carefully add 15 mL conc. HNO_3 to deionized water and make up to1 L.

F.2.1.4 **5 % (w/v) $KMnO_4$ solution**, dissolve 5 g potassium permanganate in deionized water and make up to 100 mL.

F.2.1.5 **5 % (w/v) $SnCl_2$**, dissolve 50 g tin (II) chlroride dihydrate ($SnCl_2$ $2H_2O$) in 10 % HCl solution and make up to 1 L with 10 % HCl solution.

F.2.2 Stock solution

The stock solution contains 1 000 mg/L Hg. The use of commercially available concentrated calibration solutions for AAS recommended.

WARNING Mercury solutions are toxic.

F.2.3 **Calibration solution** 1 mg Hg/L (in 1.5 % HNO_3 stabilized by the addition of a few drops of 5 % $KMnO_4$ solution).

F.2.3.1 Aliquots for calibration: 100, 200, 500 μL

F.2.3.2 Corresponding to: 100, 200, 500 ng Hg

F.2.3.3	Diluent:	1.5 % (v/v) nitric acid or 1.5 % (v/v) hydrochloric acid
F.2.3.4	Calibration volume:	10 mL

F.2.4 Reductant solution: 5 % $SnCl_2$ $2H_2O$ in 10 % HCl solution.

F.2.5 Instrument conditions

F.2.5.1	Analytical wavelength:	253.6 nm
F.2.5.2	Slit width and height:	0.7 nm low
F.2.5.3	Radiation source:	Electrodeless discharge lamp or hollow cathode lamp for Hg
F.2.5.4	QTA heating:	No flame required. If condensation in the QTA is a problem, heat the QTA gently by mounting an infrared lamp above it.
F.2.5.5	Prepared measurement volume:	10 mL minimum to 50 mL maximum.
F.2.5.6	Pre-reaction purge time:	Approx. 5 s
F.2.5.7	Post-reaction purge time:	Approx. 50 s
F.2.5.8	Characteristic mass:	4.68 ng Hg for 1 % absorption (A = 0.0044).
F.2.5.9	Characteristic concentration:	0.468 μg / L / 1 % absorption for 10 mL calibration volume.
F.2.5.10	Characteristic concentration check:	250 μL of the 1000 mg/L Hg stock solution (250 ng) give an absorbance of approx. A = 0.2.

F.2.6 Notes

F.2.6.1 Stabilize stock and calibration solutions by adding $KMnO_4$ solution. Do not use KI solution since iodide interferes in the release of mercury.

F.2.6.2 Stabilize all solutions in the reaction flask by adding 1 drop of 5 % (w/v) $KMnO_4$ solution before starting the determination.

Annex G
(normative)

Microbiological examination

G.1 Outline of the method

The test consists of plating a known dilution of the sample or any digest agar medium (soyabean 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.

G.2 Apparatus

G.2.1 Tubes, of resistant glass, provided with closely fitting metal caps.

G.2.2 Autoclaves, of sufficient size. They shall keep uniform temperature within the chamber up to and including the sterilizing temperature of 122 °C. They shall 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 valves.

G.2.3 Petri dishes, of 100 mm diameter and 15 mm depth. The bottom of the dishes shall be free from bubbles and scratches and shall be flat so that the medium is of uniform thickness throughout the plate.

G.2.4 Colony counter, an approved counting aid, such as a 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.

G.3 Media and buffer

G.3.1 Soyabean casein digest agar media, dissolve 1.5 g of pancreatic digest of casein, 5 g of papic digest of soyabean meal; and 5 g of sodium chloride in 100 mL of distilled water contained in a 2-litre beaker by heating in a water-bath. Add 15 g of powdered agar and continue boiling until the agar is completely digested. Adjust the pH to 7.5 with sodium hydroxide solution.

Distribute in 20 mL quantities; close the tubes with metal cups and autoclave at 122 °C for 20 min. After auto-claving, store the tubes in a cool place and use them within 3 weeks.

G.3.2 Stock solution pH phosphate buffer, dissolve 34 g of monobasic potassium in about 500 mL of water contained in a 100 mL volumetric flask. Adjust the pH to 7.2 ± 0.1 by the addition of sodium hydroxide solution (4 %). Add water to volume and mix. Sterilise at 122 °C for 20 min, store under refrigeration.

G.3.3 Dilute phosphate buffer solution pH 7.2, dilute 1 mL of stock solution with distilled water in the ratio of 1:800. Fill 50 mL each in conical flasks of 100 mL capacity. Plug the flasks with cotton and sterilize at 122 °C for 20 min.

G.4 Sterilization of apparatus

G.4.1 Tubes, these shall be sterilized in the autoclave at a temperature of 122 °C and 1.05 kg/cm pressure for 20 min or in the hot air oven at 180 °C for one hour.

G.4.2 Petri-dishes, these shall be packed in drums and autoclaved at 122 °C and 1.85 kg/cm pressure for 20 min or individually wrapped in kraft paper and sterilized in hot oven at 160 °C for one hour.

G.4.3 Pipettes, these shall be placed in pipette cones (copper, stainless steel or aluminium) after plugging the broader and with, cotton and sterilized in the autoclave at 122 °C and 1.05 kg/cm pressure for 20 min or at 160 °C for one hour in hot air oven.

G.5 Procedure

G.5.1 Melt a sufficient number of soyabean casein digest agar medium tubes in hot waterbath and transfer while hot into a constant temperature water-bath maintained at 48 °C ± 2 °C.

G.5.2 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) soyabean casein digest agar medium over it, and rotate the plates to mix thoroughly. Incubate the plates at 32 °C for 72 h in an inverted position.

G.6 Expression of results

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

Annex H
(normative)

Determination of *pseodomonas aeruginosa, staphylococcus aureus* and *candida albicans* in cosmetic products

H.1 Introduction

This method is obtained from the AOAC official method 998.10 entitled, "Efficacy of preservation of non-eye area water-miscible cosmetic and toiletry formulations".

Acknowledgement of microbiological techniques is required for these procedures. 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.

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.

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.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 10-20 « l inoculating loops.

H.3.10 Vortex mixer.

H.3 Reagents

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.3.1 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.3.2 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 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.3.3 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.3.4 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 p H should be 6.2 ± 0.2 at 25 °C. Cool in slanted position. Use for yeast culture maintenance and inoculum preparation.

H.3.5 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.3.6 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.3.7 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 1L. Dispense into suitable containers and sterilize by autoclaving at 121 °C for 15 min.

H.3.8 Barium sulphate standard No. 2

H.3.8.1 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.3.8.2 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.3.8.3 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.3.9 Barium sulphate standard No. 7, use solutions from H.3.8. Mix 0.7 mL of solution H.3.8.1, with 9.3 mL of solution H.3.8.2, in a screw-capped test tube. Cap tightly and store in the dark at room temperature.

H.4 Micro-organisms

H.4.1	*Staphylococcus aureus*	—	ATCC 6538
H.4.2	*Staphycloccuss epidermidis*	—	ATCC12228
H.4.3	*Klebsiella pneumoniae*	—	ATCC10031
H.4.4	*Escherichia coli*	—	ATCC 8739
H.4.5	*Enterobacter gergoviae*	—	ATCC 33028
H.4.6	*Pseudomonas aeruginosa*	—	ATCC 9027
H.4.7	*Burkholderia cepacia*	—	ATCC25416
H.4.8	*Acinetobacter baumannii*	—	ATCC 19606
H.4.9	*Candida albicans*	—	ATCC10231
H.4.10	*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.5 Product quality check

H.5.1 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.5.2 Pipette 1.0 mL of the 1:10 dilution into each of 4 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.5.3 Invert and incubate 2 plates at 35 °C ± 2 °C for 48 h and 2 plates at 25 °C ± 2 °C for 5 days.

H.5.4 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.5.5 Save plates to be used for the neutralization validation in M by refrigerating.

H.6 Product preparation

H.6.1 Measure 20 mL sterile saline into 4 sterile jars, H.3.1. Cap tightly and store at room temperature.

H.6.2 Weigh 20 g product into each of 4 sterile jars, H.3.1. Cap tightly and store at room temperature.

H.7 Bacterial inocula preparation

H.7.1 Streak each bacteria culture, H.4.1 - H.4.10 onto a nutrient agar, H.3.3, slant. Incubate 48 at 35 ± 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.7.2 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.3.8, direct microscopic count, turbidimetry, absorbance, or other method correlated to an aerobic plate count (APC), H.12. Perform an APC, H.12, on each suspension to confirm standardization.

H.8 Fungal inoculum preparation

H.8.1 *Streak C. albicans,* H.4.9, on 3 slants of Y/M agar, H.3.4. 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.8.2 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.3.9, direct microscopic count, turbidimetry, absorbance, or other method that has been correlated to an APC, H.12. Perform an APC, H.12, on the suspension to confirm standardization.

H.8.3 *Streak A. Niger*, H.4.10, 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.12, 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.12, before each use.

H.9 Inoculum pools

H.9.1 Pool equal parts of the S. *aureus* and S . *epidermidis* suspensions, H.7.2 in a sterile container to make incoculum pool 1: Gram-postive cocci.

H.9.2 Pool equal parts of the K *pneumoniae* , E . *coli*, and E . *gergoviae* suspensios, H.7.2, in a sterile container to make inoculum pool . 2: Gram- negative fermentors.

H.9.3 Pool equal parts of the P. *aeruginosa*, B .*cepacia* and A. *baumanii* suspensions, H.7.2, in a sterile container to make Inoculum Pool 3: Gram — negative nonfermentors.

H.9.4 Pool equal parts of C. *Albicans, H.8.2*, and A. *Niger,* H.8.3, suspensions in a sterile container to make innoculum pool 4: Fungi.

H.9.5 Use organism pools immediately or refrigerate them at 2 °C - 5 °C for more than 72 h.

H.10 Inoculation

H.10.1 Inoculate each of the four 20.0 mL aliquots of sterile saline, H.6.1, with 0.2 mL of its respective inoculum pool, H.9.1 - H.9.4. Mix thoroughly. Use these suspensions to determine inoculums counts (see Ka).

H.10.2 Inoculate each of the four 20 g products suspensions, F b) with 2.0 mL of its respective inoculums pool I a) – d). 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.11 Sampling intervals

H.11.1 Sample each inoculated saline suspension, H.10.1, for APC, H.12, within 1 h after inoculation to obtain inoculum count.

H.11.2 Test each inoculated product, H.10.2 for APC, H.12, at 7, 14 and 28 days after inoculation to obtain product interval count.

H.12 Aerobic Plate Count (APC)

H.12.1 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.12.2 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.12.3 Using a 2.2 mL pipette, aseptically pipette 1.0 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 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 - 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.12.4 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.12.5 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. Reincubate 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.13 Neutralization check

Make a 1:10 000 dilution in sterile saline of pools 1, 2 and 3, H.9.1 - H.9.3, and a 1: 1000 dilution of pool 4, H.9.4. Streak each dilution for isolation with a 10 μ l loop on the plates saved from H.5.5. If plates are not usable due to either desiccation or surface growth, repeat section H.5, and streak freshly prepared plates. Incubate as in H.12.4 - H.12.5

H.14 Data analysis

H.14.1 Product quality check, H.5.4, must be found to contain, <100 cfu/g to proceed with the challenge test.

H.14.2 Inoculums counts, H.11.1, 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.14.3 Neutralization check, H.13, must 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 for assistance.

H.14.4 Calculate the percentage reduction:

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

H.14.5 The test product is considered adequately preserved if

a) Bacteria show at least 99.9 % (3 log) reduction within 1 week following challenge and remain at or below that level there after, and

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

Annex K
(normative)

Determination of hydroquinone content

Identification and determination of hydroquinone, hydroquinone monomethylether, hydroquinone monoethylether and hydroquinone monobenzylether in cosmetic products

K.2 Determination using High Performance Liquid Chromatography (HPLC)

K.2.1 Scope and field of application

This method specifies a procedure for the determination of hydroquinone, hydroquinone monomethylether, hydroquinone monoethylether and hydroquinone monobenzylether in cosmetic products for lightening the skin.

K.2.2 Principle

The sample is extracted with a water/methanol mixture under gentle heating to melt any lipid material. Determination of the analytes in the resulting solution is performed by reversed phase liquid chromatography with UV-detection.

K.2.3 Reagents

K.2.3.1 All reagents must be of analytical quality. Water used must be distilled water, or water of at last equivalent purity.

K.2.3.2 Methanol

K.2.3.3 Hydroquinone

K.2.3.4 Hydroquinone monomethylether

K.2.3.5 Hydroquinone monoethylether

K.2.3.6 Hydroquinone monobenzylether (monobenzone)

K.2.3.7 Titrahydrofuran, HPLC grade

K.2.3.8 Water/methanol mixture 1:1 (v/v), mix 1 volume of water and 1 volume of methanol (A.2.3.2)

K.2.3.9 Mobile phase: Tetrahydrofuran/water mixture 45:55 (v/v), mix 45 volumes of tetrahydrofuran (A.2.3.7) and 55 volumes of water

K.2.3.10 Reference solution

Weigh 0.06 g hydroquinone (A.2.3.3), 0.08 g hydroquinone monomethylether (A.2.3.4); 0.10 g hydroquinone monoethylether (A.2.3.5) and 0.12 g hydroquinone monobenzylether (A.2.3.6) into a 50 mL volumetric flask. Dissolve and make up to volume with methanol (A.2.3.2). Prepare the reference solution by diluting 10.00 mL of this solution to 50.00 mL with water/methanol mixture (A.2.3.8). These solutions must be freshly prepared.

K.2.4 Apparatus

Normal laboratory equipment and:

K.2.4.1 Waterbath, capable of maintaining a temperature of 60 °C.

K.2.4.2 High-performance liquid chromatograph, with a variable-wavelength UV-detector and 10-µl injection loop.

K.2.4.3 Analytical column

Stainless steel chromatographic column, length 250 mm, internal diameter 4.6 mm, packed with Zorbax phenyl (chemically bonded phenethylsilane on Zorbax SIL, end-capped with trimethylchlorosilane); particle size 6 µm; or equivalent. Do not use a guard column, except phenyl guard; or equivalent.

K.2.4.4 Filter paper, diameter 90 mm, Schleicher and Schull, Weissband No. 5892, or equivalent.

K.2.5 Procedure

K.2.5.1 Sample preparation

Weigh to three decimal places 1 g ± 0.1 g (a gram) of sample into a 50 mL volumetric flask. Disperse the sample in 25 mL water/methanol mixture (A.2.3.8). Close the flask and shake vigorously until a homogeneous suspension is obtained. Shake for at least 1 minute. Place the flask in a waterbath (A.2.4.1) kept at 60 °C to enhance the extraction. Cool the flask, and make up to volume with water/methanol (A.2.3.8). Filter the extract using a filter paper (A.2.4.4). Perform the HPLC determination within 24 h of preparing the extract.

K.2.5.2 High performance liquid chromatograph

K.2.5.2.1 Adjust the flow rate of the mobile phase (A.2.3.9) to 1.0 mL/min and set the detector wavelength to 295 nm.

K.2.5.2.2 Inject 10 µl of the sample solution obtained as described in section A.2.5.1; and record the chromatogram. Measure the peak areas. Perform a calibration as described under A.2.5.2.3. Compare the chromatograms obtained for sample and standard solutions. Use the peak areas and the response factors (RF) calculated under section A.2.5.2.3 to calculate the concentration of the analytes in the sample solution.

K.2.5.2.3 Calibration

Inject 10 µl of the reference solution (A.2.3.10) and record the chromatograph. Inject several times until a constant peak area is obtained.

Determine the Response Factor RF;

$$\frac{RF_i}{C_i} = P_i$$

in which:

P_i is the peak area for hydroquinone, hydroquinone monomethylether, hydroquinone monoethylether or hydroquinone monobenzylether; and

C_i is the concentration (g/50 mL) in the reference solution (3.10) of hydroquinone, hydroquinone monomethylether, hydroquinone monoethylether or hydroquinone monobenzylether.

Ascertain whether the chromatograms obtained for a standard solution and the ample solution meet the following requirements:

* the peak separation of the worst separated pair shall be at least 0.90. (For definition of peak separation; see Figure K.1).

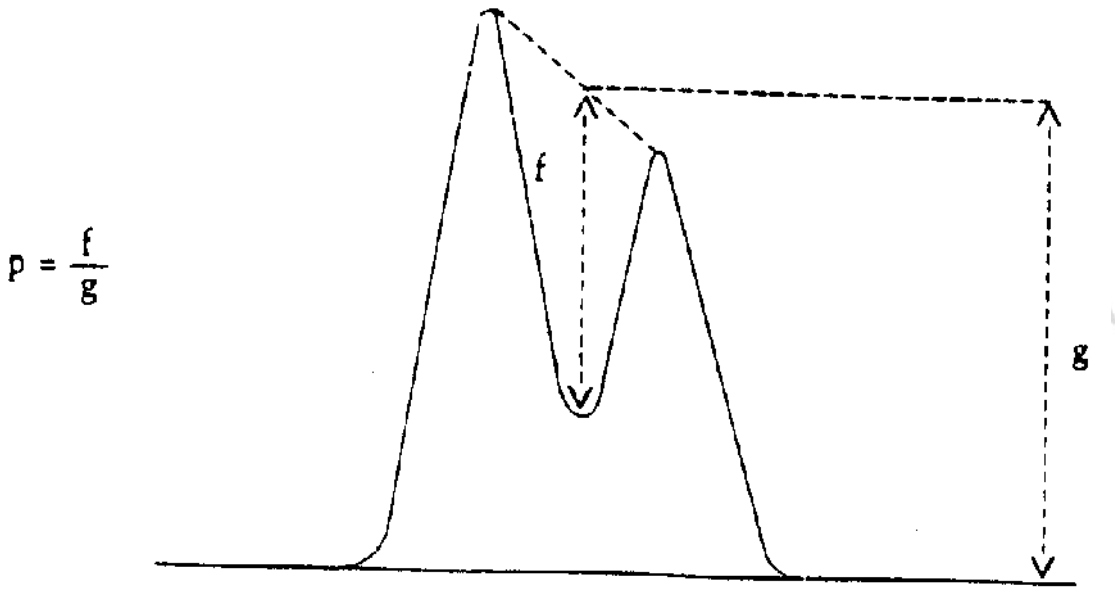

Figure K.1 — Peak separation

If the required separation is not achieved, either a more efficient column should be used, or the mobile phase composition should be adjusted until the requirement is met.

* The asymmetry factor A_s of all peaks obtained shall range between 0.9 to 1.5. (For definition of the peak asymmetry factor, see Figure K.2). To record the chromatogram for the determination of the asymmetry factor a chart speed of at least 2 cm/minute is recommended.

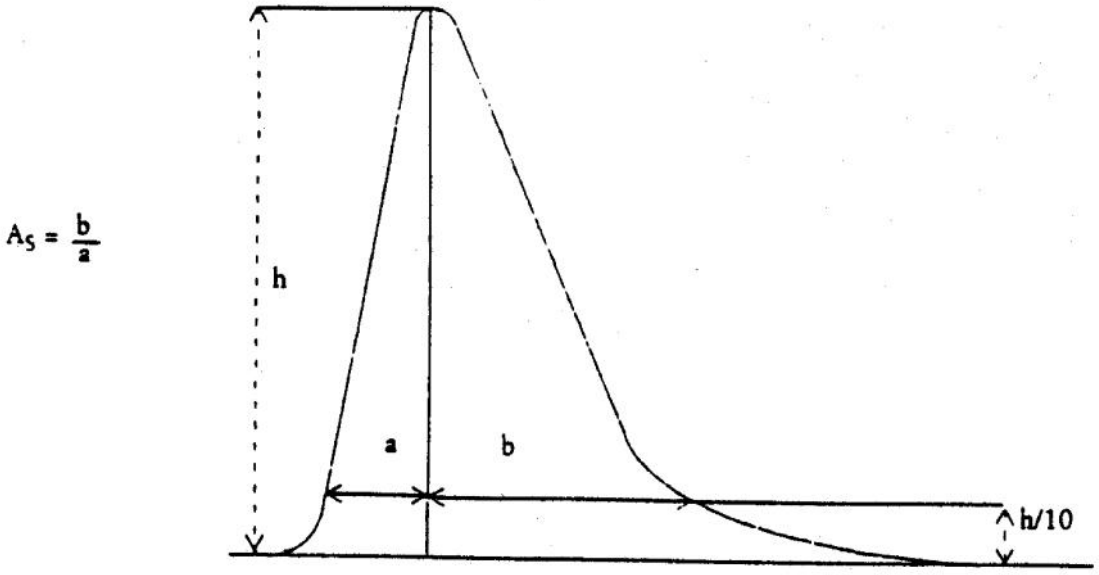

Figure K.2 — Peak symmetry factor

* a steady baseline shall be obtained.

K.2.6 Calculation

Use the areas of the analyte peaks to calculate the concentration(s) of the analyte(s) in the sample; as a percentage by mass, (x) using the formula:

$$\%\ X\ (m\ X\ m) = \frac{b_i}{RF_i oa}$$

in which:

a is the mass of the sample, in grams; and

b_i is the peak area of analyte I, in the sample.

K.2.7 Repeatability [1)]

K.2.7.1 For a hydroquinone content of 2.0 % the difference between the results of two determinations carried out in parallel on the same sample should not exceed an absolute value of 0.13 %.

K.2.7.2 For a hydoquinone monomethylether content of 1.0 % the difference between the results of two determinations carried out in parallel on the same sample should not exceed an absolute value of 0.1 %.

K.2.7.3 For a hydroquinone monoethylether content of 1.0 % the difference between the results of two determinations carried out in parallel on the same sample should not exceed an absolute value of 0.11 %.

[1)] ISO 5725 Accuracy (trueness and precision) of measurement methods and results.

K.2.7.4 For a hydroquinone monobenzylether content of 1.0 % the difference between the results of two determinations carried out in parallel on the same sample should not exceed an absolute value of 0.11 %.

K.2.8 Reproducibility[1)]

K.2.8.1 For a hydroquinone content of 2.0 % the difference between the results of two determinations carried out on the same sample under different conditions (different laboratories, different operators, different apparatus and or different time) should not exceed an absolute value of 0.37 %.

K.2.8.2 For a hydroquinone monomethylether content of 1.0 % the difference between the results of two determinations carried out on the same sample under different conditions (different laboratories, different operators, different apparatus and/or different time) should not exceed an absolute value of 0.21 %.

K.2.8.3 For a hydroquinone monomethylether content of 1.0 % the difference between the results of two determinations carried out on the same sample under different conditions (different laboratories, different operators, different apparatus and/or different time) should not exceed an absolute value of 0.19 %.

K.2.8.4 For a hydroquinone monobenzylether content of 1.0 % the difference between the results of two determinations carried out on the same sample under different conditions (different laboratories, different operators, different apparatus and/or different time) should not exceed an absolute value of 0.11 %.

K.2.9 Remarks

K.2.9.1 When a hydroquinone content considerably higher than 2 % is found and an accurate estimate of the content is required, the sample extract (5.1) should be diluted to a similar concentration as would be obtained from a sample containing 2 % hydroquinone, and the determination repeated.

(In some instruments the absorbance may be out of the linear range of the detector for high hydroquinone concentrations)

K.2.9.2 Interferences

The method described above allows the determination of hydroquinone and its ethers in a single isocratic run. The use of the phenyl column assures sufficient retention for hydroquinone, which cannot be guaranteed when a C 18 column is used with the mobile phase described.

However, this method is prone to interferences by a number of para-bens. In such cases the determination should be repeated employing a different mobile phase/stationary phase system. Suitable methods may be found in references 2 and 3, viz:

(2) Column: Zorbax ODS, 46 cm x 25 cm, or equivalent
Temperature: 36 °C
flow: 1.5 mL/min
mobile phase:
for hydroquinone: methanol/water 5/95
for hydroquinone monomethylether: methanol/water 30/70 (v/v)
or hydroquinone monobenzylether: methanol/water 80/20 (v/v)[2)]

(3) Column: Spherisorb S5-ODS, or equivalent
mobile phase: water/methanol 90/10 (v/v)
flow: 1.5 mL/min

These conditions are suitable for hydroquinone [3)].

[2)] M. Herpol-borremans and M.O. Masse, Identification et dosage par HPLC de I'hydroquinone et ses ethers dans les produit cosmetiques, Int. Doc. Inst. d'Epidemiologie.

[3)] J.Firth and I. Rix, Determination of Hydroquinone in skin toning cream, analyst (1986), 111, p. 129.

Annex L
(normative)

Antibacterial test

L.1 Procedure

Prepare nutrient agar for bacterial growth by dispersing 28 g of nutrient agar powder in 1 litre of de ionized water. Allow to soak for 10 min, swirl to mix and then heat gently with stirring to ensure uniformity. Sterilize by autoclaving for 15 min at 121 °C, cool at 47 °C, mix well and then pour to sterilized petridishes. Leave it to solidify undisturbed. Plant *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans* on the so prepared nutrient agar in petridishes.

Meanwhile, prepare filter paper discs and sterilize them by autoclaving. Dip in various samples. Place in the petri dishes containing bacteria culture agar mixture. Incubate the petri dishes at 35 °C for 48 hours. Determine bacteria growth inhibition zones.

L.2 Antifungal test

Dissolve potato dextrose agar (39 g) in 1 dm^3 of distilled water. Use the same procedure as for bacterial test (L.1) above. Test the cream against fusarium fungi. Obtain the results after 4 days and the temperature of incubation should be 25 °C.

L.3 Results

The inhibition zone shall be at least 0.2 mm in diameter.

