SLOVENSKI STANDARD
SIST EN 16956:2017

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Kozmetika - Analizne metode - Metoda HPLC/UV za identifikacijo in določevanje hidrokinona, etrov hidrokinona in kortikosteroidov v kozmetičnih izdelkih za beljenje kože

Cosmetics - Analytical methods - HPLC/UV method for the identification and assay of hydroquinone, ethers of hydroquinone and corticosteroids in skin whitening cosmetic products

Kosmetische Mittel - Untersuchungsverfahren - HPLC/UV Verfahren für die Identifizierung und Bestimmung von Hydrochinon, Hydrochinonethern und Kortikosteroiden in hautaufhellenden kosmetischen Mitteln

Cosmétiques - Méthodes analytiques - Méthode de CLHP couplée à la détection UV pour l'identification et l'analyse de l'hydroquinone, de ses éthers et des corticostéroïdes dans les produits cosmétiques éclaircissants de la peau

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Cosmetics - Analytical methods - HPLC/UV method for the identification and assay of hydroquinone, ethers of hydroquinone and corticosteroids in skin whitening cosmetic products

Cosmétiques - Méthodes analytiques - Méthode de CLHP couplée à la détection UV pour l'identification et l'analyse de l'hydroquinone, de ses éthers et des corticostéroïdes dans les produits cosmétiques éclaircissants de la peau

Kosmetische Mittel - Untersuchungsverfahren - HPLC/UV Verfahren für die Identifizierung und Bestimmung von Hydrochinon, Hydrochinonethern und Kortikosteroiden in hautaufhellenden kosmetischen Mitteln

This European Standard was approved by CEN on 19 June 2017.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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European foreword

This document (EN 16956:2017) has been prepared by Technical Committee CEN/TC 392 “Cosmetics”, the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by March 2018, and conflicting national standards shall be withdrawn at the latest by March 2018.

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

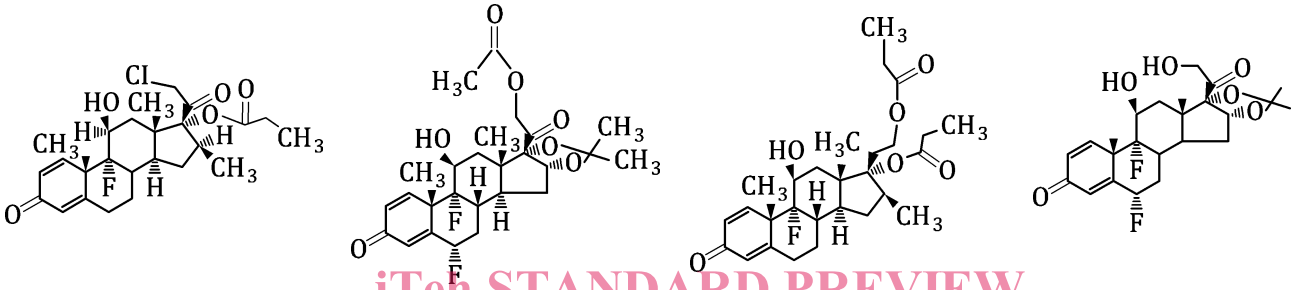
The existing peer review validation data for hydroquinone are preliminary and will be supplemented by inter-laboratory test data if available.

According to the CEN-CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

Hydroquinone is not allowed for use in cosmetic products for skin whitening and depigmentation of dermal spots or imperfections. Due to its cytotoxic effects its use has been regulated. Hydroquinone and 3 of its ethers (hydroquinone monomethylether (MME), hydroquinone monoethylether (MEE) and hydroquinone monobenzylether (MBE)) are regulated by the cosmetic regulation 1223/2009. Nowadays the use of these substances is prohibited in skin whitening cosmetic products.

Depigmentation is a side effect of topical steroids, in this way corticosteroids might be used as compounds in products illegally sold as cosmetics. Corticosteroids most commonly found in these products are clobetasol propionate, fluocinonide, betamethasone dipropionate, and fluocinolone acetonide (see Figure 1). Corticosteroids are listed in Regulation 1223/2009 Annex II "List of substances prohibited in cosmetic products" (reference number 300), and their use is also prohibited in cosmetic products.



a) Clobetasol propionate b) Fluocinonide c) Betamethasone dipropionate d) Fluocinolone Acetonide

Figure 1 — Corticosteroids most commonly found in illegal cosmetics

All these substances work on the same principle as hydroquinone which mainly consists of inhibition of melanin synthesis.

The cosmetic directive 95/32/EC [2] gives an analytical method for the assay of hydroquinone and 3 of its ethers (hydroquinone monomethylether (MME), hydroquinone monoethylether (MEE) and hydroquinone monobenzylether (MBE)) in cosmetic products for lightening the skin. In order to update and extend this official method to the identification and assay of corticosteroids in cosmetic products, this standard describes an HPLC/UV method for the identification and assay of hydroquinone, ethers of hydroquinone and corticosteroids in cosmetic products.

1 Scope

This European Standard specifies a HPLC/UV method for the identification and quantification of hydroquinone, 3 ethers of hydroquinone and 4 corticosteroids most frequently found in illegally sold skin whitening cosmetic products: clobetasol propionate, betamethasone dipropionate, fluocinonide and fluocinolone acetonide.

This standard also gives HPLC/UV methods for the identification of 38 corticosteroids that may be found in skin whitening cosmetic products (see Annex D).

This standard is not dedicated to artificial nail products or soaps.

2 Principle

The sample is extracted by a mixture of water/methanol and gently warmed in order to extract compounds present in the product. The obtained mixture is filtered. The quantitation of present compounds in solution is made by reversed phase HPLC with DAD (Diode Array Detector) detection.

3 Reagents

If not otherwise specified, analytical-grade chemicals shall be used; the water shall be distilled or of a corresponding purity. "Solution" shall be understood as an aqueous solution unless otherwise specified.

3.1 Methanol, HPLC grade.

3.2 Water, HPLC grade.

3.3 Extraction solution, methanol/water (1/1).

Mix 500 ml of methanol (3.1) and 500 ml of water (3.2) in a 1 000 ml conical flask.

3.4 Compounds considered, see Table 1.

Table 1 — Compounds considered

Compound	CAS	Manufacturer[a]	Purity %	Used method – clause
Alclometasone dipropionate (ACD)	66734–13–2	USP	99,2	Annex D
Amcinonide (AMC)	51022–69–6	Sigma	97,9	Annex D
Beclomethasone dipropionate (BCD)	5534-09-8	Sigma	99,0	Annex D
Betamethasone acetate (BMA)	987–24–6	Sigma	98,6	Annex D
Betamethasone (BM)	378–44–9	Sigma	98,4	Annex D
Betamethasone dipropionate (BMD)	5593–20–4	Sigma	98,6	2/ Annex D
Betamethasone valerate (BMV)	2152–44–5	Sigma	98,1	Annex D
Budesonide (BUD)	51333–22–3	Ph. Eur.	99,7	Annex D
Clobetasol propionate (CP)	25122–46–7	Sigma	98,8	2/ Annex D
Clocortolone pivalate (CLP)	34097–16–0	USP	98,9	Annex D
Cortisone (CS)	53–06–5	Sigma	98,3	Annex D
Desonide (DSN)	638–94–8	Cil	98,0	Annex D

Compound	CAS	Manufacturer[a]	Purity %	Used method – clause
Desoximetasone (DXM)	382–67–2	Sigma	99,0	Annex D
Dexamethasone phosphate (DMPS)	2392–39–4	Sigma	100,2	Annex D
Dexamethasone acetate (DMA)	1177–87–3	Sigma	99,0	Annex D
Diflorasone diacetate (DFD)	33564–31–7	USP	99,8	Annex D
Diflucortolone valerate (DFCV)	59198–70–8	Schering	/	Annex D
Difluprednate (DFP)	23674–86–4	Sigma	99,5	Annex D
Flumethasone pivalate (FMP)	2002–29–1	Farmabios	100,4	Annex D
Fluocinolone acetonide (FCA)	67–73–2	Sigma	99,6	2/ Annex D
Fluocinonide (FCAA)	356–12–7	Sigma	99,0	2/ Annex D
Fluocortolone –hexanoate (FCH)	303–40–2	Schering	/	Annex D
Fluocortolone pivalate (FCP)	29205–06–9	Ph. Eur.	/	Annex D
Flurandrenolide (FDL)	1524–88–5	USP	/	Annex D
Halcinonide (HAL)	3093–35–4	Sigma	99,0	Annex D
Hydrocortisone (HC)	3093–25–4	Sigma	98,0	Annex D
Hydrocortisone aceponate (HCAP)	74050–20–7	Toronto Research Chemicals	98,0	Annex D
Hydrocortisone butyrate (HCB)	13609–67–1	Sigma	99,1	Annex D
Hydrocortisone valerate (HCV)	57524–89–7	Sigma	99,0	Annex D
Methylprednisolone acetate (MPLA)	53–36–1	USP	99,8	Annex D
Mometasone furoate (MMF)	83919–23–7	USP	99,8	Annex D
Prednicarbate (PCN)	73771–04–7	Ph. Eur.	/	Annex D
Prednisolone (PL)	50–24–8	Aventis Pharma	98,0	Annex D
Prednisolone acetate (PLA)	52–21–1	Dr Ehrenstrofer	99,4	Annex D
Prednisolone hexanoate (PLH)	69164–69–8	Schering	/	Annex D
Prednisolone sulfobenzoate Na (PLSB)	630–67–1	Pharmaceutical drug	/	Annex D
Triamcinolone (TRI)	124–94–7	Sigma	98,2	Annex D
Triamcinolone acetonide (TRA)	76–25–5	Sigma	99,6	Annex D
Hydroquinone (HQ)	123–31–9	Sigma	> 99,9	2/ Annex D
Hydroquinone monomethylether (MME)	150–76–5	Sigma	99,7	2/ Annex D
Hydroquinone monoethylether (MEE)	622–62–8	Sigma	99,9	2/ Annex D
Hydroquinone monobenzylether (MBE)	103–16–2	Sigma	99,5	2/ Annex D

[a] Examples of manufacturer.

3.5 Mobile phase for HPLC, mobile phase A: methanol (3.1); mobile phase B: water (3.2).

3.6 Reference solutions.

Methanol (3.1) is used as a solvent for the preparation of stock solutions. Standard solutions are prepared by dilution in a methanol / water solution (3.3).

3.6.1 Standard Stock Solution of hydroquinone and its 3 ethers (between 1,2 mg/ml and 2,4 mg/ml).

Weigh approximately into a 25 ml volumetric flask:

— 0,03 g of hydroquinone;

— 0,04 g of monomethylether of hydroquinone;

— 0,05 g of monoethylether of hydroquinone;

— 0,06 g of monobenzylether of hydroquinone.

Firstly, dissolve in 15 ml of methanol (3.1), if necessary use a shaker and then fill up to the calibration mark with methanol. This solution shall be prepared daily.

3.6.2 Standard Stock Solutions (1 mg/ml) and Standard Working Solution of 4 most frequently found corticosteroids (10 µg/ml).

Weigh separately and approximately into a 10 ml volumetric flask:

— 10 mg of betamethasone dipropionate;

— 10 mg of clobetasol propionate;

— 10 mg of fluocinonide;

— 10 mg of fluocinolone acetonide.

Firstly, dissolve in 5 ml of methanol (3.1), if necessary use a shaker and then fill up to the calibration mark with methanol. Theses 4 solutions can be stored during 6 months at 4 °C.

Then prepare a daughter solution of 4 corticosteroids by introducing 1 ml of each standard stock solution with a glass pipette in a 100 ml volumetric flask. Fill up to the mark with mixture methanol/water (3.3). These solution can be stored during 6 weeks at 4 °C.

3.6.3 Calibration Solutions of hydroquinone and its 3 ethers

The calibration solutions shall be freshly prepared (see Table 2). With a glass pipette, introduce the volume of standard stock solution (3.6.1.) in a volumetric flask then fill up to the mark with the mixture methanol/water (3.3).

Table 2 — Calibration solutions

Number of calibration solution	Volume of Standard Stock Solution ml	Volume of flask ml	Concentration of calibration solutions µg/ml
1	0,5	100	6 – 8 – 10 – 12
2	1,0	50	24 – 32 – 40 – 48
3	5,0		120 – 160 – 200 – 240
4	10,0		240 – 320 – 400 – 480
5	20,0		480 – 640 – 800 - 960

3.6.4 Calibration Solutions of 4 most frequently found corticosteroids

The calibration solutions shall be freshly prepared (see Table 3). With a glass pipette, introduce the volume of standard daughter solution (3.6.2) in a volumetric flask then fill up to the mark with the mixture methanol/water (3.3).

Table 3 — Calibration solution

Number of calibration solution	Volume of Standard Stock Solution ml	Volume of flask ml	Concentration of calibration solutions µg/ml
6	5	100	0,5
7	5	50	1,0
8	10		2,0
9	20		4,0
10	25		5,0

4 Apparatus and equipment

In addition to the usual laboratory equipment, the following is required.

4.1 Analytical balance, with a precision of 0,1 mg.

4.2 Laboratory shaker.

4.3 Water-bath, allowing to heat at 60 °C.

4.4 High Performance Liquid Chromatography, consisting of:

— sampling device;

— pump system with gradient function;

— degasser;

— column oven;

— photodiode array detector;

— evaluation system.

4.5 Membrane filter, for sample filtration e.g. polypropylene, 0,45 µm pore size.

4.6. Analytical separation column, e.g. Thermo Fisher Scientific Hypurity Aquastar C18[1)] length = 0,25 m, internal diameter = 4,6 mm, diameter particle size = 5 µm.

4.7 Paper filter, for sample filtration, e.g. filter quality medium folded 210 mm diameter.

5 Procedure

5.1 Sample preparation

Weigh accurately 0,5 g of sample in a volumetric flask of 100 ml. Add 50 ml of extraction solution (3.3) and shake it with a laboratory shaker until a homogeneous suspension is formed. Place the mixture into a water-bath at 60 °C for 5 min to improve solution. If necessary repeat shaking with a laboratory shaker. Cool the flask to room temperature and adjust to 100 ml with the extraction mixture (3.3). Filter the extract through a paper filter and then through a membrane filter (0,45 µm). Inject the filtrate within the following 24 h into a HPLC system according to 5.2.

5.2 Liquid chromatography measurement conditions

When using the apparatus (4.4) and column (4.6), the following conditions have shown to be useful:

— Flow rate: 1,5 ml/min

— Acquisition time: 25 min

— Injection volume: 10 µl

— Oven temperature: 36 °C ± 2 °C

— Detection: Diode Array Detection with wavelength: 240 nm (corticosteroids) and 295 nm (hydroquinone and its ethers)

— Mobile phases: Mobile Phase A: Methanol

Mobile Phase B: Water

See Table 4 for gradient separation.

1) This is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product. Equivalent products may be used if they can be shown to lead to the same results.

Table 4 — Gradient separation

Time min	Volume fraction Mobile Phase A %	Volume fraction Mobile Phase B %
0	5	95
20	80	20
21	5	95
25	5	95

5.3 Detection

The detection and quantitative determination can be performed by DAD (λ = 240 nm and λ = 295 nm).

6 Evaluation

6.1 Identification

Hydroquinone, ethers of hydroquinone and corticosteroids are identified by comparing the retention times and the UV spectra of the sample with those of standard solution substances.

Table 5 gives chromatographic parameters for the identification of compounds using the analytical method proposed in 5.2. Annex A gives chromatograms of standard solutions at the two studied wavelengths.

Table 5 — Chromatographic parameters for the identification of compounds

Compound	Rt min	λ nm
Hydroquinone	4,24	295
Hydroquinone monomethylether (MME)	9,34	295
Hydroquinone monoethylether (MEE)	12,08	295
Hydroquinone monobenzylether (MBE)	17,54	295
Fluocinolone acetonide (FCA)	17,73	240
Fluocinonide (FCAA)	19,60	240
Clobetasol propionate (CP)	20,55	240
Betamethasone dipropionate (BMD)	21,44	240

6.2 Quantitative determination

The quantitative determination is done by linear regression based on peak areas of the external standard solutions. The calibration curve shall be linear and the correlation coefficient shall be upper or equal to 0,995.