SLOVENSKI STANDARD
SIST EN 17156:2019

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Kozmetika - Analizne metode - Metoda LC/UV za identifikacijo in kvantitativno določevanje 22 organskih UV-filtrov, ki se v EU uporabljajo v kozmetičnih izdelkih

Cosmetics - Analytical methods - LC/UV method for the identification and quantitative determination in cosmetic products of the 22 organic UV filters in use in the EU

Kosmetische Mittel - Untersuchungsverfahren - LC/UV Verfahren für die Identifizierung und qualitative Bestimmung von den 22 in der EU verwendeten organischen UV-Filtern in kosmetischen Produkten

Cosmétiques - Méthodes analytiques - Procédé CL/UV pour l'identification et la détermination quantitative des 22 filtres UV organiques utilisés dans les produits cosmétiques au sein de l'UE

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Cosmetics - Analytical methods - LC/UV method for the identification and quantitative determination in cosmetic products of the 22 organic UV filters in use in the EU

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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European foreword

This document (EN 17156:2018) 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of an identical text or by endorsement, at the latest by June 2019 and conflicting national standards shall be withdrawn at the latest by June 2019.

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Introduction

In order to protect human skin from the deleterious UV radiation of sunlight, the so-called UV filters have been used as active ingredients in the formulation of sunscreen cosmetic products. These active compounds are of organic or inorganic nature, and have the capacity to absorb and/or reflect, respectively, this UV radiation. Nowadays, they are not only added to those cosmetics intended specifically for sun protection but also in all type of daily products such as moisturizers, after shave products, shampoos, anti-aging creams, make-up products, etc.

The compounds that can be used as UV filters in cosmetics and their maximum allowed concentrations are regulated in order to ensure user's safety. Currently, the European Union (EU) 1223/2009 Regulation permits the use of 27 compounds as UV filters [1], the names of which are listed in Table 1. Among these 27 UV filters, titanium dioxide and zinc oxide are of inorganic nature, and among the remaining 25, 7 are highly polar and can be grouped in the 'water-soluble' group, whereas the other 18 are low polar and can be grouped in the 'fat-soluble' group. All these compounds are included in the formulations of the different cosmetic products consumed in the EU framework, with the only exceptions of BCSA and PBC which are not being currently used.

Besides, errors during the manufacturing process of cosmetics may cause a lower concentration in the final product than that formulated. This might affect the efficacy of the product since the real Sun Protection Factor could be lower than that labelled.

Therefore, reliable and practical analytical methods are needed in order to ensure compliance with the EU Regulation and thus protect user's safety, but also to ensure product's efficacy.

In this sense, with the aim of implementing a broad-spectrum analytical method to improve and facilitate the quality control of the cosmetic industry, this European Standard presents an analytical method for the quantification of 22 organic UV filters. They constitute all the organic UV filters allowed and in use in the EU when this standard was validated. Note that TBT is not included since it was later approved. The presented method, besides good analytical characteristics, is simple, low cost, rapid and both user- and environmentally-friendly. The method is based on different analytical methods previously published by the Research Group on Quality Control of Cosmetic Products of the University of Valencia [2], [3].

Table 1 — List of the UV filters permitted in cosmetic products under the EU Regulation

EU Reference number [a]	Name of Common Ingredients Glossary [b]	Acronym [c]
2	Camphor Benzalkonium Methosulfate	CBM
3	Homosalate	HMS
4	Benzophenone-3	BZ3
6	Phenylbenzimidazole Sulfonic Acid	PBSA
7	Terephthalylidene Dicamphor Sulfonic Acid	TDSA
8	Butyl Methoxydibenzoylmethane	BMDM
9	Benzylidene Camphor Sulfonic Acid	BCSA
10	Octocrylene	OC
11	Polyacrylamidomethyl Benzylidene Camphor	PBC
12	Ethylhexyl Methoxycinnamate	EHMC
13	PEG-25 PABA	P25
14	Isoamyl p-Methoxycinnamate	IMC
15	Ethylhexyl Triazone	EHT
16	Drometrizole Trisiloxane	DTS
17	Diethylhexyl Butamido Triazone	DEBT
18	4-Methylbenzylidene Camphor	MBC
20	Ethylhexyl Salicylate	EHS
21	Ethylhexyl Dimethyl PABA	EHDP
22	Benzophenone-4	BZ4
23	Methylene Bis-Benzotriazolyl Tetramethylbutylphenol	MBBT
24	Disodium Phenyl Dibenzimidazole Tetrasulfonate	PDTA
25	Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine	BEMT
26	Polysilicone-15	P15
27	Titanium Dioxide	TiO_2
28	Diethylamino Hydroxybenzoyl Hexyl Benzoate	DHHB
29	Tris-biphenyl triazine	TBT
30	Zinc Oxide	ZnO

[a] Order number given according to the EU 1223/2009 Regulation.

[b] According to the Annex VI of the European Union (EU) 1223/2009 Regulation.

[c] Acronyms used in this standard.

1 Scope

This document specifies an analytical method, based on liquid-chromatography (LC) with ultraviolet/visible spectrometry (UV/Vis) detection for the detection and quantitative determination of 22 organic UV filters in use in the EU framework. This method has been validated for emulsion-based cosmetic products, lip-balms, lotions and waters.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

No terms and definitions are listed in this document.

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

- IEC Electropedia: available at http://www.electropedia.org/
- ISO Online browsing platform: available at http://www.iso.org/obp

4 Principles

The cosmetic sample is weighed and solved in ethanol. For difficult-to-solve samples, the use of ultrasound or vortex can help. After that, depending on the UV filters to be determined, aliquots are taken and diluted with a mixture of ethanol:acetate buffer, ethanol or tetrahydrofuran for the determination of the water-soluble group, the fat-soluble group or P15 (which is a polymer), respectively.

Regarding the water-soluble group, the 6 UV filters are measured by employing a reversed-phase column and a mobile phase of ethanol and acetate buffer. Regarding the fat-soluble group, the 15 UV filters are determined by employing a reversed-phase column and a mobile phase of ethanol and aqueous formic acid containing hydroxypropyl-beta-cyclodextrin as mobile phase modifier. Finally, for P15 determination, a size-exclusion column and tetrahydrofuran as mobile phase are used.

5 Reagents

If not otherwise specified, analytical-grade chemicals shall be used.

5.1 Ethanol (EtOH), LC-grade.

5.2 Tetrahydrofuran (THF), LC-grade.

5.3 Deionized water, 18,2 MΩ·cm resistivity.

5.4 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD), 1 309 g/mol molecular weight.

5.5 Formic acid, 98 %, d = 1,22 g/ml.

5.6 Glacial acetic acid, > 99 %, d = 1,05 g/ml.

5.7 Sodium hydroxide, pellets.

5.8 Sodium hydroxide, 10 % (w/w) aqueous solution.

Weigh 10 g of sodium hydroxide (5.7) and dissolve in 100 ml of deionized water (5.3).

5.9 Acetate buffer, 1 % (v/v) solution, pH = 4,75.

Take 10 ml of glacial acetic acid (5.6) and dilute with approximately 900 ml of deionized water (5.3). Adjust with the aid of pH-meter up to pH 4,75 by adding sodium hydroxide solution (5.8). Complete up to 1 l with deionized water (5.3).

5.10 Formic acid, 1 % (v/v) solution, containing 20 mmol HP-β-CD.

Weigh 26,2 g of HP-β-CD (5.4). Add 10 ml of formic acid (5.5), and complete up to 1 l with deionized water (5.3).

5.11 UV filter standards, see Table 2.

Table 2 — UV filter standards

Type	UV filter	CAS
Water-soluble UV filters	Disodium Phenyl Dibenzimidazole Tetrasulfonate (PDTA)	180898–37–7
	Phenylbenzimidazole Sulfonic Acid (PBSA)	27503–81–7
	Terephthalylidene Dicamphor Sulfonic Acid (TDSA) (triethanolamine salt)	90457–82–2
	Benzophenone-4 (BZ4)	4065–45–6
	Camphor Benzalkonium Methosulfate (CBM) (29 % aqueous solution)	52793–97–2
	PEG-25 PABA (P25)	116242–27–4
Fat-soluble UV filters	Benzophenone-3 (BZ3)	131–57–7
	Isoamyl p-Methoxycinnamate (IMC)	71617–10–2
	4-Methylbenzylidene Camphor (MBC)	36861–47–9
	Diethylamino Hydroxybenzoyl Hexyl Benzoate (DHHB)	302776–68–7
	Octocrylene (OC)	6197–30–4
	Ethylhexyl Dimethyl PABA (EHDP)	21245–02–3
	Butyl Methoxydibenzoylmethane (BMDM)	70356–09–1
	Ethylhexyl Methoxycinnamate (EHMC)	5466–77–3
	Ethylhexyl Salicylate (EHS)	118–60–5
	Homosalate (HMS)	118–56–9
	Diethylhexyl Butamido Triazone (DEBT)	154702–15–5
	Ethylhexyl Triazone (EHT)	88122–99–0
	Drometrizole Trisiloxane (DTS)	155633–54–8
	Methylene Bis-Benzotriazolyl Tetramethylbutylphenol (MBBT)	103597–45–1
	Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine (BEMT)	187393–00–6
Polymeric UV filter	Polysilicone-15 (P15)	207574–74–1

5.12 UV filters stock solutions

Prepare a 500 µg/ml multicomponent stock solution of the 6 water-soluble UV filters in deionized water. For this purpose, weigh the appropriate amount of each standard (5.11) in a 25 ml volumetric flask and add approximately 10 ml of deionized water (5.3). In case any of the substances is not solubilized, add a few drops of 10 % sodium hydroxide solution (5.8) until complete dissolution. Finally, fill up to the mark with deionized water (5.3). This solution should be monthly prepared, stored at 4 °C and protected from light until use.

Prepare a 500 µg/ml multicomponent stock solution of the fat-soluble UV filters in EtOH, except for MBBT and BEMT, which are prepared at 100 µg/ml due to their limited solubility. For this purpose, weigh the appropriate amount of each standard (5.11) in a 25 ml volumetric flask and dissolve with approximately 10 ml of EtOH (5.1). Then fill up to the mark with EtOH (5.1). This solution should be monthly prepared, stored at 4 °C and protected from light until use.

Prepare a 1 000 µg/ml P15 solution in EtOH. For this purpose, weigh the appropriate amount of P15 (5.11) in a 25 ml volumetric flask and dissolve with approximately 10 ml of EtOH (5.1). Then fill up to the mark with EtOH (5.1). This solution should be monthly prepared, stored at 4 °C and protected from light until use.

5.13 Calibration standard solutions

For the preparation of the water-soluble UV filters calibration solutions, transfer 0,2 ml, 0,4 ml, 0,6 ml, 0,8 ml and 1,0 ml of the 500 µg/ml multicomponent stock solution (5.12) to five 10 ml volumetric flasks, respectively. Add 3 ml of EtOH (5.1) and acetate buffer solution (5.9) to each volumetric flask up to the mark. These 30:70 EtOH:acetate buffer (v/v) solutions contain 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml of each target compound, respectively. These solutions should be weekly prepared, stored at 4 °C and protected from light until use.

For the preparation of the fat-soluble UV filters calibration solutions, transfer 0,2 ml, 0,4 ml, 0,6 ml, 0,8 ml and 1,0 ml of the 500 µg/ml multicomponent stock solution (5.12) and 1 ml, 2 ml, 3 ml, 4 ml and 5 ml of the 100 µg/ml solution of MBBP and BEMT to five 10 ml volumetric flasks, respectively. Add EtOH (5.1) to each volumetric flask up to the mark. These ethanolic solutions contain 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml of each target compound, respectively. These solutions should be weekly prepared, stored at 4 °C and protected from light until use.

For the preparation of the P15 calibration solutions, transfer 0,2 ml, 0,4 ml, 0,6 ml, 0,8 ml and 1,0 ml of the 1 000 µg/ml P15 stock solution (5.12) to 10 ml volumetric flasks, respectively. Add either EtOH (5.1) or THF (5.2) to each volumetric flask up to the mark. These solutions contain 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml of P15, respectively. These solutions should be weekly prepared, stored at 4 °C and protected from light until use.

5.14 Working standard solutions

Calibration standard solutions (5.13) are daily transferred to 1,5 ml injection vials. Once sealed, they are ready to be injected into the LC-system.

6 Apparatus and equipment

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

6.1 Membrane filter, in the form of a disposable syringe filter, pore width 0,45 µm.

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

6.3 Ultrasonic water bath (or vortex mixer).

6.4 pH-meter.

6.5 LC-UV/Vis system, equipped with pump system with gradient function, degasser, autosampler, thermostated column oven, UV/Vis detector[1)] and data management station.

6.6 Analytical separation column

For the determination of both water-soluble and fat-soluble group Purospher® STAR RP-18 Endcapped[2)] (125 mm length, 4 mm i.d., 5 µm particle size) or equivalent can be used.

For the determination of P15 PLgel Mixed D[2)] (300 mm length, 7,5 mm i.d., 5 µm particle size) or equivalent can be used.

7 Procedure

7.1 Sample preparation

Homogenize the sample depending on its nature (i.e. shake creams, milks and lotions manually; cut lip balms in tiny pieces, mix and knead again). Then, in triplicate, weigh 0,02 g to 0,20 g of the sample, depending of the expected amount of UV filters, into a 25 ml volumetric flask, then dissolve and dilute with EtOH (5.1). In case of difficult-to-solve samples, an ultrasonic water bath (6.3) may be used to disperse the sample. Take different aliquots to carry out the determination of the different groups of the target compounds.

For the determination of the water-soluble UV filters, transfer 1 ml to 3 ml of the above-mentioned ethanolic sample stock solution to a 10 ml volumetric flask. Add EtOH (5.1) to complete a total volume of 3 ml, and then fill up to the mark with acetate buffer solution (5.9). Transfer this solution to a 1,5 ml injection vial, previously filtered through a membrane filter (6.1) if necessary.

For the determination of the fat-soluble UV filters, transfer 1 ml to 3 ml of the above-mentioned ethanolic sample stock solution to a 10 ml volumetric flask. Fill up to the mark with EtOH (5.1). Transfer this solution to a 1,5 ml injection vial previously filtered through a membrane filter (6.1) if necessary.

For the determination of P15, transfer 1 ml to 3 ml of the above-mentioned ethanolic sample stock solution to a 10 ml volumetric flask. Fill up to the mark with either EtOH (5.1) or THF (5.2), as in Calibration standard solutions (5.13). Transfer this solution to a 1,5 ml injection vial, previously filtered through a membrane filter (6.1) if necessary.

All these solutions should be prepared and protected from light until injection in the same working session.

7.2 LC-UV/Vis measurement conditions

When using the LC-UV/Vis system (6.5) and column (6.6) for water-soluble UV-filters determination, the conditions in Table 3 have shown to be suitable. When using LC systems with different delay volumes the gradient time tables should be accordingly adjusted.

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1) For identification purposes, diode-array detector is recommended.

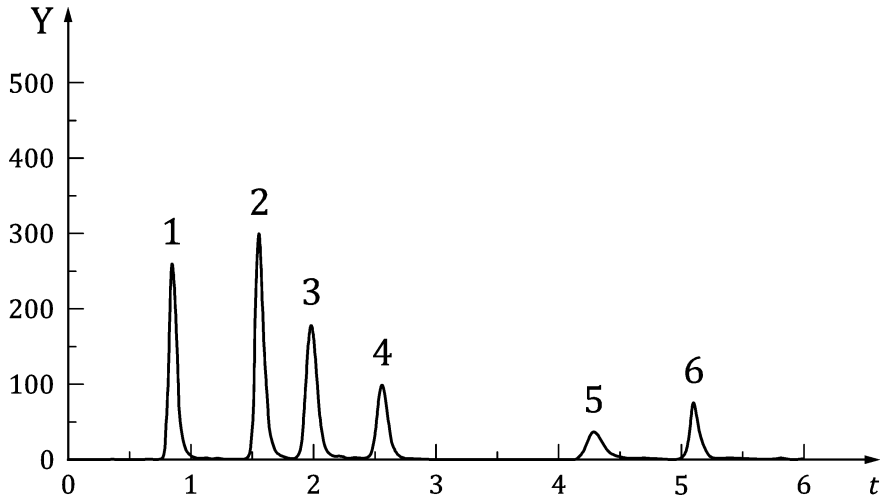
2) Purospher® STAR RP-18 Endcapped and PLgel Mixed D are examples of suitable products available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by CEN of these products. Equivalent products may be used if they can be shown to lead to the same results.

Table 3 — LC-UV/Vis conditions for water-soluble UV-filters determination

Column	Purospher® STAR RP-18 Endcapped or equivalent		
Injection volume	20 µl		
Column oven	45 °C		
Mobile phase gradient time table	Time min	EtOH %, v/v	1 % acetate buffer pH 4,75 solution %, v/v
	0,0	30	70
	3,0	30	70
	3,5	100	0
	6,0	100	0
	6,5	30	70
	10,0	30	70
Flow rate	1 ml/min		
Detection wavelength	313 nm [a]		

[a] When a diode-array detector is used, the wavelength of maximum absorbance of each compound could be used.

An example of a chromatogram, obtained using the LC-UV/Vis conditions described in Table 3, is shown in Figure 1.



Key

Y	absorbance in mAU	1	PDTA (0,84 min)	3	TDSA (1,98 min)	5	CBM (4,28 min)
t	time in min	2	PBSA (1,55 min)	4	BZ4 (2,56 min)	6	P25 (5,10 min)

Figure 1 — Chromatogram obtained for a standard solution of the water-soluble UV filters (20 µg/ml) using the conditions described in Table 3