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BSI Standards Publication

Cosmetics — Analysis of cosmetic products — Screening for UV-filters in cosmetic products and quantitative determination of 10 UV-filters by HPLC.

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National foreword

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The UK participation in its preparation was entrusted to Technical Committee CW/217, Cosmetics.

A list of organizations represented on this committee can be obtained on request to its secretary.

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Cosmétiques - Analyse des produits cosmétiques - Détection des filtres UV dans les produits cosmétiques et détermination quantitative de 10 filtres UV par CLHP

Kosmetische Mittel - Untersuchung von kosmetischen Mitteln - Screening und quantitative Bestimmung von 10 UV-Filtern in Sonnenschutzmitteln, HPLC-Verfahren

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Foreword

This document (EN 16344:2013) 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of an identical text or by endorsement, at the latest by January 2014, and conflicting national standards shall be withdrawn at the latest by January 2014.

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Introduction

Reference is made to the relevant annex of the Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products:

Annex VI List of UV-filters allowed in cosmetic products.

1 Scope

This European Standard specifies a multi-screening method using reversed-phase HPLC for the detection of UV-filters listed in the cosmetic regulations. The method is applicable for the quantitative determination of 10 UV-filters, which are mainly used in emulsion-based cosmetic products and sunscreen sprays particularly with regard to the maximum concentration listed in the cosmetic regulation.

Other analytical methods for the qualification and quantification of UV-filters may be used if they lead to comparable results.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1
UV-filters
organic chemical compounds that absorb ultraviolet light and inorganic substances that reflect, scatter and absorb UV light

Note 1 to entry: The UV-filters and UV-absorber of this method are only organic chemical compounds and are used in sunscreen products to protect the skin against UV radiation.

3 Principle

The UV-filters are extracted with an acetone/methanol mixture. For the qualitative detection of the listed UV-filters and the quantitative determination of the 10 validated UV-filter reversed phase HPLC with UV (DAD) detection is used. The method is also applicable for the quantification of the other listed UV-filters after proper validation.

Quantitative determination of samples containing the following UV-filters require the use of additional extraction methods and determinations:

— Terephthalylidene Dicamphor Sulfonic Acid (TDSA) and Disodium Phenyl Dibenzimidazole Tetrasulfonate (DPDT) are additionally extracted with methanolic-aqueous sodium hydroxide solution.

— Methylene Bis-benzotriazolyl Tetramethylbutylphenol (MBBT) and Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine (BEMT) are additionally extracted with a mixture of tetrahydrofuran/acetone.

In the case of an unsatisfactory peak shape, Butyl Methoxydibenzoylmethane (BMDM) is additionally extracted with a mixture of acetone/methanol/EDTA.

The quantitative determination is made by means of RP-HPLC with UV (DAD). The UV-spectra are compared with the reference spectra in a database.

The concentration of each UV-filter determined in accordance with this method is reported in g/100 g.

This method has been tested in an inter-laboratory test on specific cosmetic matrix (q.v. Annex A). The user should verify the performance of the method in their laboratory for each different matrix and pay particular attention to the recommended quality control elements.

4 Reagents

4.1 General

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

4.2 Methanol (MeOH), HPLC grade.

4.3 Acetone, HPLC grade.

4.4 Tetrahydrofuran (THF), HPLC grade.

4.5 Ammonia solution, mass fraction ω = 25 g/100 g.

4.6 Sodium hydroxide solution, molar concentration c = 1 mol/l.

4.7 Ethylenediaminetetraacetic acid (EDTA) disodium salt dihydrate ($Na_2EDTA \cdot 2H_2O$, CAS 6381-92-6, purity > 99 %).

4.8 EDTA solution

Weigh 1,8 g of EDTA disodium salt dihydrate (4.7) into a 100 ml volumetric flask and fill up to the calibration mark with water.

4.9 Ethanol, HPLC grade.

4.10 Lauryl Trimethyl Ammonium Bromide (LTAB, synonym: dodecyltrimethylammonium bromide. CAS 1119-94-4), if possible HPLC quality (purity $\geq$ 98 %).

4.11 Ammonium bromide (CAS 12124-97-9, purity $\geq$ 99 %).

4.12 Reference substances

Table 1 — Polar UV-filters (calibration solution in methanol)

	EU[a]	Abbrev.	INCI[b] and other common names
4.12.1	A2	CBM	Camphor Benzalkonium Methosulfate, CAS 52793-97-2
4.12.2	A6	PBSA	Phenylbenzimidazole Sulfonic Acid (2-phenylbenzimidazole-5-sulfonic acid), CAS 27503-81-7
4.12.3	A7	TDSA	Terephthalylidene Dicamphor Sulfonic Acid, CAS 90457-82-2, present as triethanolamine salt (molecular weight m = 860 g/mol), free acid (molecular weight m = 562 g/mol)
4.12.4	A22	B-4/5	Benzophenone-4/5 (2-hydroxy-4-methoxybenzophenone-5-sulfonic acid, Sulisobenzone), CAS 4065-45-6
4.12.5	A24	DPDT	Disodium Phenyl Dibenzimidazole Tetrasulfonate, CAS 180898-37-7
4.12.6	A28	DHHB	Diethylamino Hydroxybenzoyl Hexyl Benzoate, CAS 302776-68-7

[a] EU = serial number in accordance with Annex VI of (EC) No 1223/2009.

[b] INCI = International Nomenclature of Cosmetic Ingredients.

Table 2 — Medium polar UV-filters (calibration solution in methanol acetone (1:1))

	EU[a]	Abbrev.	INCI[b] and other common names
4.12.7	A4	B-3	Benzophenone-3 (oxybenzonum, 2-hydroxy-4-methoxy-benzophenone), CAS 131-57-7
4.12.8	A10	OC	Octocrylene (2-ethylhexyl-2-cyano-3,3-diphenylacrylate), CAS 6197-30-4
4.12.9	A12	EHMC	Ethylhexyl Methoxycinnamate (octylmethoxycinnamate), CAS 5466-77-3
4.12.10	A14	IMC	Isoamyl p-Methoxycinnamate, CAS 71617-10-2
4.12.11	A18	MBC	4-Methylbenzylidene Camphor (3-(4-methylbenzylidene)-dl-camphor), CAS 36861-47-9
4.12.12	A19	3-BC	3-Benzylidene Camphor, CAS 15087-24-8
4.12.13	A21	EHDP	Ethylhexyl Dimethyl PABA (2-ethylhexyl-4-dimethylaminobenzoate), CAS 21245-02-3

[a] EU = serial number in accordance with Annex VI of (EC) No 1223/2009.

[b] INCI = International Nomenclature of Cosmetic Ingredients.

Table 3 — Non polar UV-filters (calibration solution in THF)

	EU[a]	Abbrev.	INCI[b] and other common names
4.12.14	A3	HMS	Homosalate (3,3,5-trimethylcyclohexylsalicylate), CAS 118-56-9
4.12.15	A8	BMDM	Butyl Methoxydibenzoylmethane (4-tert-butyl-4'-methoxydibenzoylmethane), CAS 70356-09-1
4.12.16	A15	EHT	Ethylhexyl Triazone (octyltriazone), CAS 88122-99-0
4.12.17	A16	DTS	Drometrizole Trisiloxane (2-benzotriazole-2-yl-methylphenol trisiloxane), CAS 155633-54-8
4.12.18	A17	DEBT	Diethylhexyl Butamido Triazone, CAS 154702-15-5
4.12.19	A20	EHS	Ethylhexyl Salicylate (2-ethylhexylsalicylate), CAS 118-60-5
4.12.20	A23	MBBT	Methylene Bis-Benzotriazol Tetramethylbutylphenol, CAS 103597-45-1
4.12.21	A25	BEMT	Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine (anisotriazine), CAS 187393-00-6

[a] EU = serial number in accordance with Annex VI of (EC) No 1223/2009.

[b] INCI = International Nomenclature of Cosmetic Ingredients.

Table 4 — Other UV-filter or UV absorber (not listed in Annex VI of the (EC) No 1223/2009)

		Abbrev.	INCI[b] and other common names
4.12.22		MA	Menthyl Anthranilate, CAS 134-09-8
4.12.23		PABA	PABA (4-aminobenzoic acid), CAS 150-13-0
4.12.24		B-1	Benzophenone-1 (2,4-dihydroxybenzophenone), CAS 131-56-6
4.12.25		B-2	Benzophenone-2 (2,2',4,4'-tetrahydroxybenzophenone), CAS 131-55-5
4.12.26		DPLT	Dimethyl-PABAmidopropyl Laurdimonium Tosylate, CAS 156679-41-3
4.12.27		B-6	Benzophenone-6 (2,2'-dihydroxy-4,4'-dimethoxybenzophenone), CAS 131-54-4
4.12.28		B-8	Benzophenone-8 (2,2'-dihydroxy-4-methoxybenzophenone, dioxybenzone),CAS 131-53-3
4.12.29		B-9	Benzophenone-9 (disodium 3,3'-carbonylbis[4-hydroxy-6-methoxybenzenesulphonate]), CAS 76656-36-5
4.12.30		B-10	Benzophenone-10 (2-hydroxy-4-methoxy-4'-methylbenzophenone), CAS 1641-17-4
4.12.31		SA	Sodium Salicylate, CAS 54-21-7

[b] INCI = International Nomenclature of Cosmetic Ingredients.

NOTE The following UV-filters listed in Annex VI of the Regulation (EC) No 1223/2009 are not part of this method:

- A 9: 3-(4'-sulfo)-benzylidene-bornan-2-one, CAS 56039-58-8
- A 11: polymer of N-(2(and 4)-(2-oxoborn-3-ylidenemethyl)benzyl)acrylamid, CAS 113783-61-2 (the substances are no longer commercially available)
- A 13: ethoxylated ethyl-4-aminobenzoate, CAS 116242-27-4 (can be determined only qualitatively)
- A 26: dimethicodiethylbenzalmalonate, CAS 207574-74-1 (cannot be determined using this standard)

4.13 Extraction solution

4.13.1 Acetone/methanol mixture (for the preparation of the reference solutions and for extraction).

Mix 500 ml of acetone (4.3) and 500 ml of methanol (4.2) in a 1 000 ml conical flask.

4.13.2 Acetone/tetrahydrofuran mixture (for extraction of non-polar UV-filters).

Mix 500 ml of acetone (4.3) and 500 ml of tetrahydrofuran (4.4) in a 1 000 ml conical flask.

4.13.3 Methanolic-aqueous sodium hydroxide solution (for extraction of polar UV-filters).

Mix 800 ml of methanol (4.2) and 200 ml of water and 10 ml of sodium hydroxide solution (4.6) in a 1 000 ml conical flask.

4.13.4 Acetone/methanol/EDTA mixture (for extraction of BMDM-containing samples with unsatisfactory peak shape).

Add 1,0 ml of EDTA solution (4.8) to 200 ml of acetone/methanol mixture (4.13.1) in a 200 ml conical flask. As the solution is supersaturated with regard to EDTA it shall be used on the same day.

4.14 Reference solutions

4.14.1 Stock solutions

The stock solutions are prepared in accordance with Table 5, Table 6 and Table 7. For each substance, weigh the initial weights with an accuracy of 0,1 mg to the mentioned volume. One stock solution is prepared per substance. For the initial weight, the purity of the substances shall be taken into account and if applicable be converted to an initial weight of 4 mg/ml of "active substance" in each case. The stock solutions can be stored for at least one month in a refrigerator between 2 °C and 8 °C in the absence of light.

Table 5 — Scheme for preparing the stock solutions of polar UV-filters

Polar UV-filter	Initial weight	Volume	Solvent
CBM (29 %) (4.12.1)	140 mg	10 ml	Methanol (4.2) (Initial weight: 140 mg, as a 29 % standard solution is used; corresponds to 40 mg of "active substance")
PBSA (4.12.2)	40 mg	10 ml	Add 2 ml of methanol (4.2) and 3 drops of ammonia (4.5). Fill up to the calibration mark with water.
TDSA (4.12.3)	60 mg	10 ml	Add 2 ml of methanol (4.2) and 3 drops of ammonia (4.5). Fill up to the calibration mark with water. (Initial weight: 60 mg because of triethanolamine salt; corresponds to 40 mg of free acid, see limit value)
B-4/5 (4.12.4)	40 mg	10 ml	Methanol (4.2)
DPDT (4.12.5)	40 mg	10 ml	Add 2 ml of methanol (4.2) and 3 drops of ammonia (4.5). Fill up to the calibration mark with water.
DHHB (4.12.6)	40 mg	10 ml	Methanol (4.2)

Table 6 — Scheme for preparing the stock solutions of medium polar UV-filters

Medium-polar UV-filter	Initial weight	Volume	Solvent
B-3 (4.12.7)	40 mg	10 ml	Acetone/methanol mixture (4.13.1)
OC (4.12.8)	40 mg	10 ml	Acetone/methanol mixture (4.13.1)
EHMC (4.12.9)	40 mg	10 ml	Acetone/methanol mixture (4.13.1)
MBC (4.12.11)	40 mg	10 ml	Acetone/methanol mixture (4.13.1)
IMC (4.12.10)	40 mg	10 ml	Acetone/methanol mixture (4.13.1)
3-BC (4.12.12)	40 mg	10 ml	Acetone/methanol mixture (4.13.1)
EHDP (4.12.13)	40 mg	10 ml	Acetone/methanol mixture (4.13.1)

Table 7 — Scheme for preparing the stock solutions of non polar UV-filters

Non-polar UV-filter	Initial weight	Volume	Solvent
HMS (4.12.14)	40 mg	10 ml	Tetrahydrofuran (4.4)
BMDM (4.12.15)	40 mg	10 ml	Tetrahydrofuran (4.4)
EHT (4.12.16)	40 mg	10 ml	Tetrahydrofuran (4.4)
DTS (4.12.17)	40 mg	10 ml	Tetrahydrofuran (4.4)
DEBT (4.12.18)	40 mg	10 ml	Tetrahydrofuran (4.4)
EHS (4.12.19)	40 mg	10 ml	Tetrahydrofuran (4.4)
MBBT (4.12.20)	40 mg	10 ml	Tetrahydrofuran (4.4)
BEMT (4.12.21)	40 mg	10 ml	Tetrahydrofuran (4.4)

4.14.2 Preparing the stock solutions for the UV-filters of Table 4 (not permitted UV-filters)

The stock solutions of UV-Filter B-1, B-2, B-6, B-8, B-10, MA and DPLT can be prepared using the acetone/methanol mixture (4.13.1) as a solvent. PABA and SA can be prepared using methanol as solvent. B-9 can be prepared according to the procedure for PBSA (4.12.2). The B-9 solution colours yellow but experiments showed that no degradation of the substance took place and the colouring of the solution did not influence the result.

4.14.3 Calibration solutions

The calibration solutions are prepared according to the scheme given in Table 8. The stock solution volumes of all UV-filters for polar (see Table 5), medium polar (see Table 6) and non-polar (see Table 7) are mixed in each case in calibration solution 1 (Cal 1) and made to the volumetric graduation mark with either methanol (MeOH), acetone/methanol mixture (MeOH/Acetone) or tetrahydrofuran (THF). Follow up calibration solutions are prepared from Cal 1. This results in a calibration within the range of 10 ng and 400 ng. A 1 µl volume is injected for the analysis. The calibration solutions may be stored for at least one week in a refrigerator between 2 °C and 8 °C in the absence of light.

Table 8 — Scheme for preparing the calibration solutions

Calibration solution	Volume	Solution	ad to	Solvent			Concentration	
				Polar	Medium-polar	Non-polar	Solution	Sample[a]
	ml		ml				ng/µl	g/100 g
1	2	Stock	20	MeOH	MeOH/Acetone	THF	400	10,0
2	5	Cal 1	10	MeOH	MeOH/Acetone	THF	200	5,0
3	2,5	Cal 1	10	MeOH	MeOH/Acetone	THF	100	2,5
4	1	Cal 1	10	MeOH	MeOH/Acetone	THF	40	1,0
5	0,5	Cal 1	10	MeOH	MeOH/Acetone	THF	20	0,5
6	0,5	Cal 1	20	MeOH	MeOH/Acetone	THF	10	0,25

[a] Referring to a sample initial weight of 100 mg.

All standard solutions shall be protected from light during the entire analysis.

4.15 Mobile phase for HPLC

4.15.1 Mobile phase A: aqueous phase

Weigh 3,0 g of LTAB (4.10), 1,0 g of ammonium bromide (4.11) and 2,5 ml of EDTA solution (4.8) into a 1 000 ml volumetric flask and fill up to the calibration mark with water.

4.15.2 Mobile phase B: organic phase

Weigh 3,0 g of LTAB (4.10) and 1,0 g of ammonium bromide (4.11) into a 1 000 ml volumetric flask and fill up to the calibration mark with ethanol (4.9).

5 Apparatus and equipment

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

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

5.2 Erlenmeyer flask, conical flask 50 ml with glass stopper.

5.3 Laboratory shaker.

5.4 Ultrasonic bath, with temperature controlled heater.

5.5 Centrifuge, at least 2 500 g.

5.6 Membrane filter, for sample filtration, e.g. PTFE, 0,2 µm pore size[1)].

5.7 High-performance liquid chromatograph consisting of:

— sampling device;

— pump system with gradient function;

— degasser;

— column oven;

— photodiode array detector (PDA);

— evaluation system.

5.8 Analytical separation column, e.g.:

Kromasil column C18, 5 µm, 125 mm, ID, e.g. 2 mm, 3 mm or 4 mm.

A pre-column packed with stationary phase similar to the analytical separation column shall be used.

6 Sampling

The sampling technique is not part of the technique specified in the official method.

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1) The ring test was performed using 0,2 µm filters.

7 Procedure

7.1 Sample preparation

7.1.1 Standard screening extraction

Weigh 90 mg to 120 mg of the sample to the nearest 0,1 mg into a 50 ml conical flask (5.2), add 25 ml of the acetone/methanol mixture (4.13.1) using a volumetric pipette and shake vigorously. Close the conical flask with the glass stopper and extract the sample for 15 min at 50 °C in the ultrasonic bath. After cooling (approximately 20 min), centrifuge the suspension for 5 min if applicable at about 2 500 *g*. Filter approximately 1 ml of the supernatant through a PTFE membrane filter (5.6). Inject the filtrate in the HPLC system.

If necessary, the weight can be adapted according to the concentration filter levels.

Care shall be taken that no solvent evaporates during the extraction. To detect any solvent losses the UV-filter benzophenone-10 (4.12.30) can be added in a concentration of w = 0,1 mg/ml to the sample extraction as an internal standard.

7.1.2 Quantitative extraction for TDSA, DPDT, BMDM, MBBT and BEMT

The extraction is carried out as described in 7.1.1; however, the extracting agents given in Table 9 shall be used for the respective analytes.

Table 9 — Solvents for the extraction of specific UV-filters

UV-filter	Solvent
MBBT/BEMT	Acetone/tetrahydrofuran mixture (4.13.2)
TDSA/DPDT	Methanolic-aqueous sodium hydroxide solution (4.13.3)
BMDM	Acetone/methanol/EDTA mixture (4.13.4)

All sample solutions shall be protected from light during the entire analysis.

7.2 High-performance liquid chromatography (HPLC)

The qualitative and quantitative analysis is performed by means of ion-pair elution reversed-phase HPLC. The following HPLC conditions can be regarded as an example of the selection of suitable working conditions. They shall be adapted to the apparatus, columns and mobile phases which are actually used.

— Injection volume: e.g. 1 µl.

— Column temperature: (30 ± 1) °C.

— Detection: 300 nm, 350 nm.

UV-spectral range: 220 nm to 420 nm.

NOTE When flushing the injection system an acetone/methanol mixture with a volume fraction of 1:1 (4.13.1) has proved useful in avoiding any carry-over which may occur in particular in the case of non-polar UV-filters (MBBT and BEMT).

When using HPLC systems with different delay volumes, the gradient shall be adjusted accordingly. Particular care shall be taken that no substance elutes during the flow gradient. This would lead to non-reproducible

results. In the following tables, three alternative gradient programmes (regarding different internal diameters (ID) of the column) are given as examples.

Table 10 — Gradient programme using a column with 2 mm ID (HPLC-System with 850 µl delay volume)

Time min	**Mobile phase A** (% aqueous)	**Mobile phase B** (% organic)	**Volume flow** ml/min
0	70	30	0,27
1	35	65	0,27
13	15	85	0,27
14	0	100	0,27
18,6	0	100	0,27
19,4	0	100	0,6
21,5	0	100	0,6
22	70	30	0,6
23	70	30	0,6
23,5	70	30	0,27
32	70	30	0,27

Table 11 — Gradient programme using a column with 3 mm ID (HPLC-system with 850 µl delay volume)

Time min	**Mobile phase A** (% aqueous)	**Mobile phase B** (% organic)	**Volume flow** ml/min
0	70	30	0,50
4,0	35	65	0,50
15,0	15	85	0,50
16,0	0	100	0,50
17,5	0	100	0,50
17,6	0	100	0,90
22,5	0	100	0,90
22,6	70	30	0,50
29,0	70	30	0,50

Table 12 — Gradient programme using a column with 4 mm ID (HPLC-system with 850 µl delay volume)

Time min	Mobile phase A (% aqueous)	Mobile phase B (% organic)	Volume flow ml/min
0	70	30	0,80
4	35	65	0,80
15	15	85	0,80
16	0	100	0,80
17	0	100	0,80
17,1	0	100	1,2
23	0	100	1,2
23,1	70	30	0,80
29	70	30	0,80

From time to time the column can be uncoupled and be backwashed with acetone or THF. If the separation efficiency is unsatisfactory the pre-column should be replaced.

8 Evaluations

8.1 Evaluation of the chromatograms

The peak areas are measured at a wavelength of 300 nm or 350 nm respectively (see Annex B).

If it is necessary, calibration can be made at any wavelength better suited and/or based on the peak height. The chromatogram peaks are allocated based on the retention times and the UV spectrum.

If the peak shape of BMDM (4.12.15) is unsatisfactory, it is extracted additionally with an acetone/methanol/EDTA mixture (4.13.4) (see Annex C).

8.2 Calculation

Quantification is done by means of a calibration line based on the peak areas or peak heights of the external standards:

$$y = m \cdot x + b \tag{1}$$

where

y is the peak area/peak height of the standard;

x is the concentration of the standard;

m is the slope of the calibration line;

b is the y-intercept of the calibration line.

Since the calibration range is relatively large (factor 40), weighting with $1/x$ (or 1/amount) has proved useful for calculating the regression line.

Based on the peak area or peak height the amount x of the analyte (in ng) is calculated from the regression line.

The UV-filter content in the sample used in g/100 g can be calculated by the following formula:

$$\omega = \frac{\frac{x}{InjV} \cdot V \cdot VF}{m \cdot 10} = \frac{x \cdot 2{,}5}{m} \quad (2)$$

where

ω is the UV-filter concentration, in g/100 g;

x is the analyte content obtained from calibration, in ng;

$InjV$ is the injection volume, in µl (e.g. 1 µl);

m is the initial weight, in mg;

V is the extraction volume, in ml (in this case: 25 ml);

VF is the dilution factor (only applicable if the sample is diluted);

10 is the conversion factor (mg/g to g/100 g).

In the case of the Octocrylene filter (OC - A10) the content is determined as an ester and it shall be converted into the content of the corresponding acid in accordance with the (EC) No 1223/2009 Annex VI. The molecular weight of the ester is 361,48 g/mol, the molecular weight of the free acid is 249,27 g/mol. This gives a conversion factor of 0,69.

8.3 Expression of results

The content is reported in g/100 g rounded to three significant figures.

9 Test report

The test report shall contain the following data:

a) information necessary for the identification of the sample (type, origin and designation of the sample);

b) a reference to this European Standard;

c) name of the laboratory performing the test;

d) the date and type of sampling procedure (if known);

e) the date of receipt and date of analysis;

f) the date of test;

g) the test results and the units in which they have been expressed;

h) justification of any deviations from this official method;

i) operations not specified in the method or regarded as optional, which might have affected the results.

Annex A
(informative)

Results of the inter-laboratory test

A.1 General

This method has been developed by the “Cosmetics” working group of the German Federal Office of Consumer Protection and Food Safety (BVL) for the purpose of implementing Section 64 of the Food and Feed Code (LFGB); it has been tested in an inter-laboratory test with a total of 10 participants.

The reliability of the method has been tested for the following UV-filters:

— Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine (BEMT, 4.12.21);

— Butyl Methoxydibenzoylmethane (BMDM, 4.12.15);

— Diethylhexyl Butamido Triazone (DEBT, 4.12.18);

— Drometrizole Trisiloxane (DTS, 4.12.17);

— Ethylhexyl Methoxycinnamate (EHMC, 4.12.9);

— Ethylhexyl Triazone (EHT, 4.12.16);

— Isoamyl p-Methoxycinnamate (IMC, 4.12.10);

— Methylene Bis-Benzotriazol Tetramethylbutylphenol (MBBT, 4.12.20);

— Octocrylene (OC, 4.12.8);

— Terephthalylidene Dicamphor Sulfonic Acid (TDSA, 4.12.3).

Sample 1: sunscreen milk, Sun Protection Factor 40.

Sample 2: sunscreen spray, Sun Protection Factor 50.

Sample 3: sunscreen product oil in water, Sun Protection Factor unknown.

Samples 1 and 2 are commercially available products and sample 3 has been specially manufactured for the inter-laboratory test.

Any trade names mentioned in this method or any product descriptions referring to individual suppliers are given only as an information for the users of this method and do not constitute an endorsement by CEN of the product mentioned. Equivalent products may be used if it can be demonstrated that they lead to the same results.

A.2 Reliability of the method

Table A.1 — Sample 1 – Extracting agent as described in 4.13.1 (acetone/methanol mixture)

Parameter	BMDM A 08	OC A 10	EHMC A 12	DEBT A 17	BEMT A 25
Number of participating laboratories	10	10	10	10	10
Number of laboratories after elimination of the outliers	10	10	10	10	9
Number of outliers	0	0	0	0	1
Target value, g/100 g	4,80	6,00	1,50	1,30	0,80
Mean value $\bar{x}$ g/100 g	4,95	6,16	1,64	1,36	0,84
Recovery, %	103,1	102,7	109,3	104,6	105,0
Repeatability limit r, g/100 g	0,24	0,33	0,09	0,07	0,04
Repeatability standard deviation s_r, g/100 g	0,08	0,12	0,03	0,03	0,01
Relative repeatability standard deviation $s_{r,rel}$, %	1,8	1,9	2,2	2,0	1,6
Reproducibility limit R, g/100 g	0,66	0,90	0,19	0,26	0,20
Reproducibility standard deviation s_R, g/100 g	0,24	0,32	0,07	0,09	0,07
Relative reproducibility standard deviation $s_{R,rel}$, %	4,9	5,4	4,5	7,2	8,7
Horrat value	1,6	1,8	1,2	1,9	2,1

Table A.2 — Sample 1 – Extraction as described in 4.13.2 (acetone/tetrahydrofuran mixture)

Parameter	BEMT A 25	BMDM A 08	EHMC A 12	DEBT A 17	MBBT A 23	OC A 10
Number of participating laboratories	10	10	10	10	10	10
Number of laboratories after elimination of the outliers	8	9	8	9	9	9
Number of outliers	2	1	2	1	1	1
Target value, g/100 g	0,80	4,80	1,50	1,30	2,00	6,00
Mean value $\bar{x}$ g/100 g	0,82	4,89	1,63	1,28	2,06	6,00
Recovery, %	102,5	101,9	108,7	98,5	103,0	100,0
Repeatability limit r, g/100 g	0,06	0,16	0,06	0,06	0,09	0,21
Repeatability standard deviation s_r, g/100 g	0,02	0,06	0,02	0,02	0,03	0,08
Relative repeatability standard deviation $s_{r,rel}$, %	2,5	1,2	1,3	1,6	1,6	1,3
Reproducibility limit R, g/100 g	0,16	0,53	0,12	0,29	0,42	0,48
Reproducibility standard deviation s_R, g/100 g	0,06	0,19	0,04	0,10	0,15	0,17
Relative reproducibility standard deviation $s_{R,rel}$, %	7,1	4,0	2,9	7,9	7,5	2,9
Horrat value	1,7	1,3	0,8	2,1	2,1	0,9

Table A.3 — Sample 1 – Extracting agent as described in 4.13.4 (acetone/methanol/EDTA mixture)

Parameter	BEMT A 25	BMDM A 08	EHMC A 12	DEBT A 17	OC A 10
Number of participating laboratories	10	10	10	10	10
Number of laboratories after elimination of the outliers	9	9	9	9	9
Number of outliers	1	1	1	1	1
Target value, g/100 g	0,80	4,80	1,50	1,30	6,00
Mean value $\bar{x}$ g/100 g	0,85	4,98	1,66	1,36	6,09
Recovery, %	106,3	103,8	110,7	104,6	101,5
Repeatability limit r, g/100 g	0,13	0,30	0,10	0,10	0,34
Repeatability standard deviation s_r, g/100 g	0,05	0,11	0,04	0,04	0,12
Relative repeatability standard deviation $s_{r,rel}$, %	5,8	2,2	2,4	2,7	2,0
Reproducibility limit R, g/100 g	0,23	0,45	0,17	0,26	0,53
Reproducibility standard deviation s_R, g/100 g	0,08	0,16	0,06	0,09	0,19
Relative reproducibility standard deviation $s_{R,rel}$, %	10,1	3,4	4,1	7,2	3,2
Horrat value	2,4	1,1	1,1	1,9	0,9

Table A.4 — Sample 2 – Extracting agent as described in 4.13.1 (acetone/methanol mixture)

Parameter	BMDM A 08	BEMT A 25	EHMC A 12	DEBT A 17	IMC A 14	OC A 10
Number of participating laboratories	10	10	10	10	10	10
Number of laboratories after elimination of the outliers	9	9	9	9	9	9
Number of outliers	1	1	1	1	1	1
Target value, g/100 g	4,8	0,80	3,00	2,00	3,00	9,00
Mean value $\bar{x}$ g/100 g	4,86	0,81	3,04	2,00	2,98	8,86
Recovery, %	101,3	101,3	101,3	100,0	99,3	98,4
Repeatability limit r, g/100 g	0,18	0,03	0,13	0,08	0,11	0,32
Repeatability standard deviation s_r, g/100 g	0,07	0,01	0,05	0,03	0,04	0,12
Relative repeatability standard deviation $s_{r,rel}$, %	1,4	1,5	1,5	1,4	1,3	1,3
Reproducibility limit R, g/100 g	0,71	0,16	0,20	0,34	0,36	0,75
Reproducibility standard deviation s_R, g/100 g	0,25	0,06	0,07	0,12	0,13	0,27
Relative reproducibility standard deviation $s_{R,rel}$, %	5,3	7,2	2,4	6,0	4,2	3,0
Horrat value	1,7	1,8	0,7	1,7	1,3	1,0

Table A.5 — Sample 2 – Extracting agent as described in 4.13.2 (acetone/tetrahydrofuran mixture)

Parameter	BEMT A 25	BMDM A 08	EHMC A 12	DEBT A 17	IMC A 14	MBBT A 23	OC A 10
Number of participating laboratories	10	10	10	10	10	10	10
Number of laboratories after elimination of the outliers	9	9	9	9	9	10	9
Number of outliers	1	1	1	1	1	0	1
Target value, g/100 g	0,80	4,8	3,00	2,00	3,00	4,5	9,00
Mean value $\bar{x}$ g/100 g	0,81	4,90	3,07	1,97	2,89	4,68	8,93
Recovery, %	101,3	102,1	102,3	98,5	96,3	104,0	99,2
Repeatability limit r, g/100 g	0,06	0,27	0,28	0,08	0,12	0,19	0,35
Repeatability standard deviation s_r, g/100 g	0,02	0,10	0,10	0,03	0,04	0,07	0,13
Relative repeatability standard deviation $s_{r,rel}$, %	2,4	2,0	3,3	1,5	1,4	1,5	1,4
Reproducibility limit R, g/100 g	0,13	0,61	0,37	0,23	0,64	1,07	0,93
Reproducibility standard deviation s_R, g/100 g	0,05	0,22	0,13	0,08	0,23	0,38	0,33
Relative reproducibility standard deviation $s_{R,rel}$, %	5,9	4,5	4,4	4,1	7,6	8,5	3,7
Horrat value	1,4	1,4	1,3	1,2	2,2	2,7	1,3

Table A.6 — Sample 2 – extracting agent as described in 4.13.4 (acetone/methanol/EDTA mixture)

Parameter	BEMT A 25	BMDM A 08	DEBT A 17	EHMC A 12	IMC A 14	OC A 10
Number of participating laboratories	10	10	10	10	10	10
Number of laboratories after elimination of the outliers	9	9	9	9	9	9
Number of outliers	1	1	1	1	1	1
Target value, g/100 g	0,80	4,8	2,00	3,00	3,00	9,00
Mean value $\bar{x}$ g/100 g	0,84	4,94	2,03	3,12	2,93	8,99
Recovery, %	105,0	102,9	101,5	104,0	97,7	99,9
Repeatability limit r, g/100 g	0,06	0,31	0,15	0,20	0,18	0,56
Repeatability standard deviation s_r, g/100 g	0,02	0,11	0,05	0,07	0,07	0,20
Relative repeatability standard deviation $s_{r,rel}$ %	2,7	2,3	2,7	2,4	2,2	2,2
Reproducibility limit R, g/100 g	0,22	0,60	0,30	0,24	0,65	0,91
Reproducibility standard deviation s_R, g/100 g	0,08	0,22	0,11	0,09	0,23	0,32
Relative reproducibility standard deviation $s_{R,rel}$, %	9,8	4,5	5,3	2,9	7,8	3,6
Horrat value	2,4	1,4	1,5	0,8	2,3	1,3

Table A.7 — Sample 3 – Extracting agent as described in 4.13.1 (acetone/methanol mixture)

Parameter	BMDM A 8	DTS A 16	EHT A 15	OC A 10	TDSA A 7
Number of participating laboratories	10	10	10	10	10
Number of laboratories after elimination of the outliers	9	9	9	9	10
Number of outliers	1	1	1	1	0
Target value, g/100 g	3,00	6,00	1,20	3,00	3,00
Mean value $\bar{x}$ g/100 g	2,97	6,28	1,25	3,03	3,08
Recovery, %	99,0	104,7	104,2	101,0	102,8
Repeatability limit r, g/100 g	0,08	0,18	0,03	0,08	0,10
Repeatability standard deviation s_r, g/100 g	0,03	0,06	0,01	0,03	0,04
Relative repeatability standard deviation $s_{r,rel}$, %	1,0	1,1	1,0	1,0	1,2
Reproducibility limit R, g/100 g	0,30	1,01	0,13	0,33	0,27
Reproducibility standard deviation s_R, g/100 g	0,11	0,36	0,05	0,12	0,10
Relative reproducibility standard deviation $s_{R,rel}$, %	3,6	6,0	3,8	4,0	3,2
Horrat value	1,1	2,0	1,0	1,2	0,9

Table A.8 — Sample 3 – Extracting agent as described in 4.13.2 (acetone/tetrahydrofuran mixture)

Parameter	BMDM A 8	DTS A 16	OC A 10
Number of participating laboratories	10	10	10
Number of laboratories after elimination of the outliers	9	9	9
Number of outliers	1	1	1
Target value, g/100 g	3,00	6,00	3,00
Mean value $\bar{x}$ g/100 g	2,85	6,11	3,03
Recovery, %	95,0	101,8	101,0
Repeatability limit r, g/100 g	0,13	0,28	0,12
Repeatability standard deviation s_r, g/100 g	0,05	0,10	0,04
Relative repeatability standard deviation $s_{r,rel}$, %	1,6	1,7	1,48
Reproducibility limit R, g/100 g	0,36	0,75	0,26
Reproducibility standard deviation s_R, g/100 g	0,13	0,27	0,09
Relative reproducibility standard deviation $s_{R,rel}$, %	4,3	4,5	3,1
Horrat value	1,3	1,5	0,9

Table A.9 — Sample 3 – Extracting agent as described in 4.13.3 (methanol/sodium hydroxide mixture)

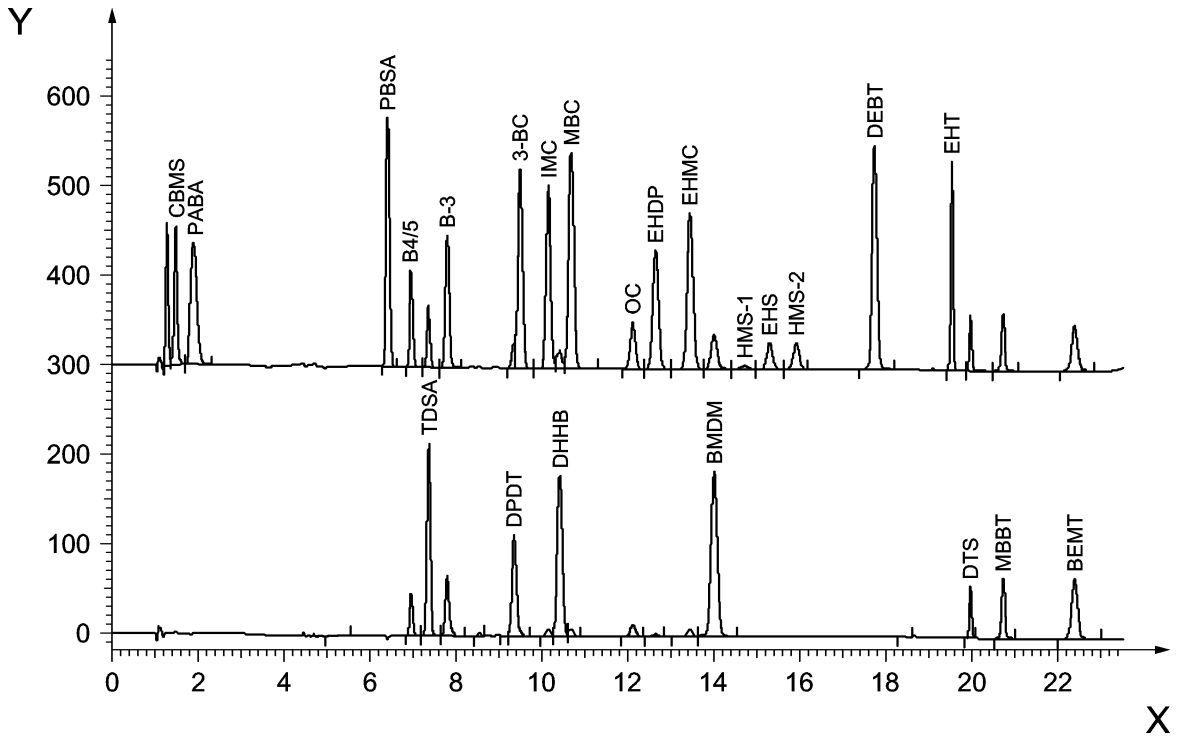
Parameter	TDSA A 7
Number of participating laboratories	10
Number of laboratories after elimination of the outliers	9
Number of outliers	1
Target value, g/100 g	3,00
Mean value $\bar{x}$ g/100 g	3,07
Recovery, %	102,2
Repeatability limit r, g/100 g	0,14
Repeatability standard deviation s_r, g/100 g	0,05
Relative repeatability standard deviation $s_{r,rel}$, %	1,6
Reproducibility limit R, g/100 g	0,50
Reproducibility standard deviation s_R, g/100 g	0,18
Relative reproducibility standard deviation $s_{R,rel}$, %	5,9
Horrat value	1,7

Table A.10 — Sample 3 – Extracting agent as described in 4.13.4 (acetone/methanol/EDTA mixture)

Parameter	BMDM A 8	DTS A 16	OC A 10	TDSA A 7
Number of participating laboratories	10	10	10	10
Number of laboratories after elimination of the outliers	9	9	9	9
Number of outliers	1	1	1	1
Target value, g/100 g	3,00	6,00	3,00	3,00
Mean value $\bar{x}$ g/100 g	3,03	6,16	3,08	3,13
Recovery, %	101,0	102,7	102,7	104,3
Repeatability limit r, g/100 g	0,22	0,49	0,14	0,13
Repeatability standard deviation s_r, g/100 g	0,08	0,18	0,05	0,05
Relative repeatability standard deviation $s_{r,rel}$, %	2,6	2,9	1,6	1,.6
Reproducibility limit R, g/100 g	0,30	0,76	0,35	0,37
Reproducibility standard deviation s_R, g/100 g	0,11	0,27	0,12	0,.13
Relative reproducibility standard deviation $s_{R,rel}$, %	3,6	4,5	4,1	4,4
Horrat value	1,1	1,5	1,2	1,3

Annex B
(informative)

Sample Chromatogram 1



Key

X time in min

Y absorption in mAU

upper line: Detection wavelength: 300 nm

lower line: Detection wavelength: 350 nm

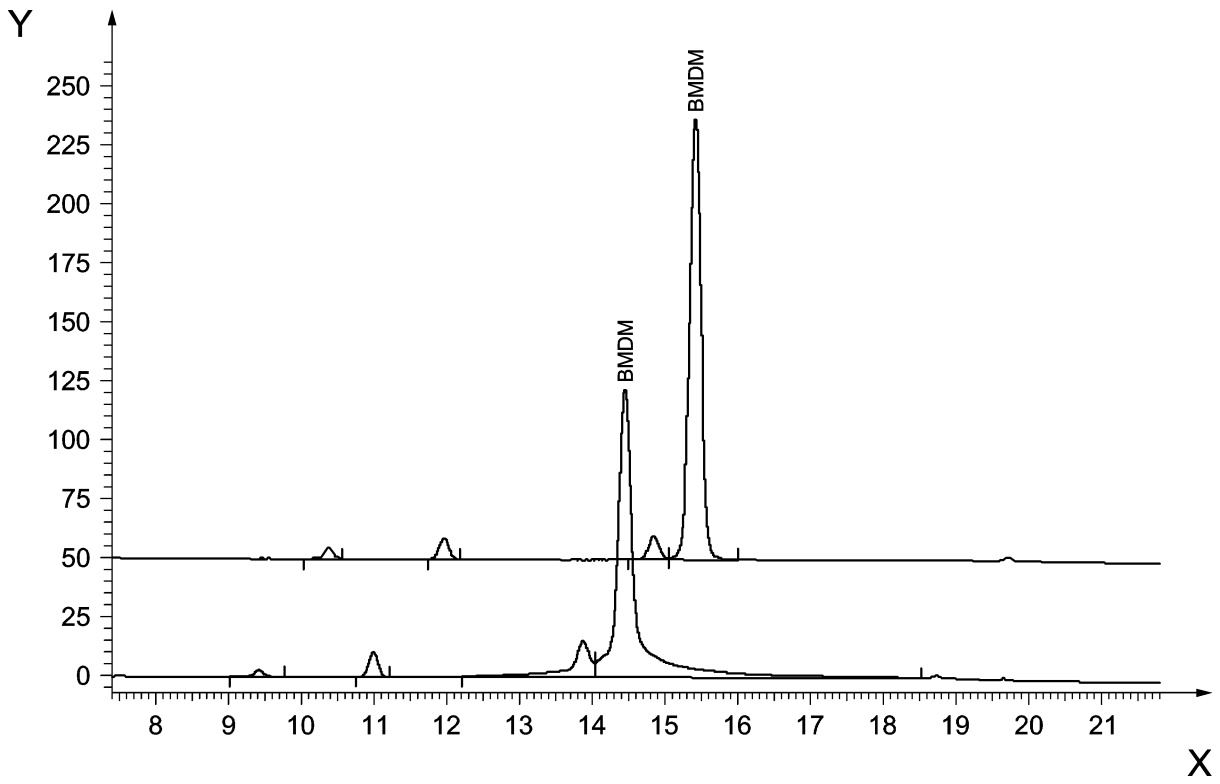
Figure B.1 — Sample Chromatogram 1 - shown here with a 150 mm × 2 mm column

Table B.1 — Retention times of the analytes in the standard mixture

UV-filter	Rt (min)	Detection wavelength (nm)
Camphor Benzalkonium Methosulfate	1,4	300
PABA	1,9	300
PEG-25 PABA	2,2 to 4,7	300
Benzophenone-2	5	350
Benzophenone-1	6,3	300
Phenyl Benzimidazole Sulfonic Acid	6,5	300
Salicylate (available only as sodium salt)	6,7	300
Benzophenone 8 (Dioxybenzone)	6,7	300
Benzophenone-4/5	7,1	300
Benzophenone-6	7,3	300
Terephthalylidene Dicamphor Sulfonic Acid	7,5	350
Benzophenone-9	7,7	300
Benzophenone-3	8	300
Benzophenone-10	9,1	300
Disodium Phenyl Dibenzimidazole Tetrasulfonate	9,6	350
Benzylidene Camphor	9,7	300
Isoamyl p-Methoxycinnamate	10,4	300
Diethylamino Hydroxybenzoyl Hexyl Benzoate	10,6	350
4-Methylbenzylidene Camphor	10,9	300
Octocrylene	12,4	300
Ethylhexyl Dimethyl PABA	12,9	300
Menthyl Anthranilate	13,3	350
Ethylhexyl Methoxycinnamate	13,8	300
Butylmethoxy Dibenzoylmethane	14,3	350
Ethylhexyl Salicylate	15,7	300
Homosalate	16,3	300
Diethylhexyl Butamido Triazone	18	300
Ethylhexyl Triazone	19,8	300
Drometrizole Trisiloxane	20,1	350
Methylene Bis-Benzotriazol Tetramethylbutylphenol	20,8	350
Bis-Ethylhexyloxyphenol Methoxyphenyl Triazine	22,5	350

Annex C
(informative)

Sample chromatogram 2



Key
X time in min
Y absorption in mAU

Figure C.1 — BMDM in a critical sample with (upper line) or without (lower line) addition of EDTA to the extracting medium

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