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Cosmetics — Analysis of cosmetic products — Quantitative determination of zinc pyrithione, piroctone olamine and climbazole in surfactant containing cosmetic anti-dandruff products

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Cosmétiques - Analyse des produits cosmétiques - Détermination quantitative de la pyrithione de zinc, de la piroctone olamine et du climbazole dans les produits cosmétiques antipelliculaires contenant des agents de surface

Kosmetische Mittel - Untersuchung von kosmetischen Mitteln - Quantitative Bestimmung von Zinkpyrithion, Pirocton-Olamin und Climbazol in tensidhaltigen kosmetischen Mitteln mit Antischuppenwirkstoffen

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Foreword

This document (EN 16342:2013) 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of an identical text or by endorsement, at the latest by November 2013, and conflicting national standards shall be withdrawn at the latest by November 2013.

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Introduction

Special hair products contain substances to help prevent dandruff. These substances mainly inhibit the development of microorganisms, which often are the cause of dandruff. The most commonly used substances are zinc pyrithione, piroctone olamine and climbazole. The substances are regulated by Council Directive of 27 July 1976 on the approximation of the laws of the member states relating to cosmetic products (EC 76/768/EEC) as well as Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. Limits for these substances are listed in the annexes regulating preservatives in cosmetic products. Zinc pyrithione is additionally listed in Annex III of both regulative documents named above.

NOTE As the Regulation (EC) 1223/2009 applies in total from 11 July 2013 and replaces Directive 76/768/EEC the following details relate only to Regulation (EC) 1223/2009.

Reference Number, maximum authorised concentration in hair products, limitations and requirements:

Annex III Regulation (EC) 1223/2009

Zinc pyrithione:	No. 101:	0,1 % leave-on hair products	Remark: For purposes other than inhibiting the development of microorganisms in the product. This purpose has to be apparent from the presentation of the product.

Annex V Regulation (EC) 1223/2009

Zinc pyrithione:	No. 8:	1,0 % hair products	Remark: Only in rinse-off products
		0,5 % other products	Remark: Not to be used in oral products
Climbazole:	No. 32:	0,5 %	
Piroctone olamine:	No. 35:	1,0 % rinse-off products	
		0,5 % other products	

1 Scope

This European Standard specifies an analytical method for the detection and quantitative determination of the following anti-dandruff agents: zinc pyrithione, piroctone olamine and climbazole in surfactant-containing cosmetic products in the concentration range from 0,1 g/100 g to 1,0 g/100 g.

NOTE The method is also suitable for the determination of ketoconazole and ciclopirox olamine (q.v. Annex A) in surfactant-containing cosmetic products and it is probably applicable for the determination of the substances in non surfactant-containing cosmetic products. For these purposes, the method has not been validated.

2 Terms and definitions

For the purposes of this document, the following term and definition applies.

2.1
anti-dandruff agents
substances, added to hair care products, active against the development of microorganism e.g. zinc pyrithione, piroctone olamine and climbazole

3 Principle

The anti-dandruff agents are extracted from the cosmetic sample matrix using dichloromethane and methanol. Each analyte present in the sample extract is separated using reversed phase HPLC with UV (DAD) detection. The quantitative determination is made using the external standard method of calibration.

4 Reagents

4.1 General

If not otherwise specified, as a minimum 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, CAS number: 67-56-1.

4.3 Dichloromethane, CAS number: 75-09-02.

4.4 Acetonitrile, CAS number: 75-05-8.

4.5 Ethylenediaminetetraacetic acid (EDTA) disodium salt dihydrate ($Na_2EDTA \cdot 2H_2O$), CAS number: 6381-92-6.

4.6 Oxalic acid dihydrate, CAS number: 6153-56-6.

4.7 Acetic acid (glacial), CAS number: 64-19-7, mass fraction w = 99,8 g/100 g.

4.8 Acetic acid, molar concentration c = 0,02 mol/l.

Weigh 1,20 g of acetic acid glacial (4.7) into a 1-l-volumetric flask and fill with water up to the calibration mark.

4.9 Methanol/acetic acid mixture

Mix 80 parts by volume of methanol (4.2) and 20 parts by volume of acetic acid (4.8).

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

4.11 Eluents

4.11.1 Eluent A: 0,002 7 mol/l of oxalic acid dihydrate (4.6) and 0,001 mol/l of EDTA (4.5) in water, pH 4,0:

Pre-dissolve 0,37 g of EDTA in water, add 0,35 g of oxalic acid dihydrate (0,025 % of oxalic acid) and adjust to pH = 4,0 with sodium hydroxide solution (4.10) using a pH-Meter. Then fill with water up to the 1000 ml mark.

4.11.2 Eluent B: Acetonitrile (4.4).

4.12 Reference substances

4.12.1 General

For the reference substances, no purity is defined. However, the purity of the reference substances shall be known to determine the definite amount of standard in the calibration solution.

4.12.2 Zinc pyrithione, CAS number: 13463-41-7.

4.12.3 Piroctone olamine, (1-hydroxy-4-methyl-6-(2,4,4-trimethylpentyl)-2-pyridone),
CAS number: 68890-66-4.

4.12.4 Climbazole, (1-(4-chlorophenoxy)-1-(imidazol-1-yl)-3,3-dimethyl-2-butanone),
CAS number: 38083-17-9.

4.13 Stock solutions

4.13.1 General

Fresh stock solutions need to be prepared each working day.

4.13.2 Zinc pyrithione stock solution, mass concentration β = 250 mg/l.

Weigh approximately 25 mg of zinc pyrithione (4.12.2) to the nearest 0,1 mg into a 100-ml-volumetric flask, dissolve in 50 ml of dichloromethane (4.3) and fill up to the mark with the methanol/acetic acid mixture (4.9).

4.13.3 Piroctone olamine and climbazole stock solution, mass concentration β = 250 mg/l.

Weigh approximately 25 mg of each piroctone olamine (4.12.3) and climbazole (4.12.4) to the nearest 0,1 mg into a 100-ml-volumetric flask and fill up to the mark with the methanol/acetic acid mixture (4.9).

4.14 Calibration solutions

Fresh calibration solutions shall be prepared each working day.

The following scheme in Table 1 for the preparation of the calibration solutions has proved useful in practice.

Together, the given amounts of the stock solutions in ml, are pipetted into 25-ml-volumetric flask and filled up to the mark with the methanol/acetic acid mixture (4.9).

For calculation the concentration of the calibration solutions have to be corrected with the known purity of the reference substances.

Table 1 — Calibration solution

Calibration solution	Zinc pyrithione (4.13.2) ml	Piroctone olamine + Climbazole (4.13.3) ml	Concentration µg/ml
1	0,5	0,5	5
2	1	1	10
3	2	2	20
4	3	3	30
5	5	5	50

5 Apparatus and equipment

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

5.2 Membrane filter, for solvent filtration, 0,45 µm pore size.

5.3 Ultrasonic bath, with temperature controlled heater.

5.4 Disposable syringes

5.5 Membrane filter, for sample filtration, e.g. PTFE, 0,45 µm pore size.

5.6 High-performance liquid chromatograph, consisting of:

— sampling device;

— pump system with gradient function;

— degasser (optionally; eluent may be degassed prior to use if the system requirements are fulfilled (q.v.));

— column oven;

— photodiode array detector (for quantitative determination without identification a multiple wavelength detector is sufficient);

— evaluation system.

5.7 Analytical reversed-phase separation column, e.g.:

Onyx Monolithic C18 100 mm × 3 mm (Phenomenex)[1)] or Chromolith RP18e, 100 mm × 3 mm (Merck)[1)].

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 standard method.

1) This is an example of a suitable product available commercially. This information is given for the convenience of users of this document 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.

7 Procedure

7.1 Sample preparation

Weigh approximately 250 mg of sample to the nearest 0,1 mg into a 50-ml-volumetric flask. Add 5 ml of dichloromethane (4.3) and 5 ml of methanol (4.2). Place the volumetric flask into a temperature controlled ultrasonic bath for 10 min and heat gently at 35 °C to 40 °C. When the sample is dissolved or homogeneously dispersed, let the sample cool down at room temperature. Fill the volumetric flask to the mark with methanol/acetic acid (4.9) and shake. Filter approximately 2 ml of methanol/acetic acid mixture through a membrane filter (5.5) into a HPLC vial, discarding the first 0,5 ml.

Fresh sample solutions shall be prepared each working day.

7.2 High-performance liquid chromatography (HPLC)

When starting measurements, examine the baseline stability and response linearity of the detector. The detector shall be able to detect the lowest calibration solution of climbazole (5 µg/ml) at a signal to noise ratio of 6:1. The same operating conditions of the HPLC System shall be maintained throughout the measurements of all sample and calibration solutions.

When using the apparatus (5.6) and the column of (5.7), the following conditions have shown useful:

- flow: 2,0 ml/min
- injection volume: 5 µl
- injector temperature: room temperature
- column temperature: 30 °C
- detection: Zinc pyrithione: detection wavelength: λ = 340 nm

  Climbazole: detection wavelength: λ = 277 nm

  Piroctone olamine: detection wavelength: λ = 305 nm
- running time: 6 min

7.3 Gradient elution

- **Eluent A:** 0,002 7 mol/l of oxalic acid + 0,001 mol/l of EDTA in water, pH 4,0 (4.11.1).
- **Eluent B:** Acetonitrile (4.4).

For the gradient elution eluents A and B are used in accordance with the volume ratios and time intervals given in Table 2, the following conditions have proved useful:

Table 2 — Gradient programme

Time min	Fraction eluent A %	Fraction eluent B %
0	85	15
2	50	50
3,5	50	50
3,6	20	80
4,6	20	80
4,7	85	15
6	85	15

It is recommended to do a blank run after each 10 sample-measurements to detect any carry-over which may have occurred. For this purpose make a solution according 7.1 without sample.

8 Evaluations

8.1 Identification

The anti-dandruff agents to be determined are identified by comparing the retention times of the sample with the calibration solutions and by comparing the spectra of the sample and the reference. A background correction shall be made if required.

8.2 Quantitative determination and calculation

Quantification is performed by means of linear regression based on the peak areas or peak heights of the external standards. The calibration curves shall be rectilinear and the correlation coefficient should be at least 0,996 or better.

The mass fraction of the anti-dandruff agent ω, in g/100 g, with respect to the sample, is calculated using the following formula:

$$\omega = \frac{\rho \cdot V \cdot VF}{m \cdot 10} \tag{1}$$

where

ω is the anti-dandruff agent content, in g/100 g;

ρ is the anti-dandruff agent content in the sample solution, in µg/ml, determined on the basis of the calibration curve;

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

V is the volume of the sample measurement solution (V = 50 ml);

m is the initial weight of the sample, in mg.

8.3 Expression of results

The content is given 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) 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

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 using a commercially available shampoo with known concentrations of the three substances.

This method is also suitable for determining the following active substances:

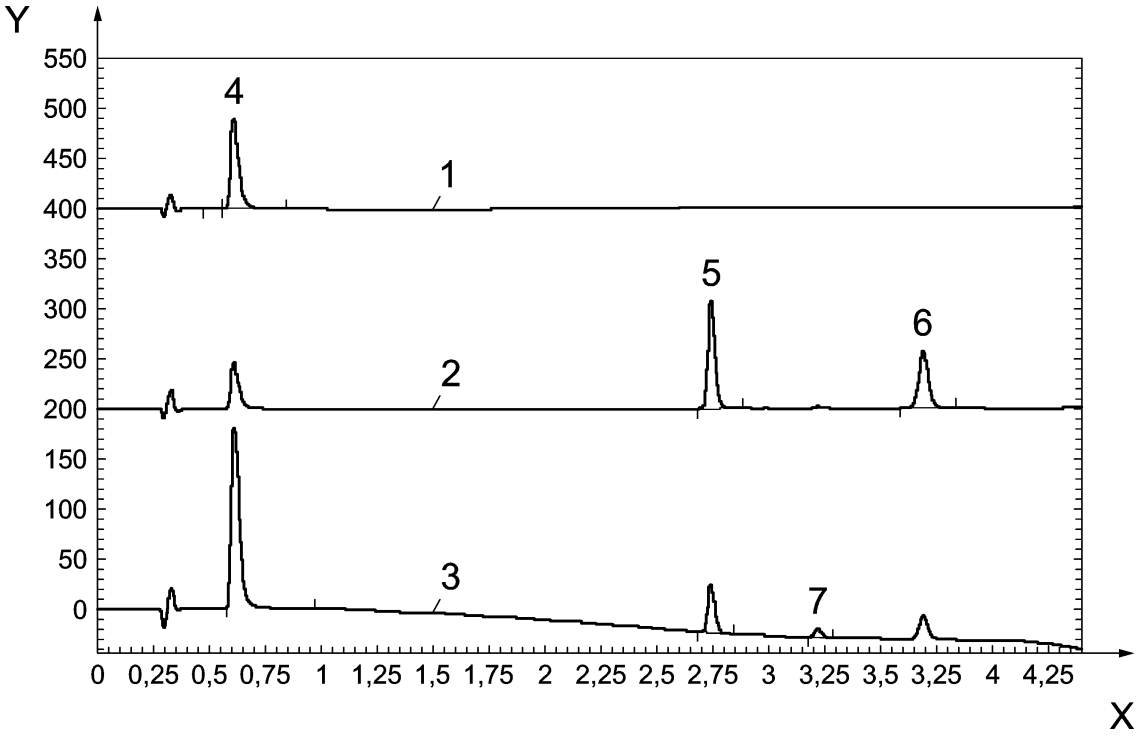
— ciclopirox olamine (6-cyclohexyl-1-hydroxy-4-methyl-2-pyridone, CAS number: 41621-49-2, detection wavelength: λ = 305 nm), and

— ketoconazole (1-(4-{4-[-2-(2,4-dichlorophenyl)-2-(imidazol-1-ylmethyl)-1,3-dioxolan-4-ylmethoxy]phenyl}piperazin-1-yl)ethanone, CAS number: 65277-42-1, detection wavelength: λ = 277 nm).

Table A.1 — Reliability of the method

Parameters	Content, in g/100 g		
	Climbazole	Piroctone olamine	Zinc pyrithione
Number of participating laboratories	10	10	10
Number of laboratories after elimination of the outliers	9	10	10
Number of outliers	1	0	0
Target value, g/100 g	0,50	0,50	0,96
Mean value $\bar{x}$, g/100 g	0,502	0,496	0,956
Recovery, %	100,4	99,2	99,6
Repeatability limit r, g/100 g	0,023	0,022	0,049
Repeatability standard deviation s_r, g/100 g	0,008	0,008	0,018
Relative repeatability standard deviation $s_{r,rel}$, %	1,7	1,6	1,8
Reproducibility limit R, g/100 g	0,080	0,062	0,123
Reproducibility standard deviation s_R, g/100 g	0,029	0,022	0,044
Relative reproducibility standard deviation $s_{R,rel}$, %	5,7	4,4	4,6
Horrat value (Horrat-R-Index)	1,3	1,0	1,1

Annex B
(informative)
Sample Chromatogram



Key

X	time in min	4	zinc pyrithione
Y	absorption in mAU	5	piroctone olamine
1	detection wavelength λ = 340 nm	6	ciclopirox olamine
2	detection wavelength λ = 305 nm	7	climbazole
3	detection wavelength λ = 277 nm		

Figure B.1 — Sample Chromatogram

Chromatogram of calibration solution 2 (concentration: 10 µg/ml; 50 ng of each substance) by using an Onyx Monolithic C18 100 mm × 3 mm.

Bibliography

[1] Gagliardi, L. et al., 1998. J. Liq. Chrom. & Rel. Technol, 21, 2365 – 2373.

[2] EC Method (01/Entr./Cos/28 v. 8/2001), Determination of Zinc Pyrithione, Piroctone Olamine and Ciclopirox Olamine in Cosmetics (04/99).

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