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**Cosmetics — Analytical methods —  
Nitrosamines: Detection and  
determination of *N*-nitrosodiethanolamine  
(NDELA) in cosmetics by HPLC, post-  
column photolysis and derivatization**

*Cosmétiques — Méthodes analytiques — Nitrosamines: Recherche et dosage de la N-nitrosodiéthanolamine (NDELA) dans les cosmétiques par CLHP, photolyse et dérivation post-colonne*  
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# Contents

Page

Foreword .....	iv
Introduction.....	v
1 Scope .....	1
2 Principle.....	1
3 Reagents.....	1
4 Apparatus .....	2
5 Sample preparation and storage .....	3
5.1 General .....	3
5.2 Standards preparation .....	4
5.2.1 Primary stock solution.....	4
5.2.2 Secondary stock solution.....	4
5.2.3 Working solutions .....	4
5.3 Sample preparation .....	4
5.3.1 General .....	4
5.3.2 SPE clean-up .....	5
5.3.3 Alternative sample preparation for samples non-dispersible in water (DCM clean-up) .....	5
6 Procedure .....	5
6.1 General .....	5
6.2 Chromatographic conditions .....	5
6.3 Set-up of the reaction system .....	6
7 Calculation of results .....	6
7.1 Calibration curve .....	6
7.2 Experimental conditions for the validity of the measurement.....	6
7.3 Calculation of concentrations.....	6
8 Test report.....	7
Annex A (informative) Examples of calibration curves and typical chromatograms .....	8
Annex B (normative) Photolysis and reaction of nitrite with Griess reagent to form the azo dye.....	11
Annex C (normative) Configuration of the post-column reactor system.....	12
Bibliography.....	13

## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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

ISO 10130 was prepared by Technical Committee ISO/TC 217, *Cosmetics*.

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## Introduction

Human exposure to *N*-nitrosamines can occur through diverse sources such as the environment, food or personal care products. As a result of their perceived carcinogenic potential on several animal species, minimization of exposure to *N*-nitrosamines is recognized as important to the preservation of human health. Among *N*-nitrosamines, *N*-nitrosodiethanolamine (NDELA) has been recognized as a potential contaminant of cosmetics.

In this context, several analytical methods have been developed to detect and determine the presence of NDELA in cosmetics. Examples of these methods are gas chromatography/thermal energy analysis, and high performance liquid chromatography coupled either with a mass spectrometry determination or with photolysis and colorimetric quantification. The latter method uses specific technology to ensure specificity towards NDELA, to minimize the risk of artefactual formation of the analyte of interest and to allow precise quantification.

This analytical method uses High Performance Liquid Chromatography (HPLC) coupled with post-column photolysis and derivatization, in order to separate and detect trace levels of NDELA from a cosmetic ingredient or product matrix with specificity for NDELA.

This International Standard refers to a collaborative study (Reference [2]) involving seven laboratories and published in 2006. Validation criteria are given in Reference [2].

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# Cosmetics — Analytical methods — Nitrosamines: Detection and determination of *N*-nitrosodiethanolamine (NDELA) in cosmetics by HPLC, post-column photolysis and derivatization

## 1 Scope

This International Standard describes a method for the detection and quantification of *N*-nitrosodiethanolamine (NDELA) in cosmetics and raw materials used in cosmetics by high performance liquid chromatography (HPLC) coupled with post-column photolysis and derivatization.

This method is not applicable to the detection and/or quantification of nitrosamines other than NDELA, nor to the detection and/or quantification of NDELA in products other than cosmetics or raw materials used in cosmetics.

If a product has the possibility of either NDELA contamination from the ingredients or NDELA formation by the composition of ingredients, the method will be applied for the testing of cosmetic products and is an alternative to ISO 15819.

This method is not applicable to matrices containing oxidation dyes.

## 2 Principle

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Extraction of the nitrosamine NDELA from cosmetic samples is carried out with water. Clean-up is performed either using solid phase extraction (SPE clean-up, see 5.3.2) with a C18 cartridge or dichloromethane (DCM clean-up, see 5.3.3) when the samples are not dispersible in water. The extracts are analysed by HPLC, post-column photolysis and derivatization. NDELA is separated from the cosmetic matrix using reversed-phase liquid chromatography. The *N*-nitroso bond is cleaved by UV photolysis with the formation of nitrite ion. According to the Griess reaction, the nitrite functional group is diazotized with sulfanilamide in an acid medium and is then coupled with *N*-(1-naphthyl)ethylenediamine dihydrochloride (NED) to form a purple-coloured azo dye that is quantitatively determined spectrophotometrically at a maximum wavelength,  $\lambda_{\max}$ , of 540 nm (see Annex B).

The presence of NDELA can be confirmed by repeating the analysis without photolysis (no nitrite ion is then produced because the *N*-nitroso bond is not cleaved). The absence of a chromatographic peak at the retention time of NDELA in the chromatogram confirms that the peak observed in the first analysis corresponds to NDELA.

## 3 Reagents

- 3.1 **Methanol**, HPLC grade.
- 3.2 **Water**, HPLC grade.
- 3.3 **Dichloromethane**, HPLC grade.
- 3.4 ***N*-Nitrosodiethanolamine**, with known purity greater than 95 %. CAS No. [116-57-7].

**3.5 Orthophosphoric acid**, 85 %, analytical grade.

**3.6 Ammonium acetate**, analytical grade.

**3.7 Ammonium acetate solution**, 1 mol/l.

Dissolve 77,08 g of ammonium acetate (3.6) in up to 1,0 l of water (3.2).

**3.8 Ammonium acetate solution**, 0,02 mol/l.

Take 20 ml of 1 mol/l ammonium acetate solution (3.7) and dilute to 1 l with water (3.2).

**3.9 N-(1-Naphthyl) ethylenediamine dihydrochloride**, with known purity greater than 98 %. CAS No. [1465-25-4].

This reagent should be sealed and stored in the absence of light.

**3.10 Sulfanilamide**, with known purity greater than 99 %. CAS No. [63-74-1].

**3.11 Griess reagent.**

Dissolve 0,25 g N-(1-naphthyl) ethylenediamine dihydrochloride (3.9) in water (3.2) and dilute to 250 ml in a volumetric flask. Dissolve 4,0 g of sulfanilamide (3.10) in 250 ml of a 5 % (mass/volume) aqueous solution of 85 % orthophosphoric acid (3.5). Mix the reagents together in an amber glass bottle and keep the mixture away from the light.

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The mixture can be used for five days and shall, in any case, remain colourless when it is stored between 2 °C and 8 °C.

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## 4 Apparatus

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Use standard laboratory glassware and equipment, and the following.

**4.1 Mechanical shaker** or **Vortex® mixer** <sup>1)</sup>.

**4.2 High performance glass vial**, 20 ml with Poly-Cone® lined urea screw caps.

**4.3 Microtube 1,5 ml**, for centrifugation in polypropylene.

**4.4 Low dead-volume mixing tee.**

**4.5 Sample processing station**, in SPE application (such as Vacmaster® or Visiprep®<sup>1)</sup>) sample processing station.

**4.6 Centrifuge**, capable of reaching not less than 20 000g with a temperature control.

**4.7 Solid-phase extraction columns**, e.g. Sep Pak®<sup>1)</sup> C18 cartridges Waters or Bakerbond®<sup>1)</sup> C18-6 ml, 500 mg reversed phase octadecylsilane bonded to silica gel 40 µm average particle diameter (APD), 60 Å.

**4.8 High performance liquid chromatograph**, equipped with a pulse damper, eluent reservoir, a pump, an injection system, data processing, e.g. an integrator, coupled with a UV/visible detector equipped with a tungsten lamp, of sensitivity 0,001 AUFS or 0,0005 AUFS (AUFS: absorbance units full scan).

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<sup>1)</sup> Poly-Cone®, Vortex® mixer, Vacmaster®, Visiprep®, Waters or Bakerbond®, Sep Pak®, Spherisorb® and Beam Boost® 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 ISO of these products.



**4.9 Griess reagent delivery pump:** low-pressure pump without pulsation, or a second liquid-chromatography (LC) pump with the same characteristics as the first may be used.

**4.10 Photolysis unit:** photochemical reactor comprising a “high-pressure” knitted PTFE reactor coil, of 5 m or 6 m × 0,3 mm internal diameter, placed around a low-pressure UV lamp emitting at 254 nm (e.g. Beam Boost®<sup>1)</sup> Photoreactors).

**4.11 Post-column module or a column oven,** equipped with a 1 ml knitted PTFE reactor coil and a low dead-volume mixing tee. The coil is convoluted in a defined manner in order to prevent band broadening (Reference [3]).

**4.12 Analytical reversed-phase HPLC separating column, C18,** e.g. Spherisorb®<sup>1)</sup> octadecylsilane (ODS) II protected with a guard column.

#### Separating column

- length: 150 mm
- internal diameter: 4,6 mm
- size of spherical particles: 5 µm

#### Guard column

- length: 10 mm
- internal diameter: 4,6 mm
- size of spherical particles: 5 µm

**4.13 Configuration of the post-column reactor system** (see Annex C).

After the injection valve, the connecting tubes shall be as short as possible.

The column is connected directly to an injection system equipped with a 50 µl or 100 µl sample loop. A photochemical reactor (1) fitted with a knitted-open tubular reactor coil and a 254 nm ultraviolet lamp is connected to the column exit. The photochemical reactor exit is connected to one branch of a mixing tee (2). The second branch of the tee is connected to the pump with the derivatization reagent (3). The third branch is connected to the tubular reactor which consists of a knitted PTFE tube under a post-column module (4). The exit of the tubular reactor is connected to the UV/visible detector (5). The eluted peaks are monitored using a microcomputer equipped with a commercial integration software package.

## 5 Sample preparation and storage

### 5.1 General

**WARNING —** Most *N*-nitrosamines are potent carcinogens and every possible precaution shall be taken to avoid human exposure.

All operations involving handling of *N*-nitrosamines or their solutions shall take place in an adequately ventilated fume hood or glove box.

Rubber surgical gloves, which are frequently employed, do not provide complete protection. They shall be removed and disposed of immediately after use and not worn for long periods.

Thought should be given to safe disposal of any solution of material containing *N*-nitrosamines.

Ultraviolet light (UV) degrades *N*-nitrosamines, so *N*-nitrosodiethanolamine and all solutions (standards/extracts) shall be stored in the absence of light between 2 °C and 8 °C.

Injection for analysis shall be made within 24 h after preparation of the extract sample.

**5.2 Standards preparation**

**5.2.1 Primary stock solution**

Prepare a primary stock standard solution (S<sub>0</sub>) of NDELA containing 1 mg/ml in water. This solution can be stored for a maximum of six months in the absence of light between 2 °C and 8 °C.

**5.2.2 Secondary stock solution**

From the primary stock solution (S<sub>0</sub>), make a 1/100 dilution in water in order to obtain a 10 µg/ml secondary stock solution (S<sub>1</sub>). A second 1/100 dilution of S<sub>1</sub> is performed in order to obtain a 100 ng/ml secondary stock solution (S<sub>2</sub>). All of the solutions are stored for a maximum of one week in the absence of light between 2 °C and 8 °C.

**5.2.3 Working solutions**

Sequentially dilute secondary stock solution (S<sub>2</sub>) with water, in order to obtain standards titrating at 1 ng/ml, 2 ng/ml, 5 ng/ml, 10 ng/ml and 20 ng/ml. (See Figure A.1.) These solutions are prepared every day and are kept in the absence of light (see Table 1).

The linearity of the standard calibration curve is checked over a 1 ng/ml to 100 ng/ml range (see Figure A.2).

Typical chromatograms are shown in Figures A.3 and A.4.

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**Table 1 — Preparation of working solutions**

Working solutions	Secondary stock solution (S <sub>2</sub> ) volume	Final volume	Final concentration	Stability	Conditions
Working solution W <sub>1</sub>	100 µl	10 ml	1 ng/ml	1 day	Stored in the absence of light
Working solution W <sub>2</sub>	200 µl	10 ml	2 ng/ml	1 day	Stored in the absence of light
Working solution W <sub>3</sub>	500 µl	10 ml	5 ng/ml	1 day	Stored in the absence of light
Working solution W <sub>4</sub>	1 ml	10 ml	10 ng/ml	1 day	Stored in the absence of light
Working solution W <sub>5</sub>	2 ml	10 ml	20 ng/ml	1 day	Stored in the absence of light

**5.3 Sample preparation**

**5.3.1 General**

Where a cosmetic product is dispersible (soluble) in water, the solid phase extraction (SPE) clean-up shall apply (see 5.3.2).

Where a cosmetic product is not dispersible in water, the dichloromethane (DCM) clean-up shall apply (see 5.3.3).

To summarize, for a cosmetic product, the criteria of dispersibility of the product shall be estimated in order to use a suitable clean-up for the product. SPE clean-up and DCM clean-up should not be carried out for the same product; the suitable clean-up shall be chosen according to the dispersibility. A product which is not dispersible in water shall be dealt with under the conditions of DCM clean-up.

### 5.3.2 SPE clean-up

Weigh about 2,0 g of the sample in a glass vial (4.2) and note the exact mass, disperse and adjust to 20 ml with water. Shake for 15 min using a mechanical shaker or for 1 min using a Vortex® mixer. Centrifuge for 10 min (SPE preparation).

Condition the solid phase extraction C18 cartridge with 3 ml of methanol, followed by 3 ml of water, with a flow rate of approximately 3,0 ml/min. Do not allow the cartridge to dry out.

Load 5 ml of the SPE preparation to the above solid phase extraction C18 cartridge and discard the first 3 ml of solution. Collect the following 2 ml (with a flow rate of approximately 3,0 ml/min) in a vial for chromatographic analysis.

If necessary, filter the collected solution through an appropriate filter.

### 5.3.3 Alternative sample preparation for samples non-dispersible in water (DCM clean-up)

Weigh about 0,4 g of the sample in a glass vial (4.2) and note the exact mass. Add 4,0 ml of dichloromethane and shake for 1 min. Add exactly 4 ml of water and shake for 5 min.

Put an aliquot part of the upper aqueous phase in a microtube (4.3).

Centrifuge at 20 000g for 10 min.

Inject the upper aqueous layer into the chromatographic system.

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## 6 Procedure

### 6.1 General

With both types of sample preparation, the final extract is analysed for NDELA by HPLC with UV photolysis and the Griess reaction.

### 6.2 Chromatographic conditions

LC pump	Pump equipped with a pulse damper
Column type	See 4.12
Guard-column type	See 4.12
<b>Injection volume</b>	50 µl or 100 µl
Mobile phase	0,02 mol/l ammonium acetate solution (3.8)
Mobile-phase flow rate	0,5 ml/min
Griess-reagent flow rate	0,5 ml/min
Temperature of post-column reactor	50 °C