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**Cosmetics — Analytical  
methods — Nitrosamines:  
Detection and determination of  
N-nitrosodiethanolamine (NDELA) in  
cosmetics by HPLC-MS-MS**

*Cosmétiques — Méthodes analytiques — Nitrosamines: Recherche  
et dosage de la N-nitrosodiéthanolamine (NDELA) dans les produits  
cosmétiques par CLHP-SM-SM*

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Case postale 56 • CH-1211 Geneva 20  
Tel. + 41 22 749 01 11  
Fax + 41 22 749 09 47  
E-mail [copyright@iso.org](mailto:copyright@iso.org)  
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## 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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see [www.iso.org/directives](http://www.iso.org/directives)).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: [Foreword - Supplementary information](#)

The committee responsible for this document is ISO/TC 217, *Cosmetics*.

This second edition cancels and replaces the first edition (ISO 15819:2008), which has been technically revised.

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## Introduction

Human exposure to N-nitrosamines can occur through diverse sources such as 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 its presence in cosmetics, such as gas chromatography/thermal energy analysis, high performance liquid chromatography (HPLC) coupled either with photolysis and colourimetric quantification or with mass spectrometry (MS) determination. This latter method uses advanced technology to ensure the maximum 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 coupled with mass spectrometry to separate and detect trace levels of NDELA from a cosmetic ingredient or product matrix with maximum specificity for NDELA.

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# Cosmetics — Analytical methods — Nitrosamines: Detection and determination of N-nitrosodiethanolamine (NDELA) in cosmetics by HPLC-MS-MS

## 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.

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 a possibility of either NDELA contamination from ingredients or NDELA formation by the composition of ingredients, the method is intended to be applied for quantitative determination of NDELA. Accordingly, the method does not apply to routine testing of cosmetic products. Because of the large variety of cosmetic products within this field of application, this method might need to be adapted for certain matrices (refer to ISO 12787).

Therefore, International Standards dedicated to alternative methods for testing nitrosamines in cosmetic products are being developed separately. Other methods can be employed provided that they are verified as to their detection of NDELA and validated in terms of recovery and quantification of the analyte.

## 2 Normative references

ISO 15819:2014

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The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*

ISO 12787:2011, *Cosmetics — Analytical methods — Validation criteria for analytical results using chromatographic techniques*

## 3 Principle

Extraction of NDELA in cosmetics samples is carried out with water in the presence of deuterated d8-NDELA used as internal standard (IS). Clean-up is performed either using solid phase extraction (SPE clean-up, see 6.3.1) with a C18 cartridge or liquid-liquid extraction using dichloromethane (DCM clean-up, see 6.3.2) when the samples are not dispersible in water. The extracts are analysed by HPLC-MS-MS (high performance liquid chromatography coupled with tandem mass spectrometric detection).

Identification of NDELA is carried out by using the molecular ion and two diagnostic ions. NDELA quantification is done by comparing the ratio of the major fragment ions of NDELA and d8-NDELA with the calibration curve.

In accordance with the ISO 12787, the absence of NDELA in the sample could be confirmed with a second analysis. A spiked preparation at a target value could be performed to evaluate the limit of detection of NDELA in the sample.

If matrix effect is observed with significant impact on the performance of the method (sensitivity, accuracy, etc.) for specific cosmetic product, standard addition calibration procedures could be utilized (refer to ISO 12787).

## 4 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and only distilled water or water of grade 1 in accordance with ISO 3696:1987. Solvents shall be of a suitable quality for HPLC-MS.

- 4.1 **Methanol (MeOH)**, HPLC-MS grade.
- 4.2 **Ethanol (EtOH)**, HPLC-MS grade.
- 4.3 **Dichloromethane (DCM)**, HPLC-MS grade.
- 4.4 **N-nitrosodiethanolamine**, with known purity greater than 95 %.
- 4.5 **d8-N-nitrosodiethanolamine**, with known purity greater than 95 %.
- 4.6 **Ammonium acetate (NH<sub>4</sub>Ac)**, analytical grade (suitable for HPLC-MS).
- 4.7 **1 mol/l ammonium acetate solution**, for the preparation of 1,0 l, dissolve 77,08 g of NH<sub>4</sub>Ac in 1,0 l of water.
- 4.8 **Eluent A: 2 mmol NH<sub>4</sub>Ac in water**, for the preparation of 1,0 l, by taking 2 ml of 1 mol/l NH<sub>4</sub>Ac (4.7) and making up to 1 l with water.
- 4.9 **Eluent B: 2 mmol NH<sub>4</sub>Ac in 90 / 10 MeOH / water v/v**, formed, for the preparation of 1,0 l, by taking 2 ml of 1 mol/l NH<sub>4</sub>Ac (4.7) and making up to 1 l with mixture of 90/10 MeOH / water v/v.

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## 5 Apparatus

Use standard laboratory glassware and equipment, with the addition of:

- 5.1 **Vortex mixer.**
- 5.2 **Sample processing station**, in SPE application [such as Vacmaster<sup>®1)</sup>] sample processing station.
- 5.3 **High speed centrifuge (ideally 20 000 G).**

NOTE If centrifuge speed below 20 000 G is used, pay attention to possible clogging problem during the SPE clean-up process. If necessary, additional filtration step with 0,2 µm pore size membrane filter could be added.

- 5.4 **Solid phase extraction columns**, e.g. Bakerbond<sup>®1)</sup> C18 — 6 ml, 500 mg reversed phase octadecylsilane bonded to silica gel, 40 APD (Average Particle Diameter), 60 Å.

### 5.5 HPLC-MS-MS equipment.

5.5.1 **High performance liquid chromatography apparatus**, consisting of an eluent reservoir, a pump, an injection system, a data processor, e.g. an integrator with plotter, coupled with tandem mass spectrometry using electrospray ionization.

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1) Vacmaster<sup>®</sup> and Bakerbond<sup>®</sup> are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.



**5.5.2 Analytical reversed phase HPLC separating column**, C18, e.g. Spherisorb<sup>®2)</sup> ODS II protected with a guard column, the dimensions of which are:

- separating column
  - length: 100 mm;
  - internal diameter: 2,1 mm;
  - size of spherical particles: 5 µm;
- guard column
  - length: 10 mm;
  - internal diameter: 2,1 – 3,0 mm;
  - size of spherical particles: 5 µm.

The use of a guard column is optional. If used, its ID is preferably same as that of separating column. The condition should be adjusted based on the brand that is used for analytical reversed phase HPLC separating column.

## 6 Sample preparation and conservation

### 6.1 General

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**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 should take place in an adequately ventilated fume hood or glove box.

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

Use safe disposal to discard any solution of material containing N-nitrosamines (such as, for example, tins or buckets for hazardous chemical waste).

N-Nitrosodiethanolamine shall be stored in the absence of light between 2 °C and 8 °C.

UV degrades N-nitrosamines, so all solutions (standards/extracts) shall be stored in such a way that deterioration and change in composition are prevented.

HPLC-MS-MS analysis should typically be carried out within 30 min of preparation of the sample extracts. If analysis is delayed, then stability should be verified.

### 6.2 Standard solutions preparation

**6.2.1** Accurately prepare a stock solution (A) of NDELA containing approximately 1,0 mg/ml in ethanol and store in the absence of light at less than –18 °C. Record the exact concentration.

**6.2.2** Accurately prepare a stock solution (d8A) of d8-NDELA containing approximately 1,0 mg/ml in ethanol and store in the absence of light at less than –18 °C. Record the exact concentration.

**6.2.3** Prepare working solutions (B, C, D, E, and F) by successive dilutions of the stock solution (A). All solutions shall be stored in the absence of light between 2 °C and 8 °C.

2) Spherisorb<sup>®</sup> 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 ISO of this product.