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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 des N-nitrosodiéthanolamines (NDELA) dans les produits cosmétiques par CLHP-SM-SM

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Foreword

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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 15819 was prepared by Technical Committee ISO/TC 217, *Cosmetics*.

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 recognised as important to the preservation of human health. Among N-nitrosamines, N-nitrosodiethanolamine (NDELA) has been recognised 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 colorimetric 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 artifactual 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.

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 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 shall be applied for quantitative determination of NDELA. Accordingly the method would not be applied in 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.

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

The following referenced documents are indispensable for the application of this document. 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*

3 Principle

Extraction of the nitrosamine NDELA in cosmetic samples is carried out with water in the presence of deuterated d8-NDELA used as internal standard. 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).

NDELA quantification is done by comparing the ratio of the major fragment ions of NDELA and d8-NDELA with the calibration curve.

Confirmation of the presence of NDELA is carried out by using the molecular ion and two diagnostic ions.

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. Solvent shall be of quality for HPLC analysis.

4.1 Methanol (MeOH), HPLC grade.

4.2 Ethanol (EtOH), HPLC grade.

4.3 Dichloromethane, HPLC 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_4Ac), analytical grade.

4.7 1 mol/l ammonium acetate solution, formed by dissolving 77,08 g of NH_4Ac in 1,0 l water.

4.8 Eluent A: 2 mmol NH_4Ac in water, formed by taking 2 ml of 1 mol/l NH_4Ac (4.7) and making up to 1 l with water.

4.9 Eluent B: 2 mmol NH_4Ac in 90 % MeOH/water, formed by taking 2 ml of 1 mol/l NH_4Ac (4.7) and adding 900 ml MeOH and 98 ml water.

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®[1]) sample processing station, IST).

5.3 Centrifuge, capable of reaching not less than 20 000 G.

5.4 Solid phase extraction columns, e.g. Bakerbond®[1]) C18 – 6 ml, 500 mg reversed phase octadecylsilane bonded to silicagel, 40 APD, 60 Å.

5.5 HPLC-MS-MS equipment consisting of:

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.

1) Vacmaster®, Bakerbond® and Spherisorb® 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.

5.5.2 Analytical reversed phase HPLC separating column, C18, e.g. Spherisorb® ODS II[1)] protected with a guard column, the dimensions of which are:

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

6 Sample preparation and conservation

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

NOTE 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.

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

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.

Injection for analysis shall be made within 30 min of preparation of the extract sample.

6.2 Standards preparation

6.2.1 Prepare stock solutions (A) of NDELA containing 1,016 mg/ml in ethanol and store them in the absence of light at −20 °C.

6.2.2 Prepare stock solutions (d8A) of d8-NDELA containing 1,016 mg/ml in ethanol and store them in the absence of light at −20 °C.

6.2.3 Prepare working solutions (B, C, D, E and F) by sequential 10-fold dilutions of the 1,016 mg/ml solution. All solutions shall be stored in the absence of light between 2 °C and 8 °C.

Working solutions	Stock or working solution volume	Water volume	Final concentration	Stability
Working solution B	100 µl of A	900 µl	0,101 645 mg/ml	1 day
Working solution C	100 µl of B	900 µl	10,164 5 µg/ml	1 day
Working solution D	100 µl of C	900 µl	1,016 45 µg/ml	1 day
Working solution E	100 µl of D	900 µl	101,645 ng/ml	1 day
Working solution F	100 µl of E	900 µl	10,164 5 ng/ml	1 day

6.2.4 Prepare d8 working solutions (d8B, d8C, d8D and d8E) by sequential 10-fold dilutions of the 1,016 mg/ml solution. All solutions shall be stored in the absence of light between 2 °C and 8 °C.

d8 working solutions	Stock or working solution volume	Water volume	Final concentration	Stability
Working solution d8B	1 ml of d8A	9 ml	0,101 645 mg/ml	1 day
Working solution d8C	1 ml of d8B	9 ml	10,164 5 µg/ml	1 day
Working solution d8D	1 ml of d8C	9 ml	1,016 45 µg/ml	1 day
Working solution d8E	1 ml of d8D	9 ml	101,645 ng/ml	1 day

6.2.5 Prepare standard solutions by dilutions of the working solutions. A standard curve from 1,0 ng/ml to 80 ng/ml is made. The internal standard d8-NDELA was at 20 ng/ml in each solution. All solutions shall be stored in the absence of light between 2 °C and 8 °C.

Standard solutions	Working solution volume	Working solution d8E	H_2O volume	Final concentration	Stability
Standard solution 1	800 µl of E	200 µl	–	81,32 ng/ml	1 day
Standard solution 2	400 µl of E	200 µl	400 µl	40,66 ng/ml	1 day
Standard solution 3	200 µl of E	200 µl	600 µl	20,33 ng/ml	1 day
Standard solution 4	100 µl of E	200 µl	700 µl	10,16 ng/ml	1 day
Standard solution 5	100 µl of F	200 µl	700 µl	1,02 ng/ml	1 day

6.3 Sample preparation

6.3.1 SPE clean-up

Weigh about 1,0 g of the sample (note the exact mass), add 400 µl of d8D working solution and adjust to 20,0 ml with water. Shake for 15 min. Centrifuge for 10 min if necessary.

Condition the solid phase extraction C18 column 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 column to dry out.

Load 5 ml of the SE preparation to the above solid phase extraction C18 column and discard the first 3 ml of solution. Collect the following 2 ml (with a flow-rate of approximately 3,0 ml/min) into a vial.

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

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

Weigh about 0,2 g of the sample (note the exact mass) into a centrifuge tube, add 800 µl of working solution d8E and shake for 1 min. Add 4 ml of dichloromethane and shake for 1 min. Add 3,2 ml of water and shake for 5 min.

Centrifuge at 20 000 G for 5 min.

A portion of the upper aqueous layer is used for chromatographic analysis.

7 Procedure

7.1 General

With both types of sample preparation, the final extract is analysed for NDELA by HPLC-MS-MS.

7.2 Chromatographic conditions

Mobile phase: Eluent A – 2 mmol NH_4Ac in water (4.8)

Eluent B – 2 mmol NH_4Ac in 90 % MeOH/water (4.9)

Gradient:

Interval min	% A	% B
0 – 7	70	30
7 – 10	70→0	30→100
10 – 17	0	100
17 – 20	0→70	100→30
20 – 30	70	30

Flow: 0,2 ml/min

Injection volume: 20 µl

Temperature column oven: 30 °C

Run injection: Every run consists of five calibration points, five sample extracts and one control sample (a standard solution). At least after every four runs (20 sample extracts) a new calibration curve is made.

The acquisition window is from 7 min to 11 min.

7.3 LC-MS-MS condition

Electrospray, positive mode.

EXAMPLE Suitable parameters for quadrupole mass-spectrometer (such as QUATTRO ULTIMA®)

Source settings

Capillary voltage 3,50 kV