SLOVENSKI STANDARD
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Kozmetika - Analizne metode - Nitrozamini: detekcija in določevanje N-nitrozodietanolamina (NDELA) v kozmetičnih izdelkih s tekočinsko kromatografijo visoke ločljivosti z masno spektrometrijsko detekcijo (HPLC-MS-MS)

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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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

[Revision of first edition (ISO 15819:2008)]

ICS 71.100.70

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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.

Revision of ISO 15819 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 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.*

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. 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 the presence 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 (LOD) 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. See ISO 12787 for reference.

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_4Ac), 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_4Ac in 1,0 l water.

4.8 Eluent A: 2 mmol NH_4Ac in water, formed, for the preparation of 1,0 l, 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 / 10 MeOH / water v/v,formed, for the preparation of 1,0 l, by taking 2 ml of 1 mol/l NH_4Ac (4.7) and making up to 1 l with mixture 90/10 MeOH / water v/v, formed by mixing 900 ml MeOH and 100 ml water.

5 Apparatus

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

5.1 Vortex mixer.

5.2 Sample processing s tation, in SPE application (such as Vacmaster®[1]) sample processing station, IST).

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.

[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.

5.4 Solid phase extr action columns, e.g. Bakerbond®[2]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.

5.5.2 Analytical reversed phase HPLC separating column, C18, e.g. Spherisorb® [2]ODS II protected with a guard column, the dimensions of which are:

separating column

- length: 100mm
- internal diameter: 4,6 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

NOTE : Condition of “the guard column” 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

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.

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.

[2] 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.

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 minutes of preparation of the sample extracts. If analysis is delayed, then stability should be verified

6.2 Standards 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 Prepareworking 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.

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

NOTE: Users can determine actual volume used for the preparation as far as final concentration is secured.

6.2.4 Prepare d8 working solutions(d8B and d8C) by sequential dilutions of the stock solution (d8A). 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	20 µl of d8A	20 ml	1,0 µg/ml	1 day
Working solution d8C	200 µl of d8B	1800 µl	100,0 ng/ml	1 day

NOTE: Users can determine actual volume used for the preparation as far as final concentration is secured.

6.2.5 Preparestandard solutions by dilutions of the working solutions. A standard curve from 1,0 ng/ml to 80 ng/ml is made, using at least five of the seven standard solutions given in the table below. A standard solution corresponding to the required limit of quantitation (or lower) should be included. 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.