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Method of Test for Nitrosamines in Cosmetics

1. Scope

This method is applicable for the determination of 8 nitrosamines (*N*-Nitrosodiethanolamine, etc. listed in the attached table) in cosmetics.

2. Method

After extraction, nitrosamines are determined by liquid chromatography/tandem mass spectrometry (LC-MS/MS).

2.1. Equipment

2.1.1. Liquid chromatograph/tandem mass spectrometer.

2.1.1.1. Ion source: atmospheric pressure chemical ionization, $APCI^+$.

2.1.1.2. Column: Nucleodur Sphinx RP, 3 μm, 3 mm i.d. × 20 cm, or an equivalent product.

2.1.2. Ultrasonicator.

2.1.3. Centrifuge: centrifugal force ≧ 3000 ×g.

2.2. Chemicals

Methanol, LC-MS grade;

Formic acid, LC-MS grade;

Deionized water, resistivity ≧ 18 MΩ·cm (at 25°C);

N-Nitrosodiethanolamine, *N*-nitrosodiethylamine, *N*-nitrosodimethylamine, *N*-nitrosodiisopropanolamine, *N*-nitrosomethylethylamine, *N*-nitrosomorpholine, *N*-nitrosopiperidine and *N*-nitrosopyrrolidine, reference standards;

N-Nitrosodiethanolamine-d_8, *N*-nitrosodiethylamine-d_4, *N*-nitrosodimethylamine-d_6, *N*-nitrosomethylethylamine-d_3, *N*-nitrosomorpholine-d_4, *N*-nitrosopiperidine-d_4 and *N*-nitrosopyrrolidine-d_4, isotope-labelled internal standards.

2.3. Apparatus

2.3.1. Volumetric flask: 5 mL, amber glass.

2.3.2. Centrifuge tube: 15 mL, amber, PP.

2.3.3. Membrane filter: 0.22 μm, PVDF.

2.4. Reagent

2.4.1. 5% methanol solution

Dilute 50 mL of methanol with deionized water to 1000 mL.

2.4.2. 5% methanol solution with 1% formic acid

Dilute 10 mL formic acid with 5% methanol solution to 1000 mL.

2.5. Mobile phase

2.5.1. Solvent A

Dilute 1 mL of formic acid with deionized water to 1000 mL, and filter with a membrane filter.

2.5.2. Solvent B

Dilute 1 mL of formic acid with methanol to 1000 mL, and mix well.

2.6. Internal standard solution preparation

Weigh accurately equivalent amount of 7 isotope-labelled internal standards into each volumetric flask, dissolve and dilute with methanol to 100 μg/mL as internal standard stock solutions. Store in a freezer at a temperature ≦－18 ℃ and protect from light. When to use, dilute appropriate volume of each internal standard stock solution, and mix with methanol to 5 μg/mL as the internal standard solution.

2.7. Standard solution preparation

Weigh accurately equivalent 10 mg of 8 reference standards into each 10-mL volumetric flask, dissolve and dilute with methanol to volume as standard stock solutions. Store in a freezer at a temperature ≦－18℃ and protect from light. When to use, dilute appropriate volume of each standard stock solution and the internal standard solution, and mix with 1% formic acid in 5% methanol solution to 0.5-50 ng/mL (containing 25 ng/mL the internal standard) as the standard solutions.

2.8. Sample solution preparation

Weigh accurately 0.25 g of the homogenized sample and transfer to a 5-mL volumetric flask. Add 25 μL of the internal standard solution and 4 mL of 1% formic acid in 5% methanol solution, vortex-mix well, and sonicate for 15 min. Add 1% formic acid in 5% methanol solution to volume, and transfer to centrifuge tube. Centrifuge at 3800 xg for 5 min. Filter the supernatant through a membrane filter, and take the filtrate as the sample solution.

2.9. Standard curve establishment

Accurately inject 10 μL of the standard solutions into LC-MS/MS separately, and operate according to the following conditions. Establish the standard curve of each nitrosamine by the ratios of the peak area of each nitrosamine to that of the internal standard vs. the concentrations of each nitrosamine.

LC-MS/MS operating conditions (note):

Column: Nucleodur Sphinx RP, 3 μm, 3 mm i.d. × 20 cm.

Column temperature: 40°C.

Sample oven temperature: 15°C.

Mobile phase: a gradient program of solvent A and solvent B is as follows.

Time (min)	Solvent A (%)	Solvent B (%)
0.0 → 3.0	95 → 95	5 → 5
3.0 → 8.0	95 → 0	5 → 100
8.0 → 10.0	0 → 0	100 → 100
10.0 → 11.0	0 → 95	100 → 5
11.0 → 16.0	95 → 95	5 → 5

Flow rate: 0.6 mL/min.

Injection volume: 10 μL.

Ionization mode: APCI+.

Capillary voltage: 3.0 kV.

Ion source temperature: 120°C.

Desolvation temperature: 450°C.

Cone gas flow rate: 150L/hr.

Desolvation flow rate: 1000L/hr.

Detection mode: multiple reaction monitoring (MRM). Detection ion pair, cone voltage (CV) and collision energy (CE) are shown in the attached table.

Note: All the parameters can be adjusted depending on the instruments used if the above conditions are not applicable.

2.10. Identification and quantification

Accurately inject 10 μL of the sample solution and the standard solutions into LC-MS/MS separately, and operate according to the conditions in section 2.9. Identify each nitrosamine based on the retention time and the relative ion intensities (note). Calculate the amount of each nitrosamine in the sample by the following formula:

$$\text{The amount of each nitrosamine in the sample (μg/kg)} = \frac{C \times V}{M}$$

where,

C: the concentration of each nitrosamine in the sample solution calculated by the standard curve (ng/mL)

V: the volume of solvent for sample extraction (5 mL)

M: the weight of sample (g)

Note: Relative ion intensities are calculated by peak areas of qualitative ions divided by peak areas of quantitative ions. Maximum permitted tolerances for relative ion intensities by LC-MS/MS are as follows:

Relative ion intensity (%)	Tolerance (%)
> 50	± 20
> 20-50	± 25
> 10-20	± 30
≦ 10	± 50

Remark

1. Limits of quantitation (LOQs) for 8 nitrosamines such as NDELA listed in the attached table are all 10 μg/kg.
2. Further validation should be performed when interference compounds appear in samples.

Reference

1. The European Directorate for the Quality of Medicines & HealthCare. 2020. Determination of polar *N*-nitrosamines in cosmetic products.
2. ISO. 2014. Cosmetics-Analytical methods-Nitrosamines: Detection and determination of N-nitrosodiethanolamine (NDELA) in cosmetics by HPLC-MS-MS. ISO 15819.
3. Ripollés, C., Pitarch, E., Sancho, J. V., López, F. J. and Hernández, F. 2011. Determination of eight nitrosamines in water at the ng L^{-1} levels by liquid chromatography coupled to atmospheric pressure chemical ionization tandem mass spectrometry. Anal. Chim. Acta 702: 62-71.

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Reference chromatogram

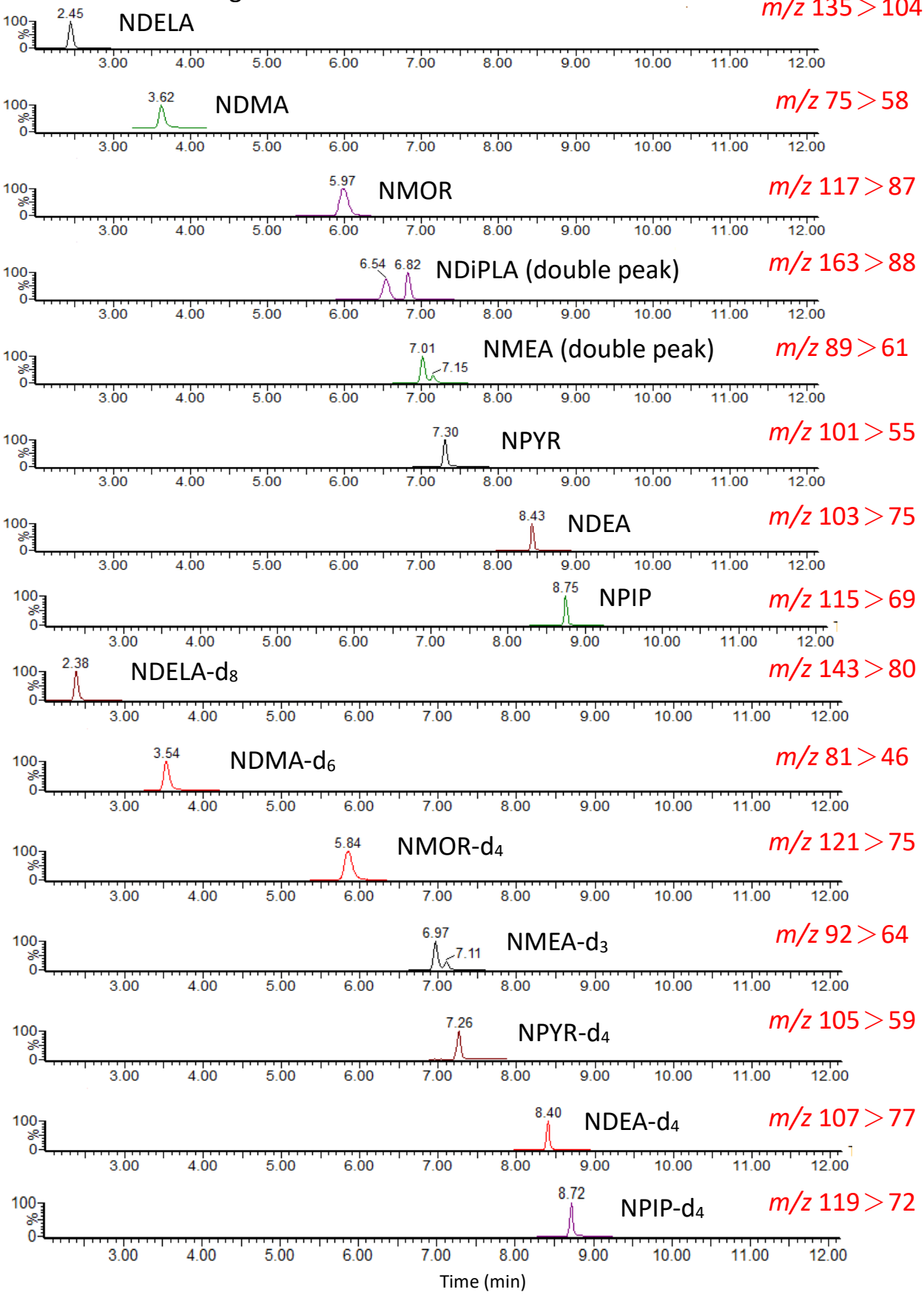

Figure. MRM chromatograms of 8 nitrosamine standards and 7 isotope-labeled internal standards analyzed by LC-MS/MS

Table、MRM parameters of 8 nitrosamines and 7 isotope-labeled internal standards

#	Analyte	Quantitative ion pair			Qualitative ion pair			Internal standard
		Precursor ion(*m/z*)>Product ion(*m/z*)	CV (V)	CE (eV)	Precursor ion(*m/z*)>Product ion(*m/z*)	CV (V)	CE (eV)	
1	*N*-Nitrosodiethanolamine (NDELA)	135 > 104	14	4	135 > 74	14	8	NDELA-d_8
2	*N*-Nitrosodiethylamine (NDEA)	103 > 75	20	10	103 > 47	20	14	NDEA-d_4
3	*N*-Nitrosodimethylamine (NDMA)	75 > 58	42	10	75 > 43	42	12	NDMA-d_6
4	*N*-Nitrosodiisopropanolamine (NDiPLA)	163 > 88	10	12	163 > 70	10	22	NMOR-d_4
5	*N*-Nitrosomethylethylamine (NMEA)	89 > 61	24	10	89 > 43	24	8	NMEA-d_3
6	*N*-Nitrosomorpholine (NMOR)	117 > 87	24	10	117 > 86	24	10	NMOR-d_4
7	*N*-Nitrosopiperidine (NPIP)	115 > 69	24	12	115 > 41	24	18	NPIP-d_4
8	*N*-Nitrosopyrrolidine (NPYR)	101 > 55	24	12	101 > 41	24	18	NPYR-d_4
I.S.	*N*-Nitrosodiethanolamine-d_8 (NDELA-d_8)	143 > 80	8	10	–	–	–	–
I.S.	*N*-Nitrosodiethylamine-d_4 (NDEA-d_4)	107 > 77	8	8	–	–	–	–
I.S.	*N*-Nitrosodimethylamine-d_6 (NDMA-d_6)	81 > 46	28	10	–	–	–	–
I.S.	*N*-Nitrosomethylethylamine-d_3 (NMEA-d_3)	92 > 64	20	10	–	–	–	–
I.S.	*N*-Nitrosomorpholine-d_4 (NMOR-d_4)	121 > 75	14	12	–	–	–	–
I.S.	*N*-Nitrosopiperidine-d_4 (NPIP-d_4)	119 > 72	14	15	–	–	–	–
I.S.	*N*-Nitrosopyrrolidine-d_4 (NPYR-d_4)	105 > 59	14	10	–	–	–	–