INTERNATIONAL STANDARD

ISO 29941

First edition
2010-12-15

Condoms — Determination of nitrosamines migrating from natural rubber latex condoms

Préservatifs — Dosage des nitrosamines migrant des préservatifs en latex de caoutchouc naturel

Reference number
ISO 29941:2010(E)

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Published in Switzerland

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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 29941 was prepared by Technical Committee ISO/TC 157, *Non-systemic contraceptives and STI barrier prophylactics*.

Condoms — Determination of nitrosamines migrating from natural rubber latex condoms

1 Scope

This International Standard specifies a test method to determine the release of *N*-nitrosamines from condoms made from natural rubber latex.

The method can also be used for other products such as probe covers, prophylactic dams, female condoms and condoms made from synthetic materials, although there was no experience of testing such products at the time of publication of this International Standard.

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, *Water for analytical laboratory use — Specification and test methods*

ISO 4074, *Natural rubber latex condoms — Requirements and test methods*

ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

3 Terms and definition

For the purposes of this document, the following terms and definitions apply.

3.1
condom
medical device used by consumers, which is intended to cover and be retained on the penis during sexual activity, for purposes of contraception and prevention of sexually transmitted infections

NOTE If a consumer could responsibly consider a device to be a condom (due to its shape, packaging, etc.), it is considered a condom for the purpose of this International Standard.

4 Principle

WARNING — Owing to their toxicity, *N*-nitrosamines can be detrimental to human health. The testing laboratories should take special care to observe the occupational health and safety standards.

N-nitrosamines are extracted into water. After having been concentrated, the water is tested for its *N*-nitrosamine content by means of gas chromatography (GC) using a chemiluminescence detector. The test is performed in an environment that is free of volatile *N*-nitrosamines.

The released *N*-nitrosamines are given in nanograms per grams (ng/g) of the sample.

5 Reagents and materials

IMPORTANT — Owing to the fact that *N*-nitrosamines are decomposed by UV light, the exposure to sunlight or fluorescent light of the standardized solutions during the preparation should be avoided. Standardized solutions, migrate solutions and water should be protected by means of aluminium foil and stored in the dark at temperatures below 5 °C.

Use only reagents of recognized analytical grade and only water conforming to ISO 3696, grade 3, unless otherwise specified.

5.1 Dichloromethane, which should be checked for the absence of *N*-nitrosamines.

5.2 Diatomaceous earth, from liquid-liquid extraction, with a specific surface area of 1 m^2/g, a pore size of 3 000 nm to 8 500 nm and a particle size of 150 µm to 650 µm.

Heat for 1 h at 200 °C and wash with dichloromethane (5.1) after cooling.

Another separating agent can be used providing it has been validated against diatomaceous earth.

5.3 *n*-Hexane.

5.4 Sodium hydroxide solution in water, c(NaOH) = 1 mol/l.

5.5 Nitrogen, with a volume fraction of at least 99,996 %.

5.6 Boiling chips.

5.7 Sintered glass frit for columns (6.3 and 6.4).

5.8 Acetone.

5.9 *N*-nitrosamine standardized solutions.

Prepare standardized solutions with known quantities of the *N*-nitrosamines to be determined in *n*-hexane (5.3) in the concentration range of 100 ng/l to 300 ng/l.

The following *N*-nitrosamines are of importance for condoms from elastomer or rubber. The list is, however, not comprehensive:

— *N*-nitrosodimethylamine (NDMA);

— *N*-nitrosodiethylamine (NDEA);

— *N*-nitrosodibutylamine (NDBA).

If there are indications of the presence of other *N*-nitrosamines relevant for toxicology or if the nature of the used vulcanization accelerators supports this indication these other *N*-nitrosamines should also be tested, examples could be:

— *N*-nitrosodibenzylamine (NDBzA);

— *N*-nitrosodiisononylamine (NDiNA), i.e. *N*-nitroso-3,3,5-trimethylhexylamine.

5.10 Solution of the internal standard of 200 ng/ml *N*-nitrosodiisopropylamin (NDiPA) in acetone (5.8).

The solution shall be free of other *N*-nitrosamines.

5.11 Water-free sodium sulfate, granulated, or a suitable phase separation filter for the Whatman apparatus.

Pre-wash 30 g of sodium sulfate with 25 ml of dichloromethane (5.1).

5.12 Ammonia solution in water, $c(NH_3) = 0{,}1$ mol/l.

5.13 Sea sand, washed with acid and calcined.

6 Apparatus

Usual laboratory apparatus and, in particular, the following:

6.1 Glass vessels washed with acidic cleansing agents, shall be treated with ammonia solution (5.12), rinsed with water and dried before being used.

6.2 Drying cabinet, adjustable to (40 ± 2) °C.

6.3 Glass column, with outlet and plug made from polytetrafluorethylene (PTFE). Length of the column: (300 ± 10) mm, inner diameter: (26 ± 1) mm.

6.4 Glass column, with outlet and plug made from polytetrafluorethylene (PTFE). Length of the column: (300 ± 10) mm, inner diameter: (15 ± 1) mm.

6.5 Kuderna-Danish vaporizer, modified with a graduated collecting vessel and an air cooler.

An alternative apparatus may be used provided that its performance has been validated against the Kuderna-Danish vaporizer.

6.6 Water bath, temperatures adjustable from 40 °C to 60 °C.

6.7 Vials, closable with vial mouth rings and *N*-nitrosamine free or PTFE coated septa.

6.8 Pliers, for closing the vials (6.7).

6.9 Fibreglass plug, washed with dichloromethane (5.1).

6.10 200 ml separating funnel.

6.11 100 ml separating funnel.

6.12 Chemiluminescence detector, of sufficient sensitivity [Thermal Energy Analyzer, TEA[1)]].

Another analysis detector may be used providing it has been validated against the TEA.

6.13 Chromatography system.

The decision regarding the selection of the chromatography system may be made by the tester. The test laboratory shall, however, provide evidence that the conditions were optimized in such a way as to achieve a sufficient separation of peaks, with the following points being observed:

— The system shall separate the *N*-nitrosamines (5.9) mentioned in this International Standard such that their peak areas can be compared with the peak area of the internal standard solution (5.10).

— The system separates *N*-nitrodimethylamine and *N*-nitrodiethylamine from the mentioned *N*-nitrosamines.

1) Thermal Energy Analyzer is an example of a suitable product available commercially. This information is given for the convenience of the users of this International Standard and does not constitute an endorsement by ISO of this product.

It is possible that two separating columns are necessary to separate all the *N*-nitrosamines and to achieve a sufficient sensitivity for NDBzA, if it is part of the test.

The following conditions have been found to be suitable for the determination of volatile *N*-nitrosamines when using gas chromatography.

EXAMPLE 1 Packed columns

Injector temperature: 200 °C

Oven temperature: 200 °C

Columns: 2,5 m to 3,0 m glass, outer diameter 1/8", packed with:

— 15 % Carbowax 20 M, TPA on Chromosorb WHP 100/120 mesh[2]; or

— 10 % Carbowax 20 M, 2 % KOH on Chromosorb WHP 100/120 mesh;

or

4,0 m to 5,0 m glass, outer diameter 1/8", packed with:

— 15 % SP 1220, 1 % H_3PO_4 on Chromosorb WAW 100/120 mesh.

Temperature of the pyrolysis oven: 480 °C

Carrier gas: Argon, helium, or nitrogen with a flow rate of (20 ± 1) ml/min

Coupling: Directly between gas chromatography column and pyrolysis oven or by applying a coupling heated to 250 °C.

As regards the determination of alkylphenyl-*N*-nitrosamines, the following conditions have been found to be suitable:

Injector temperature: 150 °C.

Oven temperature: between 120 °C and 130 °C.

Columns: 2 m glass, outer diameter 1/4", inner diameter 2,0 mm, packed with:

— 10 % OV-101 on Chromosorb WHP 80/100 mesh; or

— 3 % OV-225 on Chromosorb WHP 80/100 mesh.

Temperature of the pyrolysis oven: 480 °C

Temperature of the coupling: 250 °C.

EXAMPLE 2 Capillary columns

Either

Injector temperature: 200 °C

Oven temperature: 60 °C, 230 °C (10 °C/min)

Column: 25,0 m quartz capillary 0,53 mm FFAP 1 µm

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2) Carbowax and Chromosorb are examples of suitable products available commercially. This information is given for the convenience of the users of this International Standard and does not constitute an endorsement by ISO of these products.

Temperature of the pyrolysis oven: 480 °C

Temperature of the coupling: 250 °C

or

Injector temperature: 50 °C, 1 min 200 °C (75 °C/min)

Oven temperature 40 °C, 7 min 60 °C (1 °C/min), 230 °C (14 °C/min)

Column: 30,0 m quartz capillary, 0,53 mm, SE-54-film of 2 µm

Temperature of the pyrolysis oven: 480 °C

Temperature of the coupling: 250 °C.

7 Procedure

7.1 Migration of the condom material

7.1.1 For this test, use only condoms that have been tested according to ISO 4074 and conform to that standard. Use the average results of the tests for length and width for the calculations (see Clause 8).

Determine the masses of three condoms.

The average mass of these three reference condoms is the basis for the quantity of intact condoms to be used in the test.

Cut each condom in half.

Weigh (5 ± 1) g of the prepared condoms to the nearest 0,05 g and transfer them to a 50 ml Erlenmeyer flask. Add 40,0 ml of water by means of a pipette. Close the flask by means of a glass stopper, shake it cautiously so that the solution covers the prepared condoms. Store the flask in the drying cabinet (6.2) for 10 min (± 30 s) at (40 ± 2) °C.

Adapt the reagent quantities and the apparatus sizes proportionally if the mass of the condom material exceeds 5 g. The quantity of added internal standard solution (5.10), however, is always 1,0 ml.

7.1.2 Decant the solution from the flask to a 50 ml measuring cylinder with glass stopper, wash the condom material with 4,0 ml of water, add it to the test solution, fill with water up to the mark and mix it for at least 1 min.

7.2 Isolation of the *N*-nitrosamines in the solution

7.2.1 General

Add 1,0 ml of the internal standard solution (5.10) and 1,0 ml of sodium hydroxide solution (5.4) to the solution in the measuring cylinder (7.1.2) by means of a pipette.

The test solution can be processed according to procedure A or B.

7.2.2 Procedure A

7.2.2.1 Fill, with (25,0 ± 0,1) g of diatomaceous earth or another suitable separating agent (5.2), the glass column [inner diameter of 26 mm; the bottom of which is closed by means of a fiberglass plug (6.9)]. Cover the top of the column with the sintered glass frit (5.7) or with a sand layer of 1 cm (5.13).

When filling the column, tap the outside gently to ensure an even distribution.