

BS ISO 29941:2010



BSI Standards Publication

# Condoms — Determination of nitrosamines migrating from natural rubber latex condoms

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**National foreword**

This British Standard is the UK implementation of ISO 29941:2010.

The UK participation in its preparation was entrusted to Technical Committee CH/157, Mechanical contraceptives.

A list of organizations represented on this committee can be obtained on request to its secretary.

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ISBN 978 0 580 65906 5

ICS 11.200

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This British Standard was published under the authority of the Standards Policy and Strategy Committee on 31 January 2011.

**Amendments issued since publication**

Date	Text affected
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**Condoms — Determination  
of nitrosamines migrating from natural  
rubber latex condoms**

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



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Published in Switzerland

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## Foreword

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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<sup>2</sup>/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,  $c(\text{NaOH}) = 1 \text{ mol/l}$ .

**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,  $c(\text{NH}_3) = 0,1 \text{ mol/l}$ .

**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  $(40 \pm 2) \text{ }^\circ\text{C}$ .

**6.3 Glass column**, with outlet and plug made from polytetrafluorethylene (PTFE). Length of the column:  $(300 \pm 10) \text{ mm}$ , inner diameter:  $(26 \pm 1) \text{ mm}$ .

**6.4 Glass column**, with outlet and plug made from polytetrafluorethylene (PTFE). Length of the column:  $(300 \pm 10) \text{ mm}$ , inner diameter:  $(15 \pm 1) \text{ mm}$ .

**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  $40 \text{ }^\circ\text{C}$  to  $60 \text{ }^\circ\text{C}$ .

**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<sup>1</sup>].

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.

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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<sup>2)</sup>; 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<sub>3</sub>PO<sub>4</sub> 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 ( $5 \pm 1$ ) 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 ( $\pm 30$  s) at ( $40 \pm 2$ ) °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 ( $25,0 \pm 0,1$ ) 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.

**7.2.2.2** Close the measuring cylinder with the solution (7.2.1) and shake it until it is homogeneous. Then, slowly transfer it to the prepared column with diatomaceous earth or equivalent material (7.2.2.1).

The test solution shall be distributed as stationary phase onto the porous matrix within a period of 10 min to 15 min. In the lower part of the column, there remains a dry zone of a width of between 50 mm and 70 mm.

**7.2.2.3** Pour 60 ml to 80 ml of dichloromethane onto the column, collect 40 ml of eluate in the Kuderna-Danish collecting vessel or an equivalent vessel (6.5) within a period of 15 min to 25 min, with the dropping speed being regulated by means of the PTFE plug.

**NOTE** During the elution with dichloromethane, the dry zone will be decreased to 15 mm to 30 mm. This process can be well observed because of the different tinges of the diatomaceous earth zone that is wet from the test solution and the diatomaceous earth zone that is wet from dichloromethane. It is important not to reach the capacity limit of this dry zone; otherwise, the test solution might contain water.

### 7.2.3 Procedure B

**7.2.3.1** Close the measuring cylinder with the solution (7.2.1), shake it until it is homogeneous; slowly pour the solution into the separating funnel (6.10).

**7.2.3.2** Add at least 20 ml of dichloromethane and shake it well for at least 1 min. After the phase separation and possible centrifuging to destroy any existing emulsion, transfer the lower phase over 30 g of pre-washed sodium sulfate or another suitable phase separating filter (5.11) to the Kuderna-Danish collecting vessel or an equivalent apparatus (6.5).

**7.2.3.3** Repeat the procedure described under 7.2.3.2 twice.

**7.2.3.4** Wash the sodium sulfate (or the suitable phase separation filter) (5.11) with 25 ml of dichloromethane (5.1) and transfer the washing solution also to the Kuderna-Danish collecting vessel or the equivalent apparatus (6.5).

## 7.3 Concentration of the *N*-nitrosamines in the solution

**7.3.1** Add 2 ml of *n*-hexane (5.3) and two or three boiling chips (5.6) to the extract in the Kuderna-Danish collecting vessel (or in the equivalent apparatus) (6.5), which was made according to procedure A or B.

Put the air cooler onto the apparatus. Concentrate the solution in the water bath (6.6) to a volume of 4 ml to 6 ml. To avoid losses of testing substance, slowly warm the water bath from  $(40 \pm 2)^\circ\text{C}$  at a rate of approximately  $2^\circ\text{C}/\text{min}$  to  $(60 \pm 2)^\circ\text{C}$ . After the solution has cooled down, rinse the walls of the vaporizer and concentration system with  $(2 \pm 0,1)$  ml of dichloromethane (5.1).

Work shall be performed in a fume hood due to the flammability of the hexane.

**7.3.2** Remove the air cooler from the Kuderna-Danish collecting vessel (or the equivalent apparatus) and concentrate the solution to  $(1 \pm 0,1)$  ml by cautiously streaming nitrogen (5.5) over it. Allow the solution to cool down to room temperature and transfer it to a vial that is closed with septum and vial mouth ring (6.7).

The nitrogen flow shall be adjusted in such a way as to create a depression of 4 mm to 5 mm on the surface of the concentrated eluate, as otherwise the liquid might spill over or the extract might get too cold.

Owing to the volatility of the *N*-nitrosamines, the volume shall not fall below the minimum volumes mentioned in 7.3.1 and 7.3.2. If the period of time between the concentration of the solution and the measuring of the *N*-nitrosamines exceeds 1 h, store the solution in the dark at a temperature below  $5^\circ\text{C}$ .

## 7.4 Blank preparation

Run the blank preparation by carrying out all the processes described below, but without condom material and during the migration step (7.1.2).

## 7.5 Gas chromatographic test

Inject between 1 µl and 10 µl each of the extract under optimized conditions (6.13) into the gas chromatograph/chemiluminescence detector system. Test the same volume of the standardized solutions (5.9) and the solution of the internal standard (5.10).

To achieve reliable results, it is recommended that the sample preparation and the measurement be carried out on the same day. If this is not possible, store the extracts and the standardized solutions in the dark at a temperature below 5 °C.

## 8 Calculation and evaluation of the results of the *N*-nitrosamine content of the solution

8.1 Calculate the mass fractions for each of the *N*-nitrosamines found according to Equations (1) and (2).

$$w = \frac{F \cdot A_{NA}}{A_{NDiPA}^R} \quad (1)$$

where

$w$  is the mass fraction of one *N*-nitrosamine migrated from the sample into the water, in nanograms per gram (ng/g), corrected with regard to the recovery rate of the added internal standard NDiPA;

$F$  is the factor to be calculated according to Equation (2);

$A_{NA}$  is the peak area of the detected *N*-nitrosamine migrated from the sample into the water;

$A_{NDiPA}^R$  is the peak area of the internal standard NDiPA, recovered in the solution.

$$F = \frac{V \cdot \rho}{m} \cdot \frac{A_{NDiPA}^I}{A_{NASTD}} \cdot \frac{V_{NASTD}}{V_{NDiPA}^I} \quad (2)$$

where

$V$  is the volume of the added NDiPA solution of the internal standard, in millilitres (ml);

$\rho$  is the concentration of identified *N*-nitrosamine in the standardized solution, in nanograms per millilitre (ng/ml);

$m$  is the mass of the sample of condom material, in grams (g);

$A_{NASTD}$  is the peak area of the identified *N*-nitrosamine in the standardized solution;

$A_{NDiPA}^I$  is the peak area of the directly injected NDiPA solution of the internal standard solution;

$V_{NASTD}$  is the injected volume of the *N*-nitrosamine standard;

$V_{NDiPA}^I$  is the injected volume of the NDiPA standardized solution (5.10), in microlitres (µl).

8.2 If desired, calculate the mass per area for each of the *N*-nitrosamines found according to Equation (3).

$$\rho_A = w \cdot \frac{m_{\text{avg}}}{2bl} \quad (3)$$

where

$\rho_A$  is the mass per area, in nanograms per square decimetre (ng/dm<sup>2</sup>);

$w$  is the mass fraction of one *N*-nitrosamine migrated from the sample into the water, in nanograms per gram (ng/g), corrected with regard to the recovery rate of the added internal standard NDiPA;

$l$  is the length of condom, in decimetres (dm);

$b$  is the width of condom, in decimetres (dm);

$m_{\text{avg}}$  is the averaged mass of one condom, in grams (g).

8.3 Calculate the total *N*-nitrosamine content by adding the results of Equation (1) for each of the individually detected *N*-nitrosamines. If, as for one certain *N*-nitrosamine, there is no measurable detector signal, i.e. a peak with its height not at least three times the noise level, this substance shall be recorded as "ND" (not detected), and its value will be considered as "Zero".

## 9 Confirmation of the detected *N*-nitrosamines

9.1 The determined *N*-nitrosamines and their quantities need to be confirmed according to one of the following procedures:

- a) by irradiating one aliquot of the remaining test solution in a clear, UV permeable glass vessel with UV light (3 h, wave length: 366 nm). Treat a standard solution (5.9) produced in the same way in a second vessel analogously. Owing to the decomposition, the CG analysis might show either no peaks or only remarkably diminished peaks for *N*-nitrosamines. The original signals were wrongly positive if the signals from the samples are not remarkably diminished after the irradiation. In this case, a further test for the determination of *N*-nitrosamines is not necessary;
- b) by applying at least one other column with a stationary phase of different polarity;
- c) by mass spectroscopy testing.

9.2 If it turns out by one of the above-mentioned procedures that some peaks cannot be put down to the presence of *N*-nitrosamines, the total content of *N*-nitrosamines needs to be re-calculated exclusively with the *N*-nitrosamine signals.

## 10 Test report

**10.1** The activities performed by the testing laboratory shall be described in a test report, in which the test results and all the other relevant information according to ISO/IEC 17025 are laid down carefully, clearly and unambiguously.

**10.2** The test report shall contain at least the following information:

- a) name and address of the testing laboratory as well as the testing place, if it is different from the address of the testing laboratory;
- b) an identification characteristic that is uniquely assigned (e.g. continual number) of the test report and for each of the pages as well as the total number of pages of the report;
- c) description and identification characteristics of the laboratory sample;
- d) description of the sampling procedure;
- e) date of receipt of the sample and date of test (dates of test) of the laboratory sample including for each sample tested, the date and time testing commenced (7.2.1) and the date and time testing was completed (7.5);
- f) identification characteristics of the testing specification or description of the applied procedure including a list of all "alternates" used and specify if procedure A or procedure B was used;
- g) the results of the tested individual *N*-nitrosamines and the total *N*-nitrosamines in nanograms per grams (ng/g);
- h) name and signature of the testing person and name and signature of the laboratory supervisor attesting to the accuracy and completeness of the report;
- i) the number and publication year of this International Standard.

## Bibliography

- [1] EN 12868, *Child use and care articles — Methods for determining the release of N-Nitrosamines and N-Nitrosatable substances from elastomer or rubber teats and soothers*









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