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British Standard

Determination of volatile nitrosamines in rubber teats for feeding bottles and babies' dummies

Part 2. Artificial saliva extraction method

Dosage des nitrosamines volatiles dans les tétines en caoutchouc pour bibérons et sucettes pour bébés
Partie 2. Méthode par extraction à la salive artificielle

Bestimmung von flüchtigen Nitrosaminen in Saugern für Sauflaschen und Saugern zur Beruhigung von Säuglingen
Teil 2. Verfahren mit künstlichem Speichel

British Standards Institution

Foreword

This Part of BS 7115 has been prepared under the direction of the Rubber Standards Committee.

Volatile nitrosamines are thought to be carcinogenic and their presence in rubber teats for feeding bottles and babies' dummies is severely controlled. The method described herein is a development of a technique referred to in legislation in West Germany.

Attention is drawn to Part 1 of BS 7115, which describes a similar analytical method comprising extraction of the nitrosamines by dichloromethane.

Compliance with a British Standard does not of itself confer immunity from legal obligations.

Contents

Method

1 Scope

This Part of BS 7115 describes a method for the determination of volatile nitrosamines in rubber teats for feeding bottles and babies' dummies by extraction with artificial saliva solution and subsequent separation and analysis by gas chromatography.

NOTE 1. The application of the method to other rubber products is being investigated.

NOTE 2. The titles of the publications referred to in this standard are listed on the inside back cover.

2 Principle

Nitrosamines are extracted from the rubber by a solution of artificial saliva. After a clean-up process the final test solution is examined for volatile nitrosamines by separation using a gas chromatography unit employing a chemiluminescence detector.

Warning. The analysis should be carried out in an atmosphere free from volatile nitrosamines.

3 Reagents

3.1 *General.* All chemicals used shall be of analytical grade and distilled water complying with grade 2 of BS 3978 shall be used throughout. In order to ensure that oxides of nitrogen are not present, the water shall be distilled from a 0.5 % (*V/V*) solution of sulphamic acid, and nitrogen gas shall then be bubbled through the solution for 10 min to ensure the removal of nitric oxide. Deionized water shall not be used since nitrosamines can be present.

3.2 *Artificial saliva test solution,* comprising 4.2 g of sodium hydrogen carbonate, 0.5 g of sodium chloride and 0.20 g of potassium carbonate dissolved in 1 L of water. Store in the dark at a temperature below 5 °C.

3.3 *Dichloromethane* (D.C.M.), distilled in glass and checked for the absence of nitrosamines in accordance with 5.1.

3.4 *Sodium sulphate,* anhydrous and free from nitrosamines.

3.5 *Antibumping granules.*

3.6 *n-Hexane.*

3.7 *Iso-octane.*

3.8 *Standard solutions of nitrosamines.*

NOTE. Nitrosamines are potentially carcinogenic and exposure should be avoided.

Warning. N-nitrosamines are degraded by ultraviolet light and exposure of extracts or standard solutions to sources such as sunlight and fluorescent tube light should be avoided.

Prepare a solution containing known amounts of each of the following nitrosamines in n-hexane (3.6) or iso-octane (3.7):

N-nitrosodimethylamine (N.D.M.A.);
N-nitrosodiethylamine (N.D.E.A.);
N-nitrosodipropylamine (N.D.P.A.);
N-nitrosodibutylamine (N.D.B.A.);
N-nitrosopiperidine (N.P.I.P.);
N-nitrosopyrrolidine;
N-nitrosomorpholine

within the concentration range 100 ng/mL to 330 ng/mL. Prepare a separate solution containing a known amount of N-nitrosoethylphenylamine within the same concentration range. Protect the solutions from light by, for example, wrapping in aluminium foil and store at a temperature below 5 °C.

3.9 *N-nitrosodipropylamine (N.D.P.A.) internal standard solution.* Prepare a 200 ng/mL solution in ethanol (3.11) and store in the dark at a temperature below -15 °C.

NOTE. See the note and warning to 3.8.

3.10 *Morpholine solution.* Dissolve 100 mg of freshly distilled morpholine in D.C.M. (3.3), dilute to 100 mL in a volumetric flask and store at a temperature below 5 °C.

3.11 *Ethanol* (min 94.7 % (*V/V*)).

4 Apparatus

4.1 *Kuderna-Danish (K-D) evaporative concentrator,* modified with a graduated collecting vessel and an air cooler with a floating or expansion sphere.

4.2 *Ampoules,* welted-edged and capable of being closed with flanged rings and PTFE-coated septa.

4.3 *Sealing tongs.*

4.4 *Chromatography*

4.4.1 *General.* A column efficient enough to obtain baseline separation of the peaks obtained in the analysis of the solutions specified in 3.8 and having the features described in 4.4.2 to 4.4.5 is required.

4.4.2 *Column.* A 2.5 m to 3 m long glass column of internal diameter 3 mm to 4 mm using a stationary phase of 15 % (*m/m*) polyethylene glycol of average molecular weight 20 000 on a 100/120 mesh diatomaceous silica (white high purity acid washed silanized) support has been found to be suitable at the following temperatures:

injector 200 °C;
column 175 °C.

4.4.3 *Helium or argon carrier gas,* flow rate 20 mL/min.

4.4.4 *Pyrolysis oven,* coupled directly to the gas chromatograph.

4.4.5 *Chemiluminescence detector.* The carrier gas is to be passed through the pyrolysis oven (4.4.4) at 450 °C to 500 °C and a cooling trap at -150 °C before entering the detector.

NOTE. Where the presence of alkyl phenylnitrosamines is suspected an injection temperature of 150 °C and an oven temperature of 120 °C to 130 °C together with a less retentive column such as cyano propyl methyl phenyl methysilicone has been found to be satisfactory.

4.4.6 *Conical flask*, 250 mL, with a ground glass joint complying with designation 24/29 of BS 572.

5 Procedure

5.1 Verification of reagent and distilled water purity

5.1.1 Extract 50 mL of distilled water with two 50 mL portions of D.C.M. and evaporate to 1 mL. The concentrated D.C.M. extract shall not contain volatile nitrosamines, when tested using the chromatography unit and conditions described in 4.4.

5.1.2 Dilute 1 mL of morpholine solution (3.10) to 100 mL with D.C.M. (3.3) and process, in the same manner as a sample extract, in accordance with 5.3.

5.1.3 Analyse in duplicate the final concentrated solution for the presence of nitrosamines in the reagents using the chromatography unit and conditions described in 4.4: the presence of N-nitrosomorpholine indicates either the presence of the nitrosamine or a nitrosating substance in the reagents. Eliminate the cause of any peaks corresponding to nitrosamines in the reagents. If more than 2 ng of N-nitrosomorpholine per 100 mL of D.C.M. is formed do not use the solvent.

5.1.4 Do not use the D.C.M. for more than 1 week without retesting for nitrosamines.

5.1.5 Prepare a blank solution of all the reagents but omit the rubber test material. If volatile nitrosamines are present when the solution is tested using the chromatography unit and conditions described in 4.4, discard the reagent solutions and repeat the test on fresh reagents until the absence of nitrosamines is indicated.

5.2 Extraction of rubber teats

5.2.1 Store extracts and standard solutions in the absence of light (see also the warning note to 3.8 and 5.3.6).

5.2.2 Remove any non-rubber components from the teats. Weigh to the nearest 0.1 g about 50 g of teats, immerse in boiling water and boil for 5 min using sufficient water to cover the teats. Remove from the water and cut each teat once longitudinally in half.

5.2.3 Place the washed teat halves in a 250 mL conical flask (4.4.6) and add 150 mL of artificial saliva test solution (3.2). Stopper, shake briefly so as to ensure that the teat halves are covered by the solution and maintain the closed flask at 40 °C for 24 h. Transfer the solution to a 250 mL separatory funnel and wash the teat halves with 10 mL of the artificial saliva test solution. Add the washings to the separatory funnel.

5.3 Concentration of the extract

5.3.1 Pipette 1.00 mL of N.D.P.A. internal standard solution (3.9) into the solution obtained in 5.2.3.

5.3.2 Add 75 mL of D.C.M. (3.3) and shake vigorously for 1 min.

5.3.3 After separation of the liquid phases, centrifuging as necessary to break emulsions, draw off the lower layer and pass it through 30 g of sodium sulphate (3.4) prewashed with 25 mL of D.C.M. into a K-D evaporative concentrator (4.1).

5.3.4 Repeat the procedure given in 5.3.2 and 5.3.3 twice more.

5.3.5 Wash the sodium sulphate with 25 mL of D.C.M. and add it to the K-D evaporative concentrator.

5.3.6 Add 2 mL of n-hexane (3.6) and two or three antibumping granules (3.5) to the extract. Concentrate to 4 mL to 6 mL in the K-D evaporative concentrator at a temperature of 60 °C to 80 °C. Rinse the K-D evaporative concentrator with 2 mL of D.C.M. Concentrate to 1 ± 0.05 mL and mix well. Transfer to a welted-edged ampoule (4.2) and stopper using the sealing tongs (4.3). If the concentrate is to be kept for longer than 1 h before analysis, store the fluid in the dark at a temperature below 5 °C.

5.4 Chromatography

Analyse 5 µL to 7 µL of the extract (see 5.3.6) in duplicate using the chromatography unit and conditions described in 4.4. Also analyse in duplicate an equal volume of the standards solutions (3.8) and of the N.D.P.A. internal standard solution (3.9). If less than 90 % of the N.D.P.A. internal standard solution is recovered, the recovery rate being determined as A_{sr}/A_r (see clause 6), repeat the complete analysis.

NOTE. It is recommended that, to obtain reliable results, the analysis should be carried out the same day as the preparation of the extract.

6 Expression of results

Take the total content of nitrosamines to be the sum of the individual contents determined.

Calculate the individual N-nitrosamine contents using the following equation:

$$Z = \frac{A_x}{A_r} \times \frac{CV}{M} \times \frac{A_{sr}}{A_{sx}}$$

where

Z is the quantity of the particular N-nitrosamine which has migrated from the teat material into the artificial saliva test solution, in ng/g, corrected with reference to the N.D.P.A. recovery rate;

A_x is the peak area for the N-nitrosamine which has migrated into the artificial saliva test solution from the test material;*

A_i is the peak area for the N.D.P.A. internal standard solution added to the solution prepared in 5.3.1;*

c is the N-nitrosamine concentration of the standard solution (see 3.8) in mg/mL;

V is the volume for analysis of the final solution, i.e. 1 mL;

M is the mass of sample (see 5.2.2), in g;

A_{si} is the peak area for the N.D.P.A. internal standard solution (3.9);*

A_{sx} is the peak area for the corresponding N-nitrosamine in the standard solution (see 3.8).*

7 Precision

Inter laboratory analyses have been carried out and repeatability and reproducibility values established in accordance with BS 5497 : Part 1.

The calculations of repeatability and reproducibility are based only on small sets of results from a limited number of laboratories and it is therefore necessary to use scientific judgement when interpreting the results.

Also due to a lack of data, values for N-nitrosopyrrolidine, N-nitrosoethylphenylamine and N-nitrosomorpholine are not available.

Table 1. Repeatability and reproducibility values

Individual nitrosamine	Repeatability value, r	Reproducibility value, R
N.D.M.A.	5.5	5.8
N.D.E.A.	2.4	3.3
N.D.B.A.	1.6	4.3
N.P.I.P	6.1	18.5

NOTE. Where N.P.I.P. is found the precision values should only be reported after triplicate results have been obtained.

8 Test report

The test report shall indicate:

(a) a full description of the samples tested;

(b) all test results, including the concentrations of individual nitrosamines found;

(c) a reference to this Part of BS 7115;

(d) comments on any factors that may have influenced the test;

(e) a statement on the precision of the results.

* A_x, A_{si} and A_{sx} are all in the same units.

Publications referred to

BS 572 Specification for interchangeable conical ground glass joints
BS 3978 Specification for water for laboratory use
BS 5497 Precision of test methods
Part 1 Guide for the determination of repeatability and reproducibility for a standard test method by inter-laboratory tests

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Committees responsible for this British Standard

The preparation of this British Standard was entrusted by the Rubber Standards Committee (RUM/-) to Technical Committee RUM/37, *upon which the following bodies were represented:*

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