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British Standard

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

Part 1. Dichloromethane extraction method

Dosage des nitrosamines volatiles dans les tétines en caoutchouc pour bibérons et sucettes pour bébés
Partie 1. Méthode au dichlorométhane

Bestimmung von flüchtigen Nitrosaminen in Saugern für Saugflaschen und Saugern zur Beruhigung von Säuglingen
Teil 1. Dichlormethan-Extraktionsverfahren

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 the USA.

Attention is drawn to Part 2 of BS 7115, which describes a similar analytical method comprising extraction of the nitrosamines by artificial saliva.

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 dichloromethane 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 dichloromethane containing a nitrosation inhibitor (propyl gallate). After a clean-up process in which the extracted nitrosamines are transferred to an aqueous matrix followed by distillation and extraction from the distillate by dichloromethane, 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 *Dichloromethane* (D.C.M.), distilled in glass and checked for the absence of nitrosamines in accordance with 5.1.

3.3 *Morpholine solution.* Dissolve 100 mg of freshly distilled morpholine in 10 mL of 1 mol/L hydrochloric acid, extract twice with 20 mL of D.C.M., neutralize the aqueous phase to pH 7 with 1 mol/L sodium hydroxide and dilute to 100 mL in a volumetric flask. Store at a temperature below 5 °C.

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

3.5 *Antibumping granules.*

3.6 *Sodium hydroxide solution, c* (NaOH) = 0.5 mol/L. Dissolve 20 g of sodium hydroxide in 1 L of water.

3.7 *Propyl gallate.*

3.8 *n-Hexane.*

3.9 *Iso-octane.*

3.10 *Barium hydroxide.*

3.11 *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 (3.8) or iso-octane (3.9):

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.

3.12 *N-Nitrosodipropylamine (N.D.P.A.) internal standard solution.* Prepare a 200 ng/mL solution in D.C.M. (3.2) and store in the dark at a temperature below - 15 °C.

NOTE. See the note and warning to 3.11.

4 Apparatus

NOTE. Dimensions of apparatus are given for guidance only. However, it is recommended that apparatus having dimensions close to those shown is used.

4.1 *Graham condenser,* complying with 8.4 of BS 5922 : 1980 and fitted with both 24/39 joints (complying with BS 572) and a jacket of length 200 mm.

4.2 *Vertical recovery bend (connecting adaptor),* complying with 1.01.19 of BS 6711 : Part 1 : 1986 and fitted with parallel 24/39 joints complying with BS 572, 200 mm apart.

4.3 *Kuderna-Danish (K-D) evaporative concentrator,* of 250 mL capacity with a 24/39 column connection and a 19/26 lower joint both complying with BS 572, complete with springs.

4.4 *K-D concentrator tube,* of 4 mL capacity fitted with a 19/26 joint complying with BS 572, graduated in 0.1 mL subdivisions from 0 to 2 mL.

NOTE. The accuracy of the graduations should be checked before use.

4.5 *Snyder fractionating column,* complying with 3.05.43 of BS 6711 : Part 1 : 1986, comprising 3 x 150 mm sections, fitted with 24/39 joints complying with BS 572 and floating balls.

4.6 *Micro Snyder fractionating column,* comprising either a 3 chamber column fitted with a 19/26 joint, complying with BS 572. or a 4.5 chamber column without floating balls.

4.7 *Soxhlet extractor*, complying with BS 2071, with dimension *A* a minimum of 160 mm, having a coarse sintered glass or cellulose extraction thimble and equipped with a 500 mL round-bottom receiving flask.

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

4.9 *Sealing tongs.*

4.10 *Chromatography*

4.10.1 *General.* A column efficient enough to obtain baseline separation of the peaks obtained in the analysis of the solutions specified in **3.11** and having the features described in **4.10.2** to **4.10.5** is required.

4.10.2 *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 relative molecular mass 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.

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

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

4.10.5 *Chemiluminescence detector.* The carrier gas is to be passed through the pyrolysis oven (4.10.4) 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 methylsilicone has been found to be satisfactory.

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 nitrosamines when tested using the chromatography unit and conditions described in **4.10.**

5.1.2 Dilute 1 mL of morpholine solution (3.3) to 100 mL with D.C.M. (3.2) 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 **4.10**: 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 **4.10**, 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 dark (see also the warning note to **3.11** and **5.3.15**).

5.2.2 Remove any non-rubber components and cut each teat into six approximately equal pieces. Transfer about 25 g of the teats, weighed to the nearest 0.1 g, to a coarse sintered glass or cellulose extraction thimble suspended in a Soxhlet extractor (**4.7**).

5.2.3 Add 170 mL of D.C.M. (**3.2**), ensuring that the D.C.M. does not siphon over into the receiving flask. Add 100 mg of propyl gallate (**3.7**), and pipette 1.00 mL of N.D.P.A. internal standard solution (**3.12**).

5.2.4 Leave overnight (16 h to 18 h) at a room temperature of 20 ± 2 °C.

5.2.5 Carefully tip the Soxhlet extractor to enable the D.C.M. to siphon over into the receiving flask. Add a further 100 mL of D.C.M. to the receiving flask and extract the sample for 1 h. Ensure that the Soxhlet extractor siphons a minimum of five times within this period.

5.2.6 Cool the Soxhlet extractor assembly to room temperature.

5.3 Concentration of the extract

Warning. Do not allow concentrates to evaporate to dryness.

5.3.1 Add 120 mL of 0.5 mol/L sodium hydroxide solution (**3.6**), 2 g of barium hydroxide (**3.10**), to prevent foaming, and antibumping granules (**3.5**) to the D.C.M. extract in the flask from the Soxhlet extractor.

5.3.2 Connect the round-bottom flask to the distillation apparatus in such a way that the vertical recovery bend (**4.2**) slopes downward towards the vertical Graham condenser (**4.1**) and loosely wrap an insulating jacket around the flask and vertical recovery bend.

5.3.3 Distill off all of the D.C.M. and collect in a suitable receiver.

5.3.4 Increase the heater setting and collect 70 mL of aqueous distillate in a graduated cylinder.

5.3.5 Add 5 mL of 0.5 mol/L sodium hydroxide solution to the aqueous distillate and transfer to a 250 mL separatory funnel.

5.3.6 Add 50 mL of D.C.M. (**3.2** or **5.3.3**) to the aqueous distillate and shake vigorously for 1 min.

5.3.7 After separation of the liquid phases, draw off the lower D.C.M. 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 (**4.3**).

5.3.8 Repeat the procedure given in 5.3.6 and 5.3.7 twice more.

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

5.3.10 Add one or two antibumping granules, attach a Snyder fractionating column (4.5) and carefully concentrate to approximately 4 mL in a water bath at 55 °C.

5.3.11 Remove the K-D evaporative concentrator from the bath and cool to room temperature.

5.3.12 Remove the lower concentrator tube (4.4) and attach a micro Snyder fractionating column (4.6) to it.

5.3.13 Add a fresh antibumping granule and evaporate the D.C.M. to not less than 0.8 mL in a water bath at 55 °C. Maintain boiling continuously and control the rate by raising or lowering the tube.

NOTE 1. Overheating and excessive accumulation of the D.C.M. in the column chamber should be avoided.

Stop the concentration when the D.C.M. level reaches 0.8 mL and do not allow the level to fall below 0.8 mL. Raise the tube with the tip still in contact with the water and check that the volume is 0.8 mL.

NOTE 2. The final concentration stage takes about 30 min.

5.3.14 Remove the apparatus from the water bath, cool to room temperature, add a few drops of D.C.M. and let the rinsings drain. Disconnect the column, adjust the volume to 1 ± 0.05 mL and mix well.

5.3.15 Transfer to a welted-edged ampoule (4.8) and stopper using the sealing tongs (4.9). 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 5.3.15) in duplicate using the chromatography unit and conditions described in 4.10. Also analyse in duplicate an equal volume of the standards solutions (3.11) and of the N.D.P.A. internal standard solution (3.12). 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 dichloromethane, 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 dichloromethane from the teat material;*

A_r is the peak area for the N.D.P.A. internal standard solution added to the solution prepared in 5.2.3;*

C is the N-nitrosamine concentration of the standard solution (see 3.11), in ng/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_{sr} is the peak area for the N.D.P.A. internal standard solution (3.12);*

A_{sx} is the peak area for the corresponding N-nitrosamine in the standard solution (see 3.11).*

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.	0.5	6.0
N.D.E.A.	2.4	3.2
N.D.B.A.	5.5	5.9
N.P.I.P.	3.7	16.7

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 include:

(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_r, A_{sx} and A_{sr} are all in the same units.

Publications referred to

572	Specification for interchangeable conical ground glass joints
BS 2071	Specification for Soxhlet extractors
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
BS 5922	Specification for glass condensers for laboratory use
BS 6711	Vocabulary relating to laboratory apparatus made essentially from glass, porcelain or vitreous silica Part 1 Names for items of apparatus

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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:

Department of Trade and Industry (Laboratory of the Government Chemist)
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