



BSI Standards Publication

Fortified milk powders, infant formula and adult nutritionals — Determination of total biotin by liquid chromatography coupled with immunoaffinity column clean-up extraction

National foreword

This British Standard is the UK implementation of [ISO 23305:2020](#).

The UK participation in its preparation was entrusted to Technical Committee AW/34, Food Technical Committee Chairmen.

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

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

© The British Standards Institution 2020
Published by BSI Standards Limited 2020

ISBN 978 0 539 02692 4

ICS 67.100.10

Compliance with a British Standard cannot confer immunity from legal obligations.

This British Standard was published under the authority of the Standards Policy and Strategy Committee on 30 April 2020.

Amendments/corrigenda issued since publication

Date	Text affected
------	---------------

**INTERNATIONAL
STANDARD**

**ISO
23305**

First edition
2020-04-14

**Fortified milk powders, infant
formula and adult nutritionals —
Determination of total biotin by
liquid chromatography coupled
with immunoaffinity column clean-
up extraction**

*Poudres de lait fortifié, formules infantiles et produits nutritionnels
pour adultes — Détermination de la teneur en biotine totale
par chromatographie liquide après une purification sur colonne
d'immunoaffinité*



Reference number
ISO 23305:2020(E)

© ISO 2020



COPYRIGHT PROTECTED DOCUMENT

© ISO 2020, Published in Switzerland

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents	Page
Foreword	iv
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	1
5 Reagents and materials	2
5.1 General.....	2
5.1.1 Laboratory reagent grade water.....	2
5.1.2 Sodium dihydrogen phosphate dihydrate (CAS # 13472-35-0).....	2
5.1.3 Disodium hydrogen phosphate dihydrate (CAS # 10028-24-7).....	2
5.1.4 Sodium hydroxide (CAS #1310-73-2).....	2
5.1.5 Methanol, HPLC grade (CAS # 67-56-1).....	2
5.1.6 Acetonitrile, HPLC grade (CAS # 75-05-8).....	2
5.1.7 Ortho-phosphoric acid, mass fraction w (H ₃ PO ₄) = 85 % (CAS # 7664-38-2).....	2
5.2 Reagent preparation.....	2
5.3 Standard preparation.....	3
5.4 Calculation of concentration.....	4
6 Apparatus	4
6.10 Horizontal shaker.....	4
6.18 Vortex mixer.....	5
7 Procedure	5
7.1 Sample preparation.....	5
7.2 Chromatography.....	6
7.3 Quality control.....	7
8 Calculations	7
9 Precision	8
9.1 General.....	8
9.2 Repeatability.....	8
9.3 Reproducibility.....	8
10 Uncertainty of measurement	8
11 Limit of quantitation	8
Annex A (informative) Example chromatograms	9
Annex B (informative) Precision data	11
Annex C (informative) Comparison between this document and EN 15607	13
Bibliography	15

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, in collaboration with AOAC INTERNATIONAL. It is being published by ISO and separately by AOAC INTERNATIONAL. The method described in this document is equivalent to the AOAC Official Method 2016.02: *Determination of Total Biotin by Liquid Chromatography Coupled with Immunoaffinity Column Cleanup Extraction: Multilaboratory Testing, Final Action 2016.02*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Fortified milk powders, infant formula and adult nutritionals — Determination of total biotin by liquid chromatography coupled with immunoaffinity column clean-up extraction

WARNING — The use of this method can involve hazardous materials, operations and equipment. This method does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

1 Scope

This document specifies a method for the quantitative determination of biotin and/or biocytin in fortified milk powders, infant formula and adult nutritionals in solid (i.e. powders) or liquid (i.e. ready-to-feed liquids and liquid concentrates) forms using liquid chromatography coupled with immunoaffinity column clean-up extraction.

Precision data from an interlaboratory study is given in [Annex B](#). A comparison between data obtained with the method in this document and [EN 15607](#) is given in [Annex C](#).

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

3.1

adult nutritional

nutritionally complete, specially formulated food, consumed in liquid form, which may constitute the sole source of nourishment, made from any combination of milk, soy, rice, whey, hydrolysed protein, starch and amino acids, with and without intact protein

3.2

infant formula

breast-milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding

[SOURCE: Codex Standard 72-1981]

4 Principle

The sample is dispersed in sodium phosphate buffer and autoclaved at $121\text{ °C} \pm 2\text{ °C}$ for 25 min. The sample is cooled to room temperature and then diluted to 100 ml in a volumetric flask. The extract is centrifuged and filtered using a glass microfibre filter. Clear filtrate is collected for clean-up and

5.2.2 Sodium phosphate buffer, $c = 0,15$ mol/l.

Weigh 9,15 g of sodium dihydrogen phosphate dihydrate and 16,31 g of disodium hydrogen phosphate dihydrate into a 1 l volumetric flask, dissolve and make up to the mark with water. Adjust the pH to 7 with 2 mol/l sodium hydroxide.

5.2.3 Phosphoric acid, $w = 0,1$ %.

Into a 1 l volumetric flask, transfer 500 ml water. Add 1,2 ml of ortho-phosphoric acid. Mix and make up to the mark with water.

5.2.4 Mobile phase A, 0,1 % phosphoric acid in water.

5.2.5 Mobile phase B, 100 % acetonitrile.

5.2.6 Mobile phase C, 80 % acetonitrile.

5.3 Standard preparation

5.3.1 Stock standard biotin, mass concentration $\rho = 100$ $\mu\text{g/ml}$.

Weigh 25 mg biotin standard in a 250 ml amber volumetric flask. Add 150 ml water and sonicate at room temperature for 90 min with occasional shaking. Make up to volume with water.

5.3.2 Stock standard biocytin, $\rho = 100$ $\mu\text{g/ml}$.

Weigh 10 mg biocytin standard in a 100 ml amber volumetric flask. Add 60 ml water and sonicate at room temperature for 90 min with occasional shaking. Make up to volume with water.

5.3.3 Mixed intermediate standard, $\rho = 100$ $\mu\text{g}/100$ ml.

Dilute 1 ml each of stock standards to 100 ml with water.

5.3.4 Calibration standard 1, $\rho = 1,0$ $\mu\text{g}/100$ ml.

Dilute 100 μl mixed intermediate standard to 10 ml with water.

5.3.5 Calibration standard 2, $\rho = 2,5$ $\mu\text{g}/100$ ml.

Dilute 250 μl mixed intermediate standard to 10 ml with water.

5.3.6 Calibration standard 3, $\rho = 5,0$ $\mu\text{g}/100$ ml.

Dilute 500 μl mixed intermediate standard to 10 ml with water.

5.3.7 Calibration standard 4, $\rho = 7,5$ $\mu\text{g}/100$ ml.

Dilute 750 μl mixed intermediate standard to 10 ml with water.

5.3.8 Calibration standard 5, $\rho = 10$ $\mu\text{g}/100$ ml.

Dilute 1 ml mixed intermediate standard to 10 ml with water.

5.3.9 Calibration standard 6, $\rho = 20$ $\mu\text{g}/100$ ml.

Dilute 2 ml mixed intermediate standard to 10 ml with water.

5.4 Calculation of concentration

The concentrations given in 5.3 are indicative only. Calculate the actual concentrations of biotin and biocytin in each calibration standard, in µg/100 ml, using [Formula \(1\)](#). Calibration standards should be bracketed at the beginning and at the end of an analytical run.

$$\rho_{(\text{biotin/biocytin})} = \frac{(m_1 \times P \times 10 \times V_{is})}{(V \times 10)} \quad (1)$$

where

- m_1 is the mass of biotin or biocytin, in mg;
- P is the percentage purity from the certificate of analysis or verified by USP/BP/Ph Eur monographs;
- V_{is} is the volume of mixed intermediate standard used for the calibration standard, in ml;
- V is the volume of stock standard, $V = 250$ ml for biotin and $V = 100$ ml for biocytin.

6 Apparatus

Usual laboratory glassware and equipment and, in particular, the following.

6.1 HPLC system, consisting of a PDA detector, low pressure gradient pump system, a sample injector unit, a degasser unit, and a column oven.

6.2 Column, Kinetex Phenyl-Hexyl (Cat. No. 00F-4495-E0, Phenomenex²⁾), 150 mm × 4,6 mm × 2,6 µm × 10 nm.

6.3 Glass microfibre filters (Cat. No. 1820-125, Whatman^{®2)}).

6.4 Immunoaffinity column pack (R-Biopharm Rhone EASI-EXTRACT[®] BIOTIN P82/P82B²⁾ or equivalent).

6.5 SPE manifold, with accessories.

6.6 Autoclave, set at 121 °C.

6.7 Centrifuge, variable speed.

6.8 Analytical balance, four decimal places.

6.9 Amber glass screw-cap bottle, 100 ml.

6.10 Horizontal shaker.

6.11 Volumetric flasks, 1 l, 250 ml, 100 ml and 10 ml.

6.12 Pipettors, calibrated, 10,0 ml, 5,0 ml, 1,0 ml, 200 µl, 100 µl and 50 µl.

6.13 Measuring cylinder, 1000 ml, 100 ml and 50 ml.

2) This is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to lead to the same results.

6.14 Reaction vial, Reacti-Vials (Cat. No. 13223, Thermo Scientific²).

6.15 Heating block, Reacti-therm, with nitrogen blow down (Thermo Scientific²).

6.16 Ultrasonic bath, set at 50 °C and room temperature.

6.17 Centrifuge tubes, 50 ml.

6.18 Vortex mixer.

6.19 Syringe filter, polytetrafluoroethylene (PTFE) 0,45 µm.

6.20 Disposable syringes, 10 ml and 1 ml.

6.21 HPLC vials, 2 ml with 200 µl glass inserts.

7 Procedure

7.1 Sample preparation

7.1.1 For mass and loading volumes for the different ranges of product, see [Table 1](#). A slurry may be used wherever product homogeneity is suspected or unknown.

For the slurry, reconstitute 25 g of powder (m_1) with warm water (~50 °C) to a total mass of 200 g (m_2). Mix thoroughly on a horizontal shaker for 20 min and then sonicate at 50 °C for 10 min. Cool to room temperature. For liquid samples, mix well to ensure homogeneity of the sample portion and weigh the specified quantity.

7.1.2 Weigh the sample/slurry (m_3) into a 100 ml amber glass screw-cap bottle. See [Table 1](#).

7.1.3 Add 0,15 mol/l sodium phosphate buffer to an approximate volume of 50 ml.

7.1.4 Swirl gently to mix.

7.1.5 Autoclave the sample preparation at 121 °C for 25 min.

7.1.6 Cool the sample to room temperature. Quantitatively transfer the extract into a 100 ml volumetric flask and make up to the mark with 0,15 mol/l sodium phosphate buffer, mix well.

7.1.7 Transfer the extract into a centrifuge tube and centrifuge the sample at 4 000 rpm for 15 min.

7.1.8 Filter the sample using the glass microfibre filter paper ([6.3](#)) and collect the filtrate.

7.1.9 Set up the SPE manifold ([6.5](#)). Attach the immunoaffinity column (IAC) connected to a 10 ml reservoir. Drain off buffer just above the gel.

7.1.10 Load the sample filtrate onto the column in accordance with [Table 1](#) and initialize the flow with the help of a vacuum pump.

7.1.11 Turn off the vacuum and let the solution pass through the column by gravity at a rate of one drop per second.

7.1.12 Wash the column by passing 10 ml of PBS (5.1.8) through the column, followed by 10 ml of water. Initialize the flow with the help of vacuum at every step and then leave it to flow by gravity.

7.1.13 Remove any residual liquid from the column by introducing a gentle vacuum.

7.1.14 Introduce a reaction vial (6.14) and elute the analyte under gravity with 2 ml methanol. Elute further with an additional 1 ml of methanol. Backflush at least three times when eluting; this can be achieved by a gentle up and down motion of the syringe plunger to maximize the elution.

7.1.15 Evaporate the eluate to dryness using a heating block (6.15) set at 85 °C ± 5 °C under a gentle nitrogen blow down.

7.1.16 Remove from the heating block and cool down to room temperature (about 15 min).

7.1.17 Re-dissolve with 1 ml water and then cap the reaction vial (6.14) and vortex for 30 s. Filter by using a syringe filter into a clean glass insert in a HPLC vial for the HPLC analysis.

Table 1 — Sample mass, dilution and loading volume

Biotin µg/100g		Sample preparation				Conc µg/100 ml	
Min.	Max.	Mass (g)	Volume (ml)	Load (ml)	Final	Min.	Max.
0,1	0,5	20	100	50	1 ml	1	5
0,5	1,0	10	100	20	1 ml	1	2
1,0	5,0	10	100	10	1 ml	1	5
5,0	50,0	2,0 (Slurry 16 g)	100	10	1 ml	1	10
50,0	100,0	1,0 (Slurry 8 g)	100	10	1 ml	5	10
100,0	400,0	0,5 (Slurry 4 g)	100	5	1 ml	2,5	10

7.2 Chromatography

7.2.1 Set-up the HPLC system with the following configuration. Examples of chromatograms of a calibration standard and an infant formula sample used in the interlaboratory study are given in [Annex A](#).

7.2.2 Mobile phase A, 0,1 % phosphoric acid.

7.2.3 Mobile phase B, 100 % acetonitrile.

7.2.4 Mobile phase C, 80 % acetonitrile.

7.2.5 Column, see [6.2](#).

7.2.6 Column temperature, 25 °C ± 2 °C.

7.2.7 Retention times, biocytin is 4,5 min to 5,5 min and biotin is 16 min to 17 min.

7.2.8 Run time, 27 min.

7.2.9 Detector, a PDA detector operating at 200 nm (spectrum scan 200 nm to 350 nm).

7.2.10 Injection volume, 100 µl.

7.2.11 Form low pressure gradients by mixing the three mobile phases, A, B and C, using the procedure given in [Table 2](#).

Table 2 — Gradient programme

Time min	Flow rate ml/min	Mobile phase A %	Mobile phase B %	Mobile phase C %
0,0	0,6	90	10	0
18,0	0,6	90	10	0
18,5	0,8	0	0	100
24,0	0,8	0	0	100
24,5	0,6	90	10	0
27,0	0,6	90	10	0

7.3 Quality control

7.3.1 Check system suitability by injecting standard 3 five times. RSD should be $\leq 2\%$.

7.3.2 Run the calibration standards at the beginning and end of the sequence (slope drift $\leq 2\%$).

7.3.4 The six-point calibration should give a correlation coefficient $\geq 0,997$.

7.3.5 Test one in five samples in duplicate. The duplicates should be within the method repeatability.

7.3.4 Analyse a reference sample (e.g. National Institute of Standards and Technology Standard Reference Material 1849a) in duplicate.

7.3.5 The identification of the biotin peak is based on the absolute retention time. A spectrum scan can be used for peak purity confirmation if required.

7.3.6 Perform three high level recoveries with every new batch of immunoaffinity columns.

8 Calculations

The chromatography software will automatically calculate the concentration of the sample, ρ_b , in $\mu\text{g}/100\text{g}$, provided the concentration of the standards (in $\mu\text{g}/100\text{ ml}$), the sample mass (m) and the dilution are entered correctly. A manual calculation can also be performed using [Formula \(2\)](#):

$$\rho_b = \frac{(S \times D)}{(a \times m)} \quad (2)$$

where

S is the sample area;

D is the dilution, 10 (= $100 \times 1 / 10$) (sample made up to 100 ml, 10 ml used for IAC clean-up to a final volume of 1 ml with water for HPLC analysis; the dilution will be 20 if 5 ml is used for IAC clean-up);

a is the valid slope calculation based on the concentration on the x-axis and the peak area on the y-axis;

m is the sample mass (powder equivalent), in g.

Calculate the powder equivalent of the sample mass m , in g, using [Formula \(3\)](#) for reconstituted powder samples:

$$m = \frac{(m_1 \times m_3)}{m_2} \quad (3)$$

where

- m_1 is the mass of powder sample, in g;
- m_2 is the mass of powder and water or total slurry mass, in g;
- m_3 is the mass of slurry taken for analysis, in g.

For ready to feed liquid samples, use the sample mass used for extraction for the calculation.

Report the results to three significant figures, using microgram-per-100-g units or convert to other units as required.

9 Precision

9.1 General

Details of the interlaboratory test of the precision of the method are summarized in [Annex B](#). The values derived from the interlaboratory test may not be applicable to analyte concentration ranges and/or matrices other than those given in [Annex B](#).

9.2 Repeatability

The difference between the results of duplicate portions of the same sample tested on the same day/batch should not exceed 6 % of the mean result.

9.3 Reproducibility

The difference between the results of duplicate determinations tested on different batches/days should not exceed 12 % of the mean result.

10 Uncertainty of measurement

Uncertainty of the method was calculated as 10 %, using an appropriate statistical procedure (square root of the sum of squares of the errors expressed as a percentage).

11 Limit of quantitation

The limit of quantitation ($x_{LOQ} = 0,1 \mu\text{g}/100 \text{ g}$) was calculated based on the lowest working standard concentration and the dilution factor using [Formula \(4\)](#):

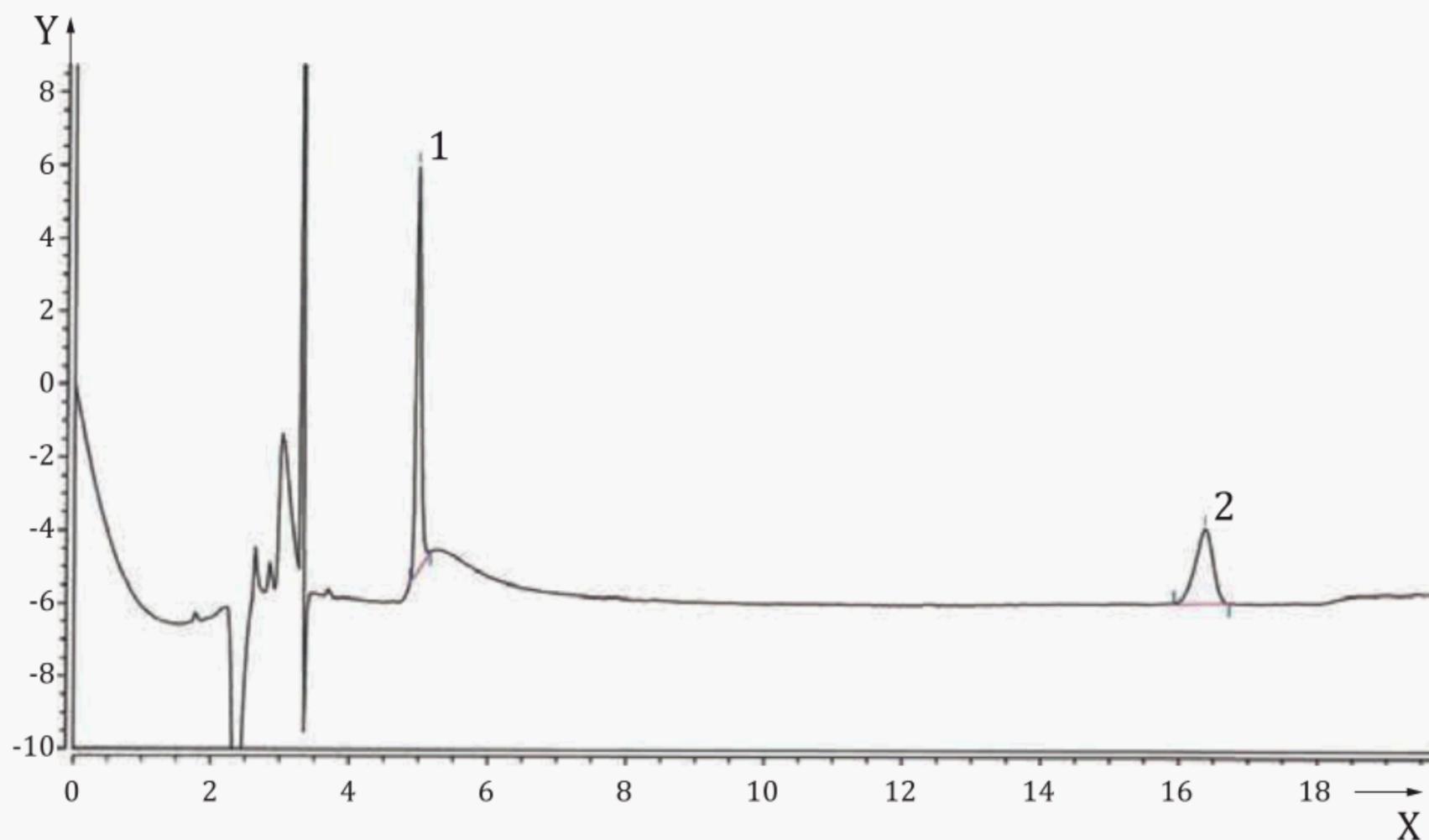
$$x_{LOQ} = \frac{(1 \times 100)}{(20 \times 50)} \quad (4)$$

where

- 1 is 1 $\mu\text{g}/100 \text{ ml}$, the lowest standard;
- 100 is the volume, in ml;
- 20 is 20 g of sample;
- 50 is the volume loaded on the immunoaffinity column, in ml;
- 1 is the final volume, in ml.

Annex A (informative)

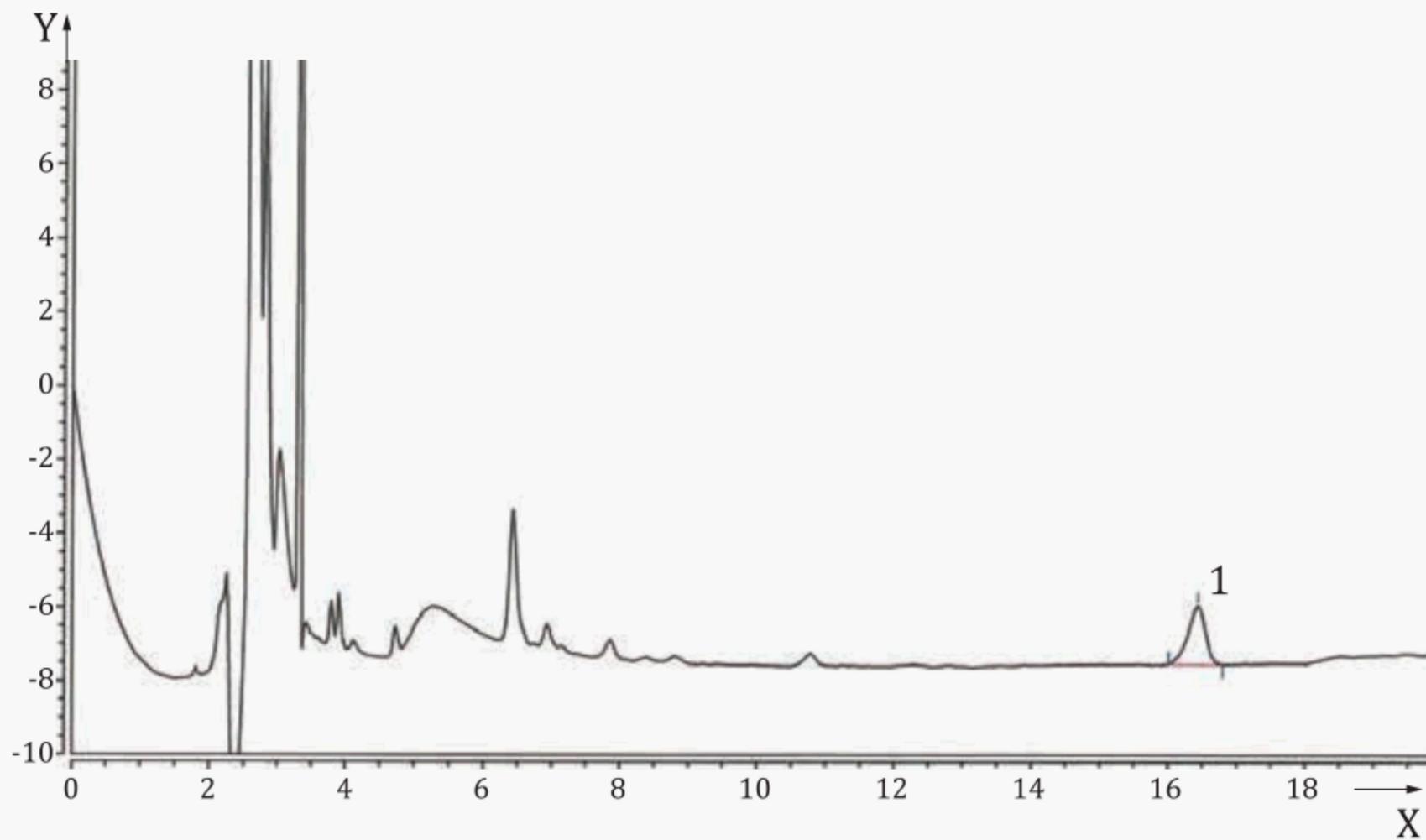
Example chromatograms



Key

- 1 biocytin, at 4,973 min
- 2 biotin, at 16,377 min
- X time, min
- Y absorbance, arbitrary units

Figure A.1 — Chromatogram calibration standard



Key

- 1 biotin, at 16,400 min
- X time, min
- Y absorbance, arbitrary units

Figure A.2 — Chromatogram of an infant formula interlaboratory study sample (8^h)

Annex B (informative)

Precision data

The data given in [Table B.1](#) were obtained in an interlaboratory study and published in 2018^[1] in accordance with [ISO 5725-2](#)^[2] and the AOAC-IUPAC Harmonized Protocol for collaborative study procedures, to assess precision characteristics of a method of analysis^[3]. The study was performed based on requirements given in Reference ^[4].

Table B.1 — Precision data for biotin

Samples	1 ^a	2 ^b	3 ^c	4 ^d	5 ^e	6 ^f	7 ^g	8 ^h	9 ⁱ	10 ^j	11 ^k	12 ^l
Year of inter-laboratory test	2017	2017	2017	2017	2017	2017	2017	2017	2017	2017	2017	2017
Number of laboratories	9	9	9	9	9	9	9	9	9	9	9	9
Number of non-compliant laboratories	0	0	0	0	0	0	0	0	0	0	0	0
Number of laboratories retained after eliminating outliers	9	9	9	9	9	9	9	9	9	9	9	9
Number of outliers (laboratories)	0	0	0	0	0	0	0	0	0	0	0	0
Number of accepted results	18	18	18	18	18	18	18	18	18	18	18	18
Mean value, \bar{x} ($\mu\text{g}/100\text{ g}$)	34,01	79,20	4,07	71,46	26,97	44,75	196,5	258,7	166,8	10,12	42,90	52,68
Repeatability standard deviation, s_r	1,88	2,09	0,22	2,77	1,83	2,58	4,68	7,26	11,74	0,58	0,97	2,71
Reproducibility standard deviation, s_R	3,22	4,07	0,31	5,54	2,36	3,11	5,97	18,19	11,74	0,67	2,45	3,10
Coefficient of variation of repeatability, $C_{V,r}$, %	5,53	2,64	5,52	3,88	6,79	5,77	2,38	2,81	7,03	5,74	2,26	5,14
Key												
a: infant formula powder partially hydrolysed milk based, b: infant elemental powder, c: infant formula RTF milk based, d: adult nutritional RTF high fat, e: infant formula powder milk based, f: infant formula powder soy based, g: NIST SRM 1849a, h: adult nutritional powder low fat, i: child formula powder, j: toddler formula powder milk based, k: infant formula powder milk based, l: adult nutritional RTF high protein.												

Samples	1^a	2^b	3^c	4^d	5^e	6^f	7^g	8^h	9ⁱ	10^j	11^k	12^l
Coefficient of variation of reproducibility, $C_{V,R}$, %	9,48	5,14	7,59	7,75	8,76	6,96	3,04	7,03	7,03	6,65	5,72	5,89
Repeatability limit, r ($r = 2,8 \times s_r$)	5,3	5,9	0,62	7,8	5,1	7,2	13,1	20,3	32,9	1,6	2,7	7,6
Reproducibility limit, R ($R = 2,8 \times s_R$)	9,0	11,4	0,87	15,5	6,6	8,7	16,7	50,9	32,9	1,9	6,9	8,7
HorRat value ^[5]	0,50	0,31	0,29	0,46	0,45	0,39	0,21	0,51	0,47	0,29	0,31	0,33

Key

a: infant formula powder partially hydrolysed milk based, b: infant elemental powder, c: infant formula RTF milk based, d: adult nutritional RTF high fat, e: infant formula powder milk based, f: infant formula powder soy based, g: NIST SRM 1849a, h: adult nutritional powder low fat, i: child formula powder, j: toddler formula powder milk based, k: infant formula powder milk based, l: adult nutritional RTF high protein.

Annex C (informative)

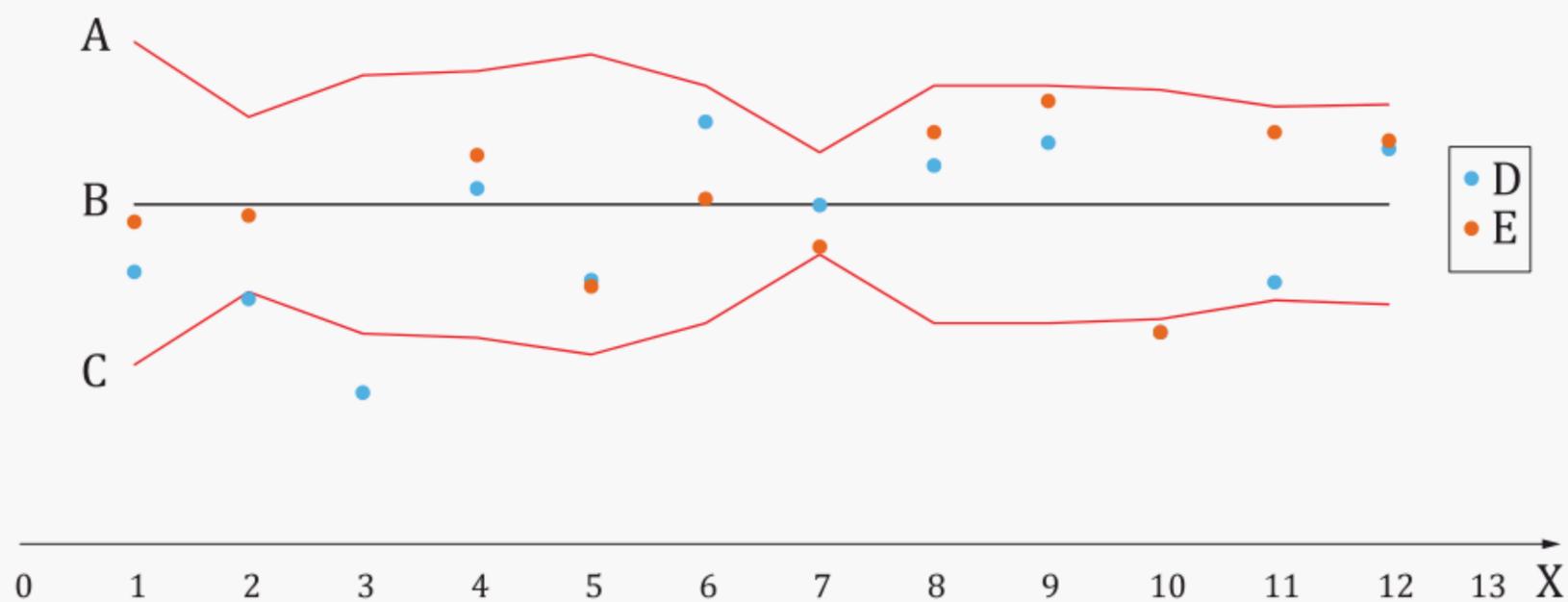
Comparison between this document and EN 15607

In order to ensure comparability of the data generated by [EN 15607](#)^[Z] and this document, data were collected by analysing the same samples with both methods. The results of this comparison are given in [Table C.1](#) and illustrated in [Figure C.1](#).

Table C.1 — Comparison of results for biotin

Sample	This document	This document	EN 15607	EN 15607
	<i>n</i> = 9 laboratories	<i>n</i> = 9 laboratories	Lab 2	Lab 4
	µg/100 g	$C_{V,R}$, %	µg/100 g	µg/100 g
SRM 1849a	196,5	3,0	196,5	187,0
Infant elemental powder	79,2	5,1	70,4	78,2
Adult nutritional RTF high protein	52,7	5,9	56,2	56,7
Infant formula powder soy based	44,8	7,0	49,2	45,1
Child elemental powder 1	10,1	6,7	8,6	8,6
Child elemental powder 2	27,0	8,8	24,6	24,4
Infant formula RTF milk based	4,1	7,6	3,2	3,8
Adult nutritional RTF high fat	71,5	7,8	72,9	75,6
Child formula powder milk based	166,8	7,0	179,0	187,0
Infant formula powder partially hydrolysed milk based	34,0	9,5	31,3	33,3
Milk-based infant formula	42,9	5,7	39,0	46,6
Adult nutritional powder low fat	258,7	7,0	270,5	280,5

In [Figure C.1](#), the values determined with [EN 15607](#) by the participating two laboratories are plotted against $\pm 2 C_{V,R}$ values as determined by the multilaboratory trial for this document with nine participating laboratories. The values of the two participating laboratories are standardized against the mean value of the multi-laboratory trial for this document. The two $C_{V,R}$ boundaries should include 95 % of the data based on this document.



Key

- | | | | |
|----|---|---|----------------|
| 1 | infant formula powder partially hydrolysed milk based | A | 2RSDR MLT |
| 2 | infant elemental powder | B | mean value MLT |
| 3 | infant formula RTF milk based | C | -2RSDR MLT |
| 4 | adult nutritional RTF high fat | D | laboratory 2 |
| 5 | infant formula powder milk based | E | laboratory 4 |
| 6 | infant formula powder soy based | | |
| 7 | NIST SRM 1849a | | |
| 8 | adult nutritional powder low fat | | |
| 9 | child formula powder | | |
| 10 | toddler formula powder milk based | | |
| 11 | infant formula powder milk based | | |
| 12 | adult nutritional RTF high protein | | |

Figure C.1 — Comparison of biotin determination

Bibliography

- [1] AOAC Official Final Action Method 2016.02. Determination of Total Biotin by Liquid Chromatography Coupled with Immunoaffinity Column Cleanup Extraction: Multilaboratory Testing, Final Action 2016.02. *J. AOAC Int.* 2018, 101(3)
- [2] [ISO 5725-2:1994](#),³⁾ *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*
- [3] AOAC INTERNATIONAL. *AOAC Official Methods Program, Associate Referee's Manual on development, Study, Review, and Approval Process*. Part IV AOAC Guidelines for Collaborative Studies. 1995, pp. 23–51
- [4] AOAC SPIFAN SMPR 2014.005 (2014)
- [5] THOMPSON M. Recent Trends in Inter-Laboratory Precision at ppb and sub-ppb Concentrations in Relation to Fitness for Purpose Criteria in Proficiency Testing. *Analyst (Lond.)*. 2000, 125 pp. 385–386
- [6] Codex Standard 72-1981, Standard for Infant Formula and Formulas for Special Medical Purposes Intended for Infants
- [7] [EN 15607:2009](#), *Foodstuffs — Determination of d-biotin by HPLC*

3) Withdrawn document.

British Standards Institution (BSI)

BSI is the national body responsible for preparing British Standards and other standards-related publications, information and services.

BSI is incorporated by Royal Charter. British Standards and other standardization products are published by BSI Standards Limited.

About us

We bring together business, industry, government, consumers, innovators and others to shape their combined experience and expertise into standards-based solutions.

The knowledge embodied in our standards has been carefully assembled in a dependable format and refined through our open consultation process. Organizations of all sizes and across all sectors choose standards to help them achieve their goals.

Information on standards

We can provide you with the knowledge that your organization needs to succeed. Find out more about British Standards by visiting our website at bsigroup.com/standards or contacting our Customer Services team or Knowledge Centre.

Buying standards

You can buy and download PDF versions of BSI publications, including British and adopted European and international standards, through our website at bsigroup.com/shop, where hard copies can also be purchased.

If you need international and foreign standards from other Standards Development Organizations, hard copies can be ordered from our Customer Services team.

Copyright in BSI publications

All the content in BSI publications, including British Standards, is the property of and copyrighted by BSI or some person or entity that owns copyright in the information used (such as the international standardization bodies) and has formally licensed such information to BSI for commercial publication and use.

Save for the provisions below, you may not transfer, share or disseminate any portion of the standard to any other person. You may not adapt, distribute, commercially exploit or publicly display the standard or any portion thereof in any manner whatsoever without BSI's prior written consent.

Storing and using standards

Standards purchased in soft copy format:

- A British Standard purchased in soft copy format is licensed to a sole named user for personal or internal company use only.
- The standard may be stored on more than one device provided that it is accessible by the sole named user only and that only one copy is accessed at any one time.
- A single paper copy may be printed for personal or internal company use only.

Standards purchased in hard copy format:

- A British Standard purchased in hard copy format is for personal or internal company use only.
- It may not be further reproduced – in any format – to create an additional copy. This includes scanning of the document.

If you need more than one copy of the document, or if you wish to share the document on an internal network, you can save money by choosing a subscription product (see 'Subscriptions').

Reproducing extracts

For permission to reproduce content from BSI publications contact the BSI Copyright and Licensing team.

Subscriptions

Our range of subscription services are designed to make using standards easier for you. For further information on our subscription products go to bsigroup.com/subscriptions.

With **British Standards Online (BSOL)** you'll have instant access to over 55,000 British and adopted European and international standards from your desktop. It's available 24/7 and is refreshed daily so you'll always be up to date.

You can keep in touch with standards developments and receive substantial discounts on the purchase price of standards, both in single copy and subscription format, by becoming a **BSI Subscribing Member**.

PLUS is an updating service exclusive to BSI Subscribing Members. You will automatically receive the latest hard copy of your standards when they're revised or replaced.

To find out more about becoming a BSI Subscribing Member and the benefits of membership, please visit bsigroup.com/shop.

With a **Multi-User Network Licence (MUNL)** you are able to host standards publications on your intranet. Licences can cover as few or as many users as you wish. With updates supplied as soon as they're available, you can be sure your documentation is current. For further information, email cservices@bsigroup.com.

Revisions

Our British Standards and other publications are updated by amendment or revision.

We continually improve the quality of our products and services to benefit your business. If you find an inaccuracy or ambiguity within a British Standard or other BSI publication please inform the Knowledge Centre.

Useful Contacts

Customer Services

Tel: +44 345 086 9001

Email: cservices@bsigroup.com

Subscriptions

Tel: +44 345 086 9001

Email: subscriptions@bsigroup.com

Knowledge Centre

Tel: +44 20 8996 7004

Email: knowledgecentre@bsigroup.com

Copyright & Licensing

Tel: +44 20 8996 7070

Email: copyright@bsigroup.com

BSI Group Headquarters

389 Chiswick High Road London W4 4AL UK