TECHNICAL SPECIFICATION

ISO/TS 16186

First edition
2012-08-15

Footwear — Critical substances potentially present in footwear and footwear components — Test method to quantitatively determine dimethyl fumarate (DMFU) in footwear materials

Chaussure — Substances critiques potentiellement présentes dans la chaussure et les composants de chaussure — Méthodes d'essai pour déterminer quantitativement le diméthylfumarate (DMFu) dans les matériaux de chaussure

Reference number
ISO/TS 16186:2012(E)

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Published in Switzerland

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In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of document:

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ISO/TS 16186 was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 309, *Footwear*, in collaboration with ISO Technical Committee TC 216, *Footwear*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

Footwear — Critical substances potentially present in footwear and footwear components — Test method to quantitatively determine dimethyl fumarate (DMFU) in footwear materials

1 Scope

This Technical Specification gives a test method for determining the amounts of dimethyl fumarate (DMFU) in footwear materials, desiccant sachets and other commodities.

The test method is not applicable to metal parts. The materials to which it is applicable are given in ISO/TR 16178:2012, Table 1.

NOTE In Europe, DMFU is prohibited in biocidal products as per Directive 98/8/EC. The substance must be used with caution to avoid any health problems in the chemistry laboratory. More recently, EU Commission Decision 2009/251/EC requires EU member states to ensure that products containing the biocide DMFU are not placed or made available on the market in the European Union. Decision 2009/251/EC establishes a maximum concentration of DMFU in products and parts of products of 0,1 mg/kg.

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 4787, *Laboratory glassware — Volumetric instruments — Methods for testing of capacity and for use*

ISO/TR 16178:2012, *Footwear — Critical substances potentially present in footwear and footwear components*

3 Principle of method

The sample is cut into small pieces and extracted with acetone in a sealed vial at a defined temperature in an ultrasonic bath. At this step, two different procedures are proposed, to be used depending on the material being tested:

a) the first procedure, without purification and concentration of the extracted solution, can be used for samples giving a simple chromatograph, for example, textiles;

b) the second procedure, with purification and concentration of the extract, can be used for samples with a complex matrix effect, such as leather.

4 Reagents

4.1 General

The substances given in Table 1 shall be used at a defined purity grade (at least 99,5 %).

Table 1 — Reagents

No	Substances	CAS[a] number
1	Dimethyl fumarate	624-49-7
2	Dimethyl maleate	624-48-6
3	d2-DMFU Dimethyl fumarate	23057-98-9
4	Acetone	67-64-1

[a] Chemical abstract service.

4.2 Stock solutions and working solutions

4.2.1 Stock solution of internal standard (1 g/l)

Weigh 10 mg of d2-DMFU into a 10 ml volumetric flask and fill to the mark with acetone. Transfer the content into an amber 10 ml vial with PTFE stopcock and keep at 4 °C.

4.2.2 Acetone working solution with internal standard (1 mg/l)

Prepare this solution by means of 1:1 000 dilution of the stock solution of internal standard with acetone.

4.2.3 Stock solution (1 g/l)

Weigh 50 mg of dimethyl fumarate and 50 mg of dimethyl maleate, with an accuracy of 0,1 mg, in a 50 ml volumetric flask, and fill to the mark with the acetone.

4.2.4 Acetone working solution of dimethyl fumarate and dimethyl maleate (1 mg/l)

Prepare this solution by means of 1:1 000 dilution of the stock solution with acetone.

5 Equipment

The usual equipment and laboratory glassware, according to ISO 4787, shall be used, in addition to the following.

5.1 Analytical balance, with a precision of at least 0,1 mg.

5.2 Glass vial that can be tightly sealed, 40 ml.

5.3 Ultrasonic bath with adjustable temperature.

5.4 PTFE membrane filter with a pore width of 0,45 µm.

5.5 Sample vials of 1 ml and PTFE-capped.

5.6 GC-MS (gas chromatograph–mass spectroscopy).

Other techniques may be used, provided it can be demonstrated that at least the same analytical performance can be obtained.

6 Sampling

In the footwear, the upper and the complex lining plus insock shall be tested.

The test piece shall consist of a single material (leather, textile or polymer).

Cut the homogenous textile, leather or polymer samples into pieces of up to 0,5 cm edge length. Desiccant samples may be used without any processing.

7 Procedures

7.1 Standard procedure

7.1.1 Extraction

Weigh 1,000 g of the sample in a 40 ml glass vial (5.2), add 1 ml of the solution of internal standard (4.2.2) and 9 ml of acetone, and seal the vial. Extract the sample at 60 °C for 1 h in an ultrasonic bath. After cooling to room temperature, filter this solution (if necessary) through a PTFE membrane filter (5.4). Transfer an aliquot of the extract to a GC-MS vial and seal with a PTFE cap.

7.1.2 Calibration solutions of dimethyl fumarate and dimethyl maleate

Prepare a working solution (1 µg/ml) by 1:1 000 dilution of the stock solution of dimethyl fumarate and dimethyl maleate (4.2.3). From this solution, mixed standards with concentrations of 0,005 µg/ml, 0,01 µg/ml, 0,05 µg/ml and 0,1 µg/ml of dimethyl fumarate and dimethyl maleate, and 0,1 µg/ml of the internal standard are made.

For this purpose, 5 µl, 10 µl, 50 µl and 100 µl of the working solution of dimethyl fumarate and dimethyl maleate (4.2.4), and 100 µl of the acetone working solution with internal standard (4.2.2) shall be filled up to 1 000 µl with acetone in sample vials.

Using this procedure, the concentration corresponding to the maximum regulation level of 0,1 mg/kg is 10 ppb.

7.2 Procedure for complex matrix

7.2.1 Extraction

Weigh 1,000 g of the sample into a 40 ml glass vial (5.2). Add 100 µl of the solution of internal standard (4.2.2) and 9,9 ml of acetone, and seal the vial. Extract the sample at 60 °C for 1 h in an ultrasonic bath.

7.2.2 Clean-up steps

Transfer 5 ml of the extract to a conical tube and reduce to around 0,2 ml under a gentle stream of nitrogen.

Reconstitute the extract to 1 ml with *n*-hexane.

Purify the extract on a Florisil cartridge (2 g/6 ml) by the following procedure.

a) **Conditioning**

   6 ml of *n*-hexane.

   Add the sample solution to the cartridge.

b) **Washing**

   2 ml of *n*-hexane.

   2 ml of *n*-hexane/acetone, of volume fraction 80/20 (V/V)

   These two fractions are discarded.

c) **Elution**

4 ml of *n*-hexane/acetone 80/20 (V/V)

The extract is reduced to 0,5 ml under a gentle stream of nitrogen, filtered (if necessary) through a PTFE membrane filter and transferred to a GC-MS vial.

7.2.3 Calibration solutions of dimethyl fumarate and dimethyl maleate

Table 2 indicates the method to be used to prepare the calibration solutions of dimethyl fumarate and dimethyl maleate.

Table 2 — Calibration solutions of dimethyl fumarate and dimethyl maleate

Standard	L1	L2	L3	L4	L5
Volume of acetone working solution of dimethyl fumarate and dimethyl maleate (4.2.4)	50 µl	100 µl	150 µl	200 µl	250 µl
Volume of acetone working solution of internal standard (4.2.2)	100 µl	100 µl	100 µl	100 µl	100 µl
Volume of acetone (volume of acetone required to make a total of 1 ml)	850 µl	800 µl	750 µl	700 µl	650 µl
Concentration of dimethyl fumarate and dimethyl maleate	50 µg/l	100 µg/l	150 µg/l	200 µg/l	250 µg/l
Concentration of the internal standard in the corresponding standard	100 µg/l	100 µg/l	100 µg/l	100 µg/l	100 µg/l

With this procedure, the concentration corresponding to the maximum regulation level of 0,1 mg/kg is 100 ppb.

8 Determination with GC-MS

8.1 Measuring method

Validated testing methods other than the one given in this Technical Specification are permitted provided a comparable precision and detection limit (see 8.3 and 8.4) can be reached.

Another requirement is sufficient separation of the dimethyl fumarate and dimethyl maleate in order to avoid false positive results by dimethyl maleate.

See Annex A for examples.

8.2 Precision

Based on a correlation study with 11 participating laboratories (see Annex B), the whole analysis, including the sample preparation and the determination by GC-MS, has a repeatability (VK) of 10 % at a concentration range between 0,7 and 0,8 mg/kg (desiccant, leather and textiles).

8.3 Detection limit

The detection limit should be lower than the legal limit of 0,1 mg/kg. It can be lowered by concentration of the sample solution or reduction of the extraction volume.

9 Test report

The test report shall include at least the following:

a) reference to this test method and ISO/TS 16186;

b) all details necessary for complete identification of the sample tested;

c) procedure used (standard or complex matrix).

d) determination of the extracted dimethyl fumarate.

e) any deviation by agreement or otherwise from the procedure specified.

Annex A
(informative)

Examples

A.1 Measuring method — Example 1

A.1.1 Measuring parameter

Column:	35 % phenyl–65 % dimethyl polysiloxane, 15 m × 0,25 mm × 0,5 µm
Carrier gas:	Helium; flow: 1 ml/min; splitless
Temperature programme:	100 °C for 1,5 min
	to 180 °C at 40 °C/min
	to 300 °C at 50 °C/min
	Isothermal: 2,5 min
Injector temperature:	250 °C
Transfer line temperature:	280 °C
Injection volume:	1 µl
MS-detection:	SIM (spectral identification method) — m/z signals, see Table A.1

A.1.2 Calibration and calculation

Table A.1 — Retention times and m/z signals

No.	Substance	Retention time	m/z
1	Dimethyl fumarate	2,27	113/59/85
2	d2-DMFU	2,27	115
3	Dimethyl maleate	2,50	113/59/85

The measurement is carried out according to Clause 8 and the data are used for quantification.

A calibration curve is set up by using the MS signals of the main mass, 113, at the different concentrations of the standards.

The quantification is conducted referring to the internal standard.

The identification of the different target compounds is carried out by comparison between the retention times and masses obtained and those of the reference substances.

A.2 Measuring method — Example 2

The DMFU is analysed by gas chromatography–mass spectrometry on a single-quad/MS use in a simultaneous SIM/SCAN mode.

Column:	DB-35MS (or equivalent 35 % phenyl–65 % dimethyl polysiloxan), 30 m × 0,25 mm × 0,25 µm
Carrier gas:	Helium
Temperature programme:	50 °C for 2 min
	to 110 °C at 6 °C/min
	to 310 °C at 30 °C/min
	Isothermal: 4 min
Injector temperature:	250 °C in splitless mode with an injection volume of 1 µl.
Transfer line:	280 °C
Injection volume:	1 µl
MS-detection:	SIM (spectral identification method) — m/z signal, see Table A.1

The single-quad MS works in simultaneous SIM/SCAN mode.

The mass SCAN range is between 50 and 160 amu.

For the SIM-mode focus on the ions, see Table A.2.

Table A.2 — Quantifier ions

Compounds	Ions
Dimethyl fumarate	113 (quantifier)
	85 (qualifier)
d2-DMFU	115 (quantifier)
Dimethyl maleate	113 (quantifier)
	85 (qualifier)