
**Footwear — Critical substances
potentially present in footwear
and footwear components —
Determination of dimethyl fumarate
(DMFU)**

*Chaussures — Substances critiques potentiellement présentes dans
les chaussures et les composants de chaussures — Détermination du
fumarate de diméthyle (DMFU)*

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Published in Switzerland

Contents

Page

Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	1
5 Reagents and materials	2
6 Apparatus	2
7 Sampling	3
8 Procedure	3
8.1 Standard procedure.....	3
8.2 Procedure for complex matrix.....	3
8.2.1 Extraction.....	3
8.2.2 Clean-up.....	4
8.3 Preparation of calibration solutions.....	4
8.4 Determination of dimethyl fumarate.....	4
9 Expression of results	4
9.1 Calculation of dimethyl fumarate in the sample.....	4
9.2 Limit of quantification (LOQ).....	5
10 Test report	5
Annex A (informative) The chromatography parameters (GC-MS)	6
Annex B (informative) The chromatography parameters (GC-MS/MS)	8
Annex C (informative) Rationale for excluding methanol as extraction solvent	10
Annex D (informative) Reliability of the method	11
Bibliography	12

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/foreword.html.

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

This first edition of ISO 16186 cancels and replaces ISO/TS 16186:2012, which has been technically revised.

The main changes compared to the previous edition are as follows:

- new [Clause 3](#), [6.6](#), [6.8](#), [6.9](#);
- gas chromatograph with tandem quadrupole mass spectrometer (GC-MS/MS);
- in [Clause 7](#), desiccant treated as a note;
- in [8.2.2](#), new clean up procedure;
- new [Annexes A](#), [B](#) and [C](#);
- Tabled [D.1](#) aligned with [Table D.2](#);
- bibliography added.

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.

Introduction

Dimethyl fumarate (DMFU) has been found to be a sensitizer at very low concentrations, producing extensive, pronounced eczema, which is difficult to treat.

There are regulations that limit the use of DMFU. For example in the EU, products, or any parts thereof, containing DMFU in concentrations greater than 0,1 mg/kg are not authorized on the market^[3].

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Footwear — Critical substances potentially present in footwear and footwear components — Determination of dimethyl fumarate (DMFU)

WARNING — The use of this document can involve hazardous materials, operations and equipment. It does not purport to address all of the safety or environmental problems associated with its use. It is the responsibility of users of this document to take appropriate measures to ensure the safety and health of personnel and the environment prior to application of the document, and to determine the applicability of regulatory limitations for this purpose.

1 Scope

This document specifies a method for the determination of the content of dimethyl fumarate (DMFU) by gas chromatograph with single quadrupole mass spectrometer (GC-MS) or tandem quadrupole mass spectrometer (GC-MS/MS).

This document is applicable to all types of footwear and footwear components except metal parts.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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*

3 Terms and definitions

No terms and definitions are listed in this document.

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/>

4 Principle

The sample is extracted using acetone (see precaution for the solvent in [Annex C](#)) at 60 °C in an ultrasonic bath. At this step, two different procedures can be used, depending on the material being tested:

- a) The “standard procedure,” without purification and concentration of the extracted solution, can be used for samples giving a simple chromatogram, for example, textile footwear components.
- b) The “procedure for complex matrix,” with purification and concentration of the extract, can be used for samples with a complex matrix effect, such as leather.

An aliquot of the extract is analysed using a gas chromatography with single quadrupole mass spectrometry (GC-MS) or tandem quadrupole mass spectrometry (GC-MS/MS).

5 Reagents and materials

The substances shall be used at a defined purity grade. If not otherwise defined, analytical reagent grade chemicals shall be used.

5.1 Acetone, CAS RN®¹⁾: 67-64-1.

5.2 n-Hexane, CAS RN®: 110-54-3.

5.3 Dimethyl fumarate (DMFU), CAS RN®: 624-49-7.

5.4 Dimethyl maleate (DMMA), CAS RN®: 624-48-6.

5.5 Internal standard, deuterated Dimethyl fumarate-2,3-d₂ (d₂-DMFU), CAS RN®: 23057-98-9.

5.6 Standard solutions.

5.6.1 Internal standard - stock solution (1 000 mg/l).

Weigh (10 ± 0,1) mg of d₂-DMFU (5.5) into a 10 ml volumetric flask (6.6) and fill to the mark with acetone (5.1). Transfer the content into an amber 10 ml vial (6.8) with cap and keep it at 4 °C.

5.6.2 Internal standard - working solution (10 mg/l).

Prepare this solution by means of 1:100 dilution of the internal standard stock solution (5.6.1) with acetone (5.1).

5.6.3 Target compounds - stock solution (1 000 mg/l).

Weigh (50 ± 0,1) mg of DMFU (5.3) and (50 ± 0,1) mg of DMMA (5.4), with an accuracy of 0,1 mg, in a 50 ml volumetric flask (6.6), and fill to the mark with the acetone (5.1).

5.6.4 Target compounds - working solution (1 mg/l).

Prepare this solution by means of 1:1 000 dilution of the standard stock solution (5.6.3) with acetone (5.1).

6 Apparatus

The usual laboratory equipment and laboratory glassware, in accordance with ISO 4787, shall be used, in addition to the following:

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

6.2 Glass vial, with screw cap that can be tightly sealed (e.g. volume of 40 ml).

6.3 Ultrasonic bath, with adjustable temperature, suitable for operation at about 60 °C.

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

6.5 GC vial, with cap (e.g. volume of 2 ml).

1) CAS Registry Number® (CAS RN®) is a trademark of CAS corporation. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

6.6 Volumetric flasks

6.7 Gas chromatograph with mass selective detector (GC-MS or GC-MS/MS).

6.8 Amber glass vial, with screw cap that can be tightly sealed (e.g. volume of 10 ml).

6.9 Activated magnesium silicate cartridge.

NOTE The two following cartridges have been found suitable.

- a) Pre-packed cartridge of FL-PR Florisil®²⁾ (170 µm, 80 Å) 2 g/12 ml.
- b) Bulk material of Florisil® 100-200 mesh, fine powder.

6.10 Nitrogen evaporator, with conical tubes and with adjustable temperature, suitable for operation up to 40 °C.

7 Sampling

The test specimen shall consist of a single material type (made of textile, leather, polymer or other organic material), which is tested separately.

Cut the homogenous material samples into pieces of about 3 mm to 5 mm edge length.

NOTE 1 Up to three test specimens (of equal mass) of the same material type can be tested together, taking into consideration the limits of detection and quantification.

NOTE 2 Desiccant can be a source of DMFU contamination of the footwear. Desiccant samples can be used without any processing.

8 Procedure

8.1 Standard procedure

Weigh $(1,0 \pm 0,1)$ g of the sample in a glass vial (6.2), record the mass to the nearest 1 mg, add 0,1 ml of the solution of internal standard working solution (5.6.2) and 9,9 ml of acetone (5.1), and seal the vial. Extract the sample at (60 ± 5) °C for (60 ± 5) min in an ultrasonic bath (6.3).

WARNING — Do not open the vial before cooling as the content can be under pressure.

After cooling to below at least 27 °C, decant the solution and, if necessary, reduce to 1,0 ml under a gentle stream of nitrogen (6.10).

Filter this solution through a PTFE membrane filter (6.4).

Transfer an aliquot of the extract to a GC vial (6.5) and seal with a cap.

8.2 Procedure for complex matrix

8.2.1 Extraction

Weigh $(1,0 \pm 0,1)$ g of the sample in a glass vial (6.2), record the mass to the nearest 1 mg, add 0,1 ml of the solution of internal standard (5.6.2) and 9,9 ml of acetone (5.1), and seal the vial. Extract the sample at (60 ± 5) °C for (60 ± 5) min in an ultrasonic bath (6.3).

2) Florisil is a registered trademark of U.S. Silica Company. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

8.2.2 Clean-up

After cooling to below at least 27 °C, transfer 5 ml of the extract to a conical tube and reduce to around 0,2 ml under a gentle stream of nitrogen (6.10).

Reconstitute the extract to 1 ml with n-Hexane (5.2).

Purify the reconstituted extract on an activated magnesium silicate cartridge (6.9) by the following procedure.

- a) Conditioning with 6 ml of n-hexane/acetone, with a volume fraction of 80/20.
- b) Decant 1 ml of the reconstituted extract on the SPE cartridge.
- c) Elute with 4 ml of n-hexane/acetone, with a volume fraction of 80/20 (V/V).
- d) Collected the eluent in a 5 ml volumetric flask, fill up to the mark with acetone.
- e) Filter the solution through a PTFE membrane filter (6.4) and transfer it to a GC-MS vial (6.5).

8.3 Preparation of calibration solutions

Prepare at least 3 calibration solutions of a mixture of dimethyl fumarate and dimethyl maleate, including internal standard, from the working solutions (5.6.4 and 5.6.2), in acetone (5.4), at suitable concentrations for the analysis.

Each solution shall contain the internal standard in a concentration that matches the internal standard concentration in the final sample extract (i.e. taking into account any volume reduction in 8.2).

An example of calibration measurement is given in [Clause A.2](#).

8.4 Determination of dimethyl fumarate

Determine the DMFU extracted in 8.1 or 8.2 by GC-MS or GC-MS/MS (6.7).

Sufficient separation of the dimethyl fumarate and dimethyl maleate is required in order to avoid false-positive results caused by dimethyl maleate.

Examples of chromatographic conditions are given in [Annexes A](#) and [B](#).

9 Expression of results

9.1 Calculation of dimethyl fumarate in the sample

Plot a calibration graph of the response against the known standard concentration (corrected for the response for the internal standard). From the calibration graph, interpolate the concentration of the dimethyl fumarate in mg/l (ρ_1).

The dimethyl fumarate level is calculated as mass portion w_s in mg/kg of the tested material according to the following formula:

$$w_s = \frac{\rho_1}{m} \times V$$

where