INTERNATIONAL STANDARD

ISO
16186

First edition
2021-05

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)

Reference number
ISO 16186:2021(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

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 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].

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-d_2 (d_2-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 d_2-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 ± 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 ± 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 registerd 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

w_s is the concentration of DFMU or DMMA in the sample, in mg/kg;

ρ_1 is the concentration of DMFU or DMMA in the sample solution, in mg/l;

m is the weight of the sample, in g;

V is the final extraction volume, in ml.

9.2 Limit of quantification (LOQ)

If the LOQ must be lower than the limit of 0,1 mg/kg (see Introduction), it can be lowered by concentration of the sample solution, see Annex D for the method reliability.

10 Test report

The test report shall include at least the following:

a) a reference to this document, i.e. ISO 16186:2021;

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

c) procedure used (with or without concentration step / with or without purification);

d) result of the extracted dimethyl fumarate, expressed in mg/kg as given in 9.1;

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

f) any unusual features observed.

Annex A
(informative)

The chromatography parameters (GC-MS)

A.1 Measurement condition of GC-MS

Column	35 % phenyl - 65 % dimethyl polysiloxane, length: 30 m, internal diameter: 0,25 mm, film thickness: 0,25 µm
Carrier gas	Helium; flow rate: 1,0 ml/min
Injector temperature	250 °C, splitless
Injection volume	1 µl
Temperature programme	50 °C for 2 min To 125 °C at 25 °C/min To 300 °C at 30 °C/min Isothermal: 1 min
Transfer line temperature	280 °C
Scan range	50 m/z to 150 m/z
Ion source temperature	230 °C
Detection mode	Selected ion monitoring (SIM) – Typical quantification ions are shown in Table A.1

A.2 Characteristic for GC-MS analysis and calibration

Table A.1 — m/z signals for quantification and qualification

No.	Substance	Quantifier/Qualifier (m/z)
1	Dimethyl fumarate (DMFU)	113/85/59[a]
2	Dimethyl fumarate-2,3-d_2 (d_2-DMFU)	115/87/59[a]
3	Dimethyl maleate(DMMA)	113/59[a]/85

[a] m/z = 59 is common ion for dimethyl fumarate and dimethyl fumarate-2,3-d_2 which should only be used as reference for qualitative analysis and should not be used for ion-ratio calculation.

The measurement is carried out according to Clauses 8 and 9 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.3 Examples of chromatograms

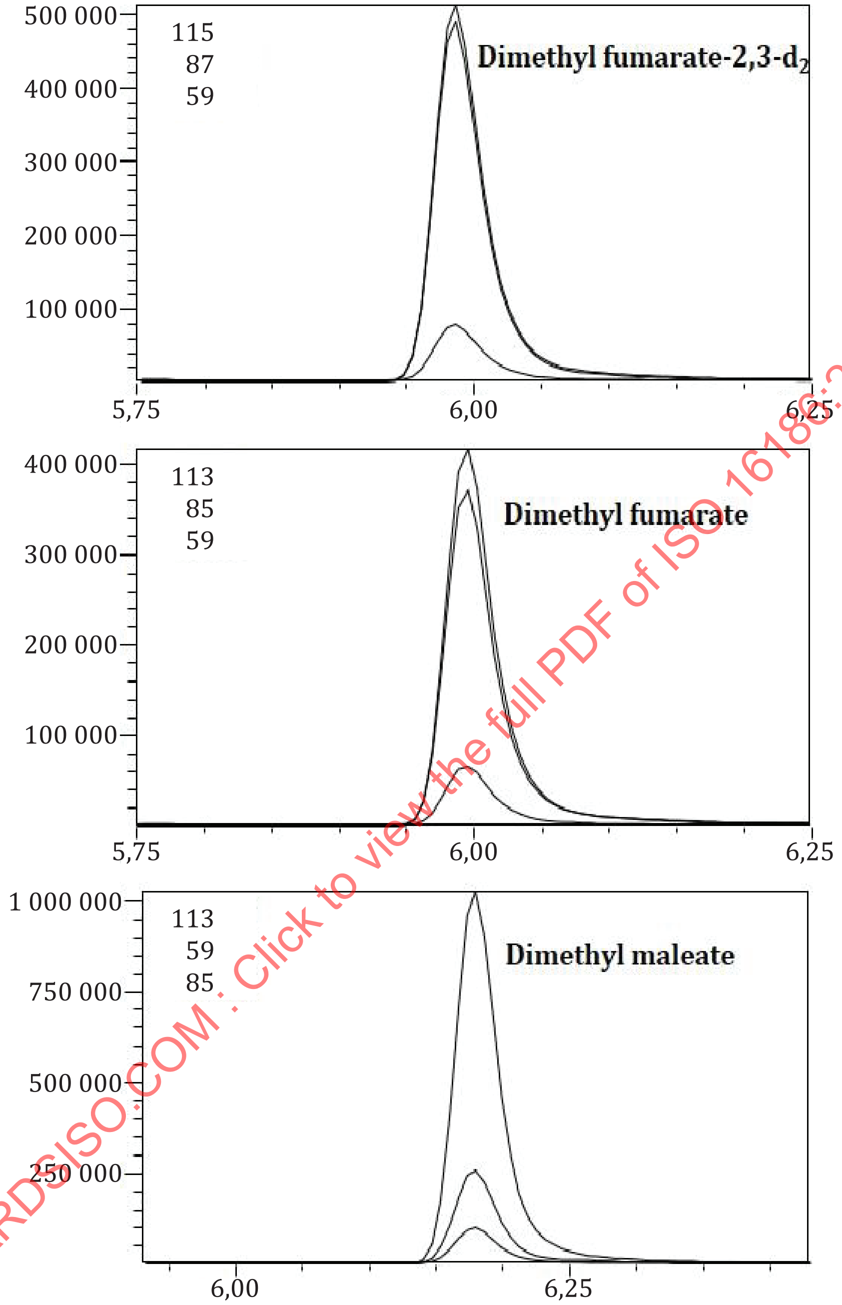


Figure A.1 — GC-MS chromatograms of d$_2$-DMFU, DMFU and DMMA

Annex B
(informative)

The chromatography parameters (GC-MS/MS)

B.1 Measurement condition of GC-MS/MS

Column	35 % phenyl - 65 % dimethyl polysiloxane, length: 30 m, internal diameter: 0,25 mm, film thickness: 0,25 µm
Carrier gas	Helium; flow rate: 1,0 ml/min
Injector temperature	250 °C, splitless
Injection volume	1 µl
Temperature programme	50 °C for 2 min To 125 °C at 25 °C/min To 300 °C at 30 °C/min Isothermal: 1 min
Transfer line temperature	280 °C
Scan range	50 *m/z* to 150 *m/z*
Ion source temperature	230 °C
Detection mode	Multiple reaction monitoring (MRM) – MRM transitions for each analytes are listed in Table B.1

B.2 Characteristic for GC-MS analysis

Table B.1 — Characteristic masses for quantification GC-MS/MS

No.	Substance	MRM transition Q1 → Q3(*m/z*)	Collision energy V
1	Dimethyl fumarate (DMFU)	113 → 85	6
		113 → 59	15
		113 → 53	15
2	Dimethyl fumarate-2,3-d_2 (d_2-DMFU)	115 → 87	9
		115 → 59	15
		115 → 54	15
3	Dimethyl maleate (DMMA)	113 → 85	9
		113 → 59	15
		113 → 53	15

B.3 Examples of chromatogram

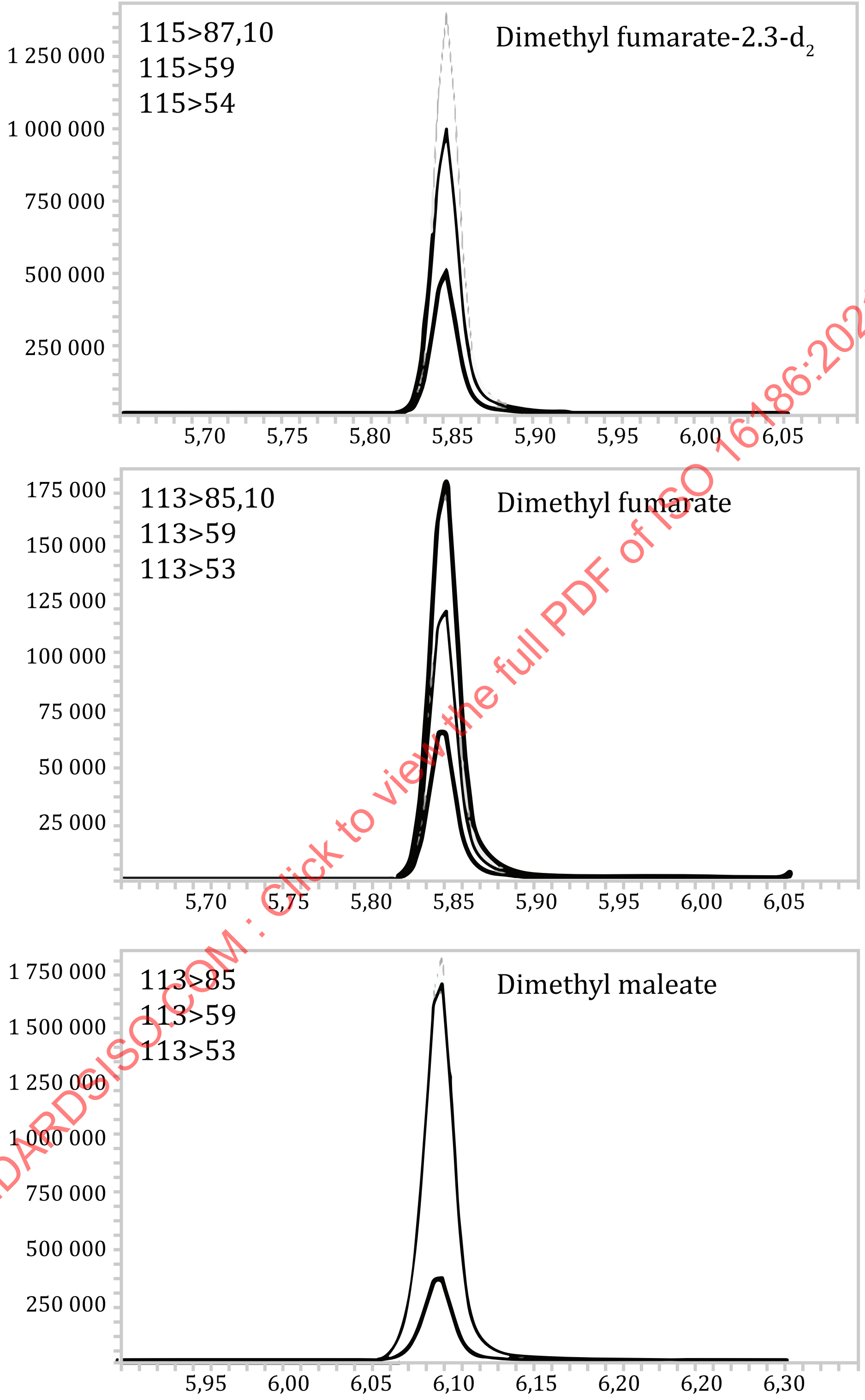
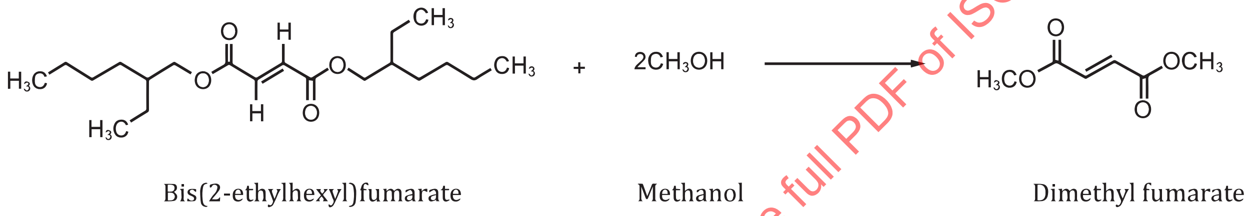


Figure B.1 — GC-MS/MS chromatograms of d_2-DMFU, DMFU and DMMA

Annex C
(informative)

Rationale for excluding methanol as extraction solvent

Dimethyl fumarate is the methyl ester of fumaric acid. When analysing dimethyl fumarate, the selection of extraction solvent is especially important. Because an alcohol and an ester react in the presence of an acid or base to form a new ester by transesterification mechanism. In particular, methanol can react with other chemicals to form a dimethyl fumarate. For example, when bis(2-ethylhexyl) fumarate (DEHF, CAS RN®: 141-02-6) as a coating agent, is present in the analysis sample, extraction with methanol can perform a transesterification reaction. The DEHF undergoes nucleophilic attack by methanol to the carbonyl carbon of the ester, which proceeds to formation of the dimethyl fumarate and Bis (2-ethylhexyl) maleate [(DEHM) CAS RN®: 142-65-2]. This specific transesterification scheme is as follows.



This phenomenon was confirmed by analysis experiments. DEHF was added into the sample without DMFU and extracted with methanol. DMFU and DEHF were detected when DEHF was added to methanol in the absence of dimethyl fumarate. This shows the conversion of DEHF to DMFU and DEHM. The relevant chromatograms are shown in Figure C.1.

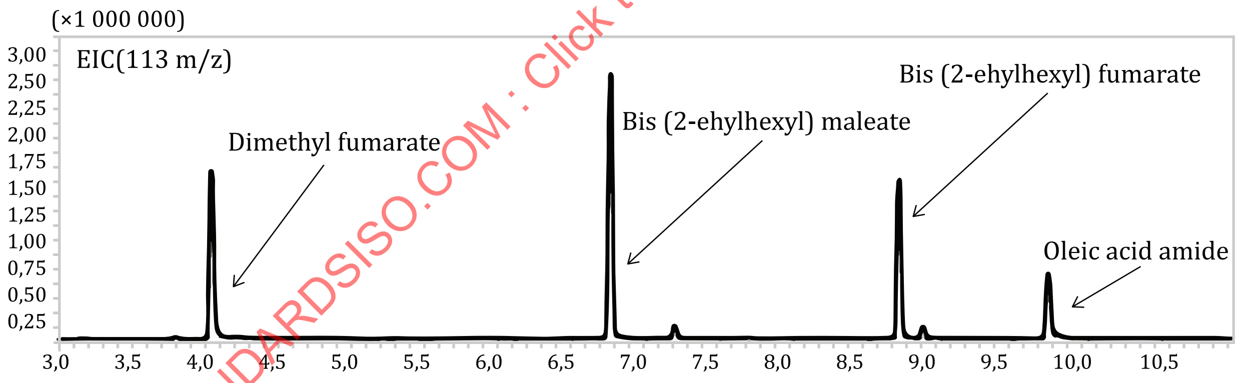


Figure C.1 — Chromatogram of DEHF conversion by methanol