

---

**EURL-MP-method\_005 (version 1)**

**Determination of erucic acid in infant formula and follow-on formula, and rapeseed oil by GC-FID**

<b>Analyte group:</b>	<b>Plant toxins</b>
<b>Analyte(s):</b>	Erucic acid
<b>Commodity group:</b>	High fat containing
<b>Comm. validated:</b>	Infant/follow-on formula and rapeseed oil
<b>Technique:</b>	Gas chromatography with flame ionisation detection (GC-FID)

**Modifications compared to previous version:**

Not applicable

**Method drafted by:**

EU Reference Laboratory for mycotoxins & plant toxins in food and feed (EURL-MP)  
Wageningen Food Safety Research (WFSR), part of Wageningen University & Research  
Akkermaalsbos 2, 6708 WB, Wageningen, the Netherlands  
[eurl.mycotoxins-planttoxins@wur.nl](mailto:eurl.mycotoxins-planttoxins@wur.nl)

**Notices:**

This method has been drafted as guidance for EU National Reference Laboratories on mycotoxins and plant toxins in food and feed. It has been produced with the utmost care. However, WFSR does not accept liability for any claims based on the contents of this document.

Any reference to specific manufacturers' products are mentioned only for the convenience of users. They do not constitute an endorsement by the EURL and do not imply exclusion of similar alternatives.

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

© 2020 Wageningen Food Safety Research, Wageningen University & Research, institute within the legal entity Wageningen Research Foundation. Reproduction is authorised provided the source is acknowledged.

*Suggested Citation: EURL-MP-method\_005 v1, 2020, Determination of erucic acid in infant formula and follow-on formula, and rapeseed oil by GC-FID, EURL mycotoxins and plant toxins, WFSR, Wageningen University & Research.*

---

<b>1. Introduction</b>	<b>3</b>
<b>2. Scope</b>	<b>3</b>
<b>3. Principle</b>	<b>3</b>
<b>4. Chemicals &amp; Reagents</b>	<b>3</b>
4.1. Chemicals	4
4.2. Reagents	4
<b>5. Equipment</b>	<b>4</b>
5.1. Laboratory balance	4
5.2. Magnetic stirring device	4
5.3. Rotary evaporator	4
<b>6. Procedure</b>	<b>4</b>
6.1. Preparation of the test sample	4
<b>7. Derivatisation and GC-FID conditions</b>	<b>4</b>
7.1. Derivatisation	4
7.2. GC-FID conditions	4
<b>8. Results</b>	<b>5</b>
<b>9. References</b>	<b>5</b>

## 1. Introduction

Erucic acid ((Z)-Docos-13-enoic acid,  $C_{22}H_{42}O_2$ , C22:1n9, CAS No 112-86-7) is a fatty acid that can be found in vegetable oils as rapeseed oil and mustard oil [1]. Maximum levels food in the EU are laid down in Regulation (EC) No 1881/2006 and amendments for erucic acid, including erucic acid bound in fat, in vegetable oils and fats placed on the market for the final consumer or for use as an ingredient in food, with specific limits for camellia oil, mustard oil and borage oil and mustard (condiment) [2]. Maximum levels for erucic acid for infant formula and follow-on formula are laid down in Delegated Regulation (EU) 2016/127 and amendments [3].

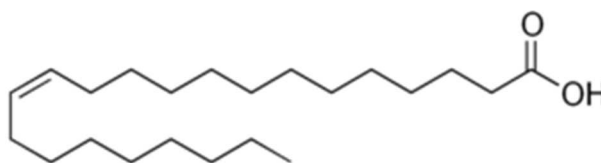


Figure 1. Chemical structure Erucic acid.

The analytical method for quantification consists of three different steps: i) extraction of the fat fraction from the matrix, ii) derivatisation of the fatty acids, and iii) identification and quantification using gas chromatography. The extraction of the fat fraction from the matrix (step i) is described in this document. The derivatisation step (step ii) is described in the harmonised method ISO 12966-2:2017 [4] and the gas chromatographic determination (step iii) is described in the harmonised method ISO 12966-4:2015 [5]. The method is commonly used to determine the concentrations of a variety of individual fatty acids, including erucic acid.

## 2. Scope

This document describes the method for extraction of fat from infant formula and follow-on formula.

## 3. Principle

Fat is extracted from the matrix by means of a mixture of chloroform and methanol (2:1, v/v). After evaporation of the extraction solvent the methyl esters are prepared by means of ISO 12966-2:2017 [4] and quantified by GC-FID according to ISO 12966-2:2015 [4].

## 4. Chemicals & Reagents

Use only reagents of recognised analytical grade unless otherwise specified.

## 4.1. Chemicals

4.1.1. **Methanol**, p.a. quality or similar

4.1.2. **Chloroform**, p.a. quality or similar

## 4.2. Reagents

### 4.2.1. Extraction solution

Mix 600 ml of chloroform (4.1.2) with 300 ml of methanol (4.1.1).

## 5. Equipment

Any reference to type and/or product is only to inform the user and to identify the equipment and does not imply exclusion of similar equipment.

### 5.1. Laboratory balance

accuracy: 0.01 g

### 5.2. Magnetic stirring device

### 5.3. Rotary evaporator

## 6. Procedure

### 6.1. Preparation of the test sample

In case of infant- and follow-on formula the fat must be extracted according to the method below, before the methylation procedure. This is not required when starting from oils or fats.

- Weigh approximately 50 g infant formula or follow-on formula in a 500 mL flask.
- Add 200 ml extraction solution (4.2.1) and mix the solution with a magnetic stirrer (5.2) during at least 2 hours at room temperature.
- Evaporate the extraction solution with a rotary evaporator (5.3) and collect the fat.
- Continue with the preparation of the FAMES according to ISO 12966-2:2017 [4] and analysis of the FAMES with GC-FID according to ISO-12966-4:2015 [5].

## 7. Derivatisation and GC-FID conditions

### 7.1. Derivatisation

ISO 12966-2:2017 [4]

### 7.2. GC-FID conditions

ISO 12966-4:2015 [5]

## 8. Results

Not applicable.

## 9. References

- [1] EFSA, Scientific Opinion on erucic acid in feed and food. EFSA Panel on Contaminants in the Food Chain (CONTAM). EFSA Journal, 2016. 14(11): 4593. pp. 173. (<https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2016.4593>).
- [2] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Communities, 2006. L364: pp. 5-24. (<https://eur-lex.europa.eu>)
- [3] Commission Delegated Regulation (EU) 2016/127 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for infant formula and follow-on formula and as regards requirements on information relating to infant and young child feeding. Official Journal of the European Union, 2016. L25. pp. 28. (<https://eur-lex.europa.eu>)
- [4] ISO 12966-2:2017 Animal and vegetable fats and oils - Gas Chromatography of fatty acid methyl esters - Part 2: Preparation of methyl esters of fatty acids. <https://www.iso.org/standard/72142.html>.
- [5] ISO 12966-4:2015 Animal and vegetable fats and oils - Gas Chromatography of fatty acid methyl esters - Part 4: Determination by Capillary Gas Chromatography. <https://www.iso.org/standard/63503.html>.