

**Animal feeding stuffs – Determination of the  
content of fatty acids –  
Part 1: Preparation of methyl esters  
(ISO/TS 17764-1:2002)**

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This Technical Specification is not a Swedish Standard. This document contains the English version of CEN ISO/TS 17764-1:2006.

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*Telefon:* 08 - 555 523 10. *Telefax:* 08 - 555 523 11  
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**CEN ISO/TS 17764-1**

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**Animal feeding stuffs - Determination of the content of fatty acids - Part 1: Preparation of methyl esters (ISO/TS 17764-1:2002)**

Aliments des animaux - Détermination de la teneur en acides gras - Partie 1: Préparation des esters méthyliques (ISO/TS 17764-1:2002)

Futtermittel - Bestimmung des Gehaltes an Fettsäuren - Teil 1: Vorbereitung von Methylestern (ISO/TS 17764-1:2002)

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**Management Centre: rue de Stassart, 36 B-1050 Brussels**

## CEN ISO/TS 17764-1:2006 (E)

### Foreword

This document (CEN ISO/TS 17764-1:2006) has been prepared by Technical Committee CEN/TC 327 "Animal feeding stuffs - Methods of sampling and analysis", the secretariat of which is held by NEN, in collaboration with Technical Committee ISO/TC 34 "Agricultural food products".

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to announce this CEN Technical Specification: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

# Animal feeding stuffs — Determination of the content of fatty acids —

## Part 1: Preparation of methyl esters

### 1 Scope

ISO/TS 17764 specifies methods for the quantitative determination of individual fatty acids and of the sum of the fatty acids (elutable fatty acids).

This part of ISO/TS 17764 specifies two methods for preparing the methyl esters of fatty acids of animal and vegetable fats, oils and fatty acid mixtures for raw materials for compound animal feeds, and fatty acids originating from fat extracts of animal feeding stuffs, including fats and fatty acid mixtures containing butyric acid.

The general method, the boron trifluoride (BF<sub>3</sub>) method, is concerned with the preparation of methyl esters of fatty acids with six or more C atoms, originating from fats, oils and free fatty acids.

The KOH/HCl method is concerned with the preparation of methyl esters of fatty acids with four or more C atoms. This method can also be used for the quantitative determination of fatty acids with a chain length shorter than ten C atoms in free fatty acid mixtures.

The methyl esters produced can be used for gas-liquid chromatography (GLC).

NOTE 1 Unsaponifiable materials are not removed and can, when present in considerable amounts, interfere with the chromatographic analysis.

NOTE 2 ISO/TS 17764-2 describes the application of gas chromatography with capillary columns and flame ionization detection for the determination of the content of fatty acids in a fat by making use of the methyl esters of the fatty acids obtained in accordance with the methods specified in this part of ISO/TS 17764.

### 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 661:1989, *Animal and vegetable fats and oils — Preparation of test sample*

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*

ISO 6492:1999, *Animal feeding stuffs — Determination of fat content*

**CEN ISO/TS 17764-1:2006 (E)****3 Fat extraction****3.1 General**

Extract the fat for determination of the content of the saponifiable fatty acids in an animal feeding stuff or mixed feed in accordance with the category of the feeding stuff, as described in ISO 6492:1999, with the following amendments.

**3.2 Samples of category A**

Perform a fat extraction in accordance with 9.5.1 of ISO 6492:1999.

Evaporate the solvent with a rotating evaporator in a water bath at a temperature of not more than 40 °C. Then dry the residue for 2 h in a vacuum drying oven at 40 °C ± 2 °C.

**3.3 Samples of category B**

Extract the fat in two steps. For the first step, proceed in accordance with 9.5.1 of ISO 6492:1999, treating the test portion as described in 9.3 of ISO 6492:1999 as a sample of category A.

Collect the fat extract in a dry flask. Allow the solvent to evaporate from the residue by exposing the thimble to the air.

Perform a hydrolysis of the residue in accordance with 9.4 of ISO 6492:1999.

After hydrolysis, dry the residue in an extraction thimble for 60 min in a vacuum drying oven at 40 °C ± 2 °C.

Extract the residue according to 9.5.1 of ISO 6492:1999.

Add the fat extract to the first extract.

Evaporate the solvent with a rotating evaporator in a water bath at a temperature of not more than 40 °C and dry the residue for 2 h in a vacuum drying oven at 40 °C ± 2 °C.

**4 Preparation of test sample of fat or fat extract**

If the fat sample or the fat extract is not completely molten, heat the sample to a temperature of not more than 10 °C above the melting temperature. See ISO 661.

**5 Method for the preparation of methyl esters of fatty acids with six or more C atoms (BF<sub>3</sub> method)****5.1 Principle**

The glycerides are saponified with methanolic sodium hydroxide. The soaps are converted to methyl esters by reaction with a boron trifluoride/methanol complex.

**5.2 Reagents**

Use only reagents and solvents of recognized analytical grade.

**5.2.1 Water**, complying with at least grade 3 in accordance with ISO 3696:1987.

**5.2.2 Heptadecanoic acid (internal standard)**, of purity at least 99 %.