

# SVENSK STANDARD

## SS-EN ISO 17180:2013



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### **Animal feeding stuffs – Determination of lysine, methionine and threonine in commercial amino acid products and premixtures (ISO 17180:2013)**

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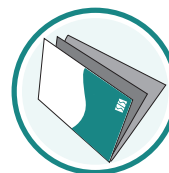
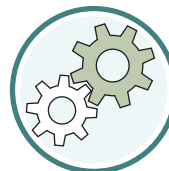
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EUROPEAN STANDARD

**EN ISO 17180**

NORME EUROPÉENNE

EUROPÄISCHE NORM

April 2013

ICS 65.120

English Version

**Animal feeding stuffs - Determination of lysine, methionine and  
threonine in commercial amino acid products and premixtures  
(ISO 17180:2013)**

Aliments des animaux - Détermination de la teneur en  
lysine, méthionine et thréonine dans les acides aminés  
industriels et les pré-mélanges (ISO 17180:2013)

Futtermittel - Bestimmung von Lysin, Methionin und  
Threonin in handelsüblichen aminosäurehaltigen Produkten  
und Vormischungen (ISO 17180:2013)

This European Standard was approved by CEN on 21 March 2013.

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EUROPÄISCHES KOMITEE FÜR NORMUNG

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## **Foreword**

This document (EN ISO 17180:2013) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 327 "Animal feeding stuffs - Methods of sampling and analysis" the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 2013, and conflicting national standards shall be withdrawn at the latest by October 2013.

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### **Endorsement notice**

The text of ISO 17180:2013 has been approved by CEN as EN ISO 17180:2013 without any modification.





# Animal feeding stuffs — Determination of lysine, methionine and threonine in commercial amino acid products and premixtures

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## 1 Scope

This International Standard specifies a method for the quantitative determination of free (non-protein-bound) lysine, methionine, and threonine in commercial products and premixtures containing more than about 10 % mass fraction of the respective amino acid. It does not distinguish between D- and L-forms.

**NOTE** For the purposes of this International Standard, the term “amino acids” used in [Clause 2](#) onwards refers to lysine, methionine, and threonine.

## 2 Principle

The samples are treated in dilute hydrochloric acid and then diluted with sodium citrate buffer. Norleucine internal standard is added and the amino acids are separated by an amino acid analyser or high performance liquid chromatography (HPLC), using a cation exchange resin and sodium citrate buffer eluent solutions. The amino acids are measured colourimetrically following post-column reaction with ninhydrin reagent or by fluorescence detection after post-column reaction with *ortho*-phthaldialdehyde (OPA).

## 3 Reagents and materials

Use only reagents of recognized analytical grade, unless otherwise specified.

**3.1 Water**, double distilled water or equivalent purity (conductivity <10 µS/cm).

**3.2 Standard substances.**

**3.2.1 Lysine·HCl crystals**, purity superior to 99 % mass fraction dried under vacuum in a desiccator for 2 days over P<sub>2</sub>O<sub>5</sub> prior to use.

**3.2.2 Threonine crystals**, purity superior to 99 % mass fraction dried under vacuum in a desiccator for 2 days over P<sub>2</sub>O<sub>5</sub> prior to use.

**3.2.3 Methionine crystals**, purity superior to 99 % mass fraction dried under vacuum in a desiccator for 2 days over P<sub>2</sub>O<sub>5</sub> prior to use.

**3.3 Norleucine crystals**, for use as internal standard, purity superior to 99 % dried under vacuum in a desiccator for 2 days over P<sub>2</sub>O<sub>5</sub> prior to use.

**3.4 Sodium hydroxide solution**,  $c(\text{NaOH}) = 7,5 \text{ mol/l}$ , for pH adjustment of sodium citrate buffer.

Carefully dissolve 300 g sodium hydroxide in water ([3.1](#)) and make up to 1 l.

**3.5 Hydrochloric acid**,  $\gamma(\text{HCl}) = 370 \text{ g/kg}$ .

**3.6 Sodium citrate dihydrate.**

**3.7 2,2'-Thiodiethanol** (thiodiglycol).

**3.8 Phenol crystals**, purity superior to 98,5 % mass fraction.

**3.9 Sodium citrate buffer**, pH 2,20.

Dissolve 19,61 g of sodium citrate dihydrate (3.6), 5 ml of thiodiglycol (3.7), 1 g of phenol (3.8) and 16,5 ml of HCl (3.5) in 800 ml of water. Adjust the pH to 2,2 with a few drops of HCl (3.5) or NaOH solution (3.4). The phenol acts to preserve the buffer solution.

Sodium citrate buffer can be also prepared from citric acid and sodium chloride.

**3.10 Hydrochloric acid solution**,  $c(\text{HCl}) = 0,1 \text{ mol/l}$ .

Take 8,2 ml of HCl (3.5), dilute with approximately 900 ml of water (3.1). Mix thoroughly and make up to 1 l with water.

**3.11 Standard solutions of amino acids.**

**3.11.1 Internal standard substance norleucine stock solution**,  $c(\text{Nle}) = 2,5 \text{ mmol/l}$ .

Dissolve 0,328 g norleucine (3.3) and transfer quantitatively with 0,1 mol/l HCl (3.10) into a 1 l volumetric flask.

Store below 5 °C for no more than 6 months.

**3.11.2 Amino acids standard stock solutions**,  $c = 2,5 \text{ mmol/l}$ .

Prepare a standard stock solution for each molecule.

Standard solutions shall contain only the amino acid being analysed, i.e. lysine, threonine or methionine. Commercially available mixed standard solutions, e.g. containing 18 amino acids, do not give optimal results.

Transfer 0,456 g lysine·HCl (3.2.1), 0,297 g threonine (3.2.2), and 0,373 g methionine (3.2.3) into a 1 l volumetric flask and make up to the mark with 0,1 mol/l HCl (3.10).

Store below 5 °C for no more than 6 months.

**3.11.3 Amino acids calibration solution.**

**3.11.3.1 Weight dilution.** Weigh 2,5ml of each amino acid stock solution  $m_{\text{aa}}$  (3.11.2) and 2,5 ml of norleucine stock solution  $m_{\text{Nle}}$  (3.11.1) into a 50 ml volumetric flask. Make up to volume with sodium citrate buffer (3.9).

Store the solution below 5 °C for not more than 1 week.

**3.11.3.2 Volume dilution.** Using a pipette (4.4) or dilutor (4.7), dilute equal volumes of amino acid stock solution (3.11.2) and norleucine solution (3.11.1) with sodium citrate buffer (3.9), e.g. 50 µl norleucine solution and 50 µl amino acid stock solution are diluted by the dilutor to 1 000 µl with sodium citrate buffer (3.9).

**3.12 Elution buffers for cation exchanger column.** Use commercially available buffers or prepare them according to the requirements specified by the analyser manufacturer. Typically three to five

buffer solutions are used containing sodium citrate or carbonate and small quantities of additives and preservatives.

**3.13 Ninhydrin or OPA reagent.** Use commercially available reagents or prepare them according to the requirements specified by the analyser manufacturer.

## 4 Apparatus

Usual laboratory apparatus and in particular the following.

- 4.1 **Glass beakers**, of capacity 1 000 ml.
- 4.2 **Volumetric flask**, of volume 50 ml, 100 ml, 500 ml, 1 000 ml.
- 4.3 **Graduated measuring cylinders**, of volume 100 ml, 1 000 ml.
- 4.4 **Graduated pipettes**, of volume 5 ml and 10 ml.
- 4.5 **Magnetic stirring plates or mechanical shaker.**
- 4.6 **Membrane filters** 0,2 µm consisting of cellulose acetate or PVDF.
- 4.7 **Dilutor**, optional for volumetric dilution.

If derivatization is done with ninhydrin, use a volume dilution ratio of 1→20. Use a higher ratio for OPA derivatization. Check the coefficient of variation (CV) of the dilution regularly with a balance; the CV shall be less than 1 %.

- 4.8 **pH meter.**
- 4.9 **Analytical balance**, readability 0,1 mg.
- 4.10 **Amino acid analyser** or equivalent **high performance liquid chromatography (HPLC)** equipment.
  - 4.10.1 **Cation exchange resin column** placed in an oven.
  - 4.10.2 **Guard column.**
  - 4.10.3 **Automatic or manual injection system**, able to inject volumes from 10 µl to 100 µl.
  - 4.10.4 **HPLC pumps** for buffers.
  - 4.10.5 **Ninhydrin or OPA post- column derivatization pump.**
  - 4.10.6 **Channel UV detector** set at 440 nm and 570 nm for ninhydrin post-column reaction or **fluorescence detector** set at excitation wavelength 330 nm and emission wavelength 460 nm for OPA post-column reaction.
  - 4.10.7 **Data acquisition and handling system** for integration of peaks.