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

*Aliments des animaux — Détermination de la teneur en lysine,
méthionine et thréonine dans les acides aminés industriels et les pré-
mélanges*





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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.

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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 P₂O₅ prior to use.

3.2.2 Threonine crystals, purity superior to 99 % mass fraction dried under vacuum in a desiccator for 2 days over P₂O₅ prior to use.

3.2.3 Methionine crystals, purity superior to 99 % mass fraction dried under vacuum in a desiccator for 2 days over P₂O₅ 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 P₂O₅ 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.