SLOVENSKI STANDARD
oSIST prEN 17294:2018

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Krma: metode vzorčenja in analize - Določevanje organskih kislin z ionsko kromatografijo in detekcijo na osnovi prevodnosti (IC-CD)

Animal feeding stuffs: Methods of sampling and analysis - Determination of organic acids by Ion Chromatography with Conductivity Detection (IC-CD)

Futtermittel - Probenahme- und Untersuchungsverfahren - Bestimmung organischer Säuren mittels Ionenchromatographie mit Leitfähigkeitsdetektion (IC-CD)

Aliments des animaux: Méthodes d’échantillonnage et d’analyse - Dosage des acides organiques par chromatographie ionique avec détection conductimétrique (CI-DC)

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

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European foreword

This document (prEN 17294:2018) 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.

This document is currently submitted to the CEN Enquiry.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

Introduction

Organic acids and their salts such as citric acid, formic acid, lactic acid, acetic acid, propionic acid, fumaric acid, benzoic acid and sorbic acid are animal feed additives which play an important role in the animal feeding by improving the animals' performance and decrease the development of (pathogenic) microorganisms in the intestine especially in the pig production. Concerning the feed legislation the substances can be used for different purposes depending on its functions and properties. According to their functional principle or their function, the relevant organic acid could be allocated within one or more of the functional groups mentioned in Annex I of Regulation (EC) no. 1831/2003 as preservative, acidity regulators, flavouring compounds, silage additives or other zoo-technical additives.

1 Scope

This document specifies a method for the determination of organic acids in animal feeding stuffs by Ion Chromatography with conductivity detection (IC-CD).

The method is intended to be used for the determination of formic acid, lactic acid, propionic acid, citric acid, fumaric acid and malic acid as active substances in feed additives, premixtures, compound feed and water and for screening of acetic acid in the same matrices. This method determines the total extractable concentration of the above mentioned organic acids and their salts.

It is advisable that the user of this standar determine the working range of the method for each organic acid. The lower limit of the working range depends on the matrix and the interferences encountered. A working range between 10 [mg/l] and 100 [mg/l] should be achievable.

The method was successfully tested in an inter-laboratory study in concentrations between 0,02 % up to 27 % of the above mentioned organic acids.

NOTE Limitation occurs during simultaneous determination of high concentration of lactic acid and low concentration of acetic acid. If the ratio of concentration of lactic acid to acetic acid exceeds factor 20, the determination of acetic acid is not guaranteed.

On the basis of the referred working range, sample weight and extraction volume, limits of quantification (LOQ), as calculated (Table 1) should be achievable.

Table 1 — Limits of quantification (LOQ)

Organic acid	LOQ [mg/kg]
Formic acid	200
Lactic acid	200
Propionic acid	200
Citric acid	200
Fumaric acid	200
Acetic acid	200
Malic acid	200

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.

EN ISO 3696:1995, *Water for analytical laboratory use - Specification and test methods (ISO 3696:1987)*

EN ISO 6498, *Animal feeding stuffs - Guidelines for sample preparation (ISO 6498)*

EN ISO 10304-1, *Water quality - Determination of dissolved anions by liquid chromatography of ions - Part 1: Determination of bromide, chloride, fluoride, nitrate, nitrite, phosphate and sulfate (ISO 10304-1)*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at http://www.electropedia.org/
- ISO Online browsing platform: available at http://www.iso.org/obp

3.1
feed additives
substances, micro-organisms or preparations, other than feed material and premixtures, which are intentionally added to feed or water

[SOURCE: Regulation (EC) No 1831/2003/Article 2/2 a [1]]

3.2
animal feeding stuffs
any substance or product,including additives, whether processed, partially processed or unprocessed, intended to be used for oral feeding to animals

[SOURCE: Regulation (EC) No 178/2002/Article 2/4 [2]]

4 Principle

The sample is extracted with water. The extract is filtrated or centrifuged and – if necessary – diluted. The amount of organic acids extracted from the sample is determined with ion chromatography (IC) in conjunction with conductivity detection (CD) using external calibration.

When using CD it is essential that the eluent shows a sufficiently low conductivity. For this reason, CD is usually combined with a suppressor device (cation exchanger), which will reduce the conductivity of the eluent and transform the sample acids into their respective salts.

The method description follows a proven approach where the chromatographic resolution *R* has to be checked to ensure that it complies with the required separation conditions in accordance with EN ISO 10304-1.

WARNING — The use of this European Standard can involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this European Standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

5 Reagents

5.1 General

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

5.2 Water

Complying with grade 1 in accordance with EN ISO 3696:1995.

5.3 Formic acids, lactic acid, propionic acid, citric acid, acetic acid, malic acid standard solution, c = 1000 [mg/l]

Single acids standard solutions with adequate and required specification are commercially available (ready-to-use solutions).

5.4 Alternative preparation of stock solution based single standard substances

NOTE Differntiation between the enantiometers (D, L, DL) are not of interest.

5.4.1 Citric acid, minimum 99 % purity

5.4.2 Malic acid, minimum 99 % purity

5.4.3 Formic Acid, minimum 98 % purity

5.4.4 Lactic acid, minimum 85 % purity

NOTE Lithium Lactate, minimum 95 % purity, can also be used.

5.4.5 Acetic acid, minimum 99 % purity

5.4.6 Fumaric acid, minimum 99 % purity

5.4.7 Propionic acid, minimum 99 % purity

5.4.8 Single standard stock solutions, app. 2500 [mg/l]

Weigh 250 mg acid (5.4.1 to 5.4.7) each into a 100 ml volumetric flask. Dissolve with approximately 80 ml water (5.2), mix and fill up to the mark with water (5.2).

For fumaric acid weigh 250 mg (5.4.6) into a 100 ml volumetric flask. Add 80 ml water (5.2) and dissolve 10 min in an ultrasonic bath at 60 °C. After cooling to room temperature fill up to the mark with water.

The maximum storage time is 6 month at 4 °C.

NOTE Addition of 1 % Isopropanol has a positive effect on the stability of this solution.

Determine the exact concentration of the stock solution using the reference standard purity value provided by the supplier:

$$C_s = \frac{m \times P}{V} \quad (1)$$

where

C_S is the experimental concentration of the organic acid in the standard stock solution, in mg/ml;

P is the purity of the organic acid standard given by the supplier in percent divided by 100, e.g. 0,98;

m is the weighed mass of the organic acid, in mg;

V is the volume of the volumetric flask, in ml.

5.5 Mixed standard solution, (app.) 100 [mg/l]

Accurately pipette 4,0 ml of each single standard stock solution (5.4.8) into a 100 ml volumetric flask and fill up to the mark with water (5.2). The exact concentration of each organic acid has to be calculated according to the real concentration of the single standard stock solutions.

In the case of ready-to-use solutions (5.3) pipette 10,0 ml each into a 100 ml volumetric flask and fill up to the mark with water (5.2).

The maximum storage time is 2 month at 4 °C.

5.6 IC Mobile phase

Degas all water used for eluent preparation.

The choice of eluent depends on the chosen column and detector (examples in Table A.1). The chosen combination of ion-chromatography column (IC-column) and eluent shall meet the resolution requirements stated in 6.11.

A selection of reagents for common eluents is given in Annex A.

6 Apparatus

6.1 General

Usual laboratory apparatus, in particular, the following.

6.2 Laboratory grinder

Laboratory grinder capable of grinding to a particle size of less than or equal to 1,0 mm.

6.3 Analytical balance

Capable of weighing to an accurancy of 0,1 mg.

6.4 Magnteic stirrer

With Polytetrafluoroethylene (PTFE)-coated stirring bar.

6.5 Ultrasonic bath

6.6 Pipettes (electronic or manual)

In the range 100 µl to 5000 µl.

6.7 Centrifuge, approx. 5000 × *g*

6.8 Folded filter, pore size 4-7 µm, (ash free paper filter)

0,45 µm (Ready-to-use filter unit with a hydrophilic, low protein-binding membrane made of regenerated cellulose)

6.9 Membrane filter, for HPLC use

6.10 Reversed phase solid phase extract (RP SPE)

Optional for protecting the IC column, e.g. OnGuard II RP (Dionex).

6.11 Ion chromatopgraph (pump, autosampler) with suppressed conductivity detection

6.11.1 IC column, with specified separation performance (6.11)

6.11.2 Precolumn

6.12 Quality requirements for the separator column

In chromatograms of samples and standard solutions (see Figure 1), the peak resolution, R, between the acid of interest and its nearest peak, shall not fall below 1.3 [see Formula (1) or Formula (2) and Figure 2].

Separation conditions shall be such that possible interfering organic acids or substances will not interfere with the organic acids of interest.

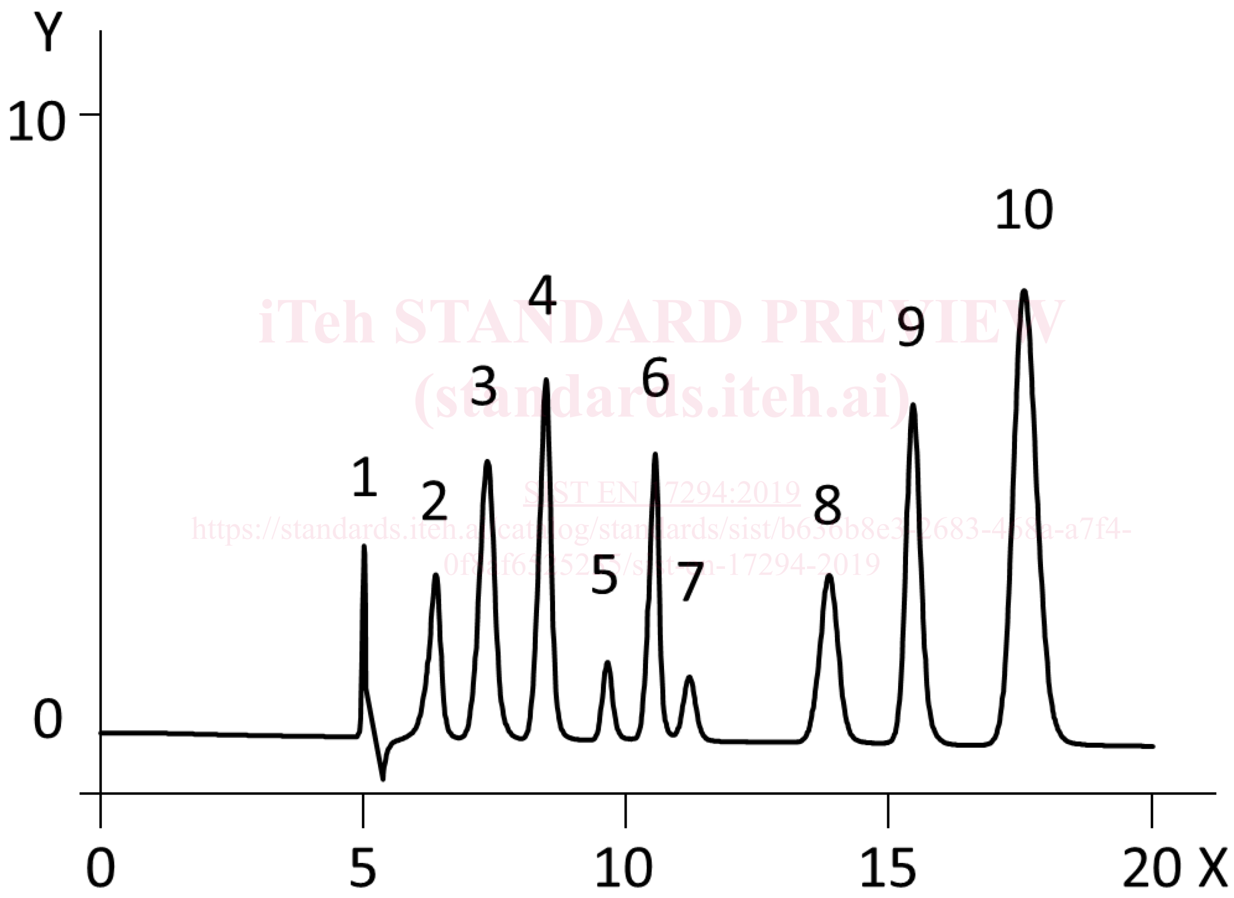

Figure 1 — Example chromatogram of organic acids with sufficient peak resolution