SLOVENSKI STANDARD
SIST EN 17294:2019

01-oktober-2019

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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65.120	Krmila	Animal feeding stuffs
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English Version

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

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Contents

Page

European foreword

This document (EN 17294:2019) 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 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of an identical text or by endorsement, at the latest by February 2020, and conflicting national standards shall be withdrawn at the latest by Feburary 2020.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

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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 animal feeding by improving the animals' performance and decreasing 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, feed materials, compound feed and water and for acetic acid in a limited manner 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 standard determines 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. It is advisable that a working range between 10 mg/l and 100 mg/l is 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) can 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, *Water for analytical laboratory use — Specification and test methods (ISO 3696)*

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 filtered 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 shall 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

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

5.1 Water, grade 1 in accordance with EN ISO 3696

5.2 Formic acids, lactic acid, propionic acid, citric acid, acetic acid, malic acid standard solution, c = 1 000 [mg/l]

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

5.3 Alternative preparation of stock solution based single standard substances

NOTE Differentiation between the enantiomers (D, L, DL) is not of interest.

5.3.1 Citric acid, minimum 99 % purity.

5.3.2 Malic acid, minimum 99 % purity.

5.3.3 Formic Acid, minimum 98 % purity.

5.3.4 Lactic acid, minimum 85 % purity.

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

5.3.5 Acetic acid, minimum 99 % purity.

5.3.6 Fumaric acid, minimum 99 % purity.

5.3.7 Propionic acid, minimum 99 % purity.

5.3.8 Single standard stock solutions, app. 2 500 [mg/l].

Weigh 250 mg acid (5.3.1 to 5.3.7) each into a 100 ml volumetric flask. Dissolve with approximately 80 ml water (5.1), mix and fill up to the mark with water (5.1).

For fumaric acid weigh 250 mg (5.3.6) into a 100 ml volumetric flask. Add 80 ml water (5.1) 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 according to Formula (1):

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

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;

NOTE For example 0,98.

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

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

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

Accurately pipette 4,0 ml of each single standard stock solution (5.3.8) into a 100 ml volumetric flask and fill up to the mark with water (5.1). The exact concentration of each organic acid shall be calculated according to the real concentration of the single standard stock solutions.

In the case of ready-to-use solutions (5.2) pipette 10,0 ml each into a 100 ml volumetric flask and fill up to the mark with water (5.1).

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

5.5 IC Mobile phase

Degas all water used for eluent preparation.

The choice of eluent depends on the chosen column and detector (examples in Annex B, Table B.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 B.

6 Apparatus

Usual laboratory apparatus, in particular, the following.

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

6.2 Analytical balance, suitable to accurately weigh between 0 g and 10 g with an accuracy of 0,1 mg.

6.3 Magnteic stirrer, with Polytetrafluoroethylene (PTFE)-coated stirring bar.

6.4 Ultrasonic bath

6.5 Pipettes (electronic or manual), in the range 100 µl to 5 000 µl.

6.6 Centrifuge, approx. 5 000 × *g*.

6.7 Folded filter, pore size 4 µm to 7 µm (ash free paper filter).

6.8 Membrane filter, for HPLC use, 0,45 µm (for example Ready-to-use filter unit with a hydrophilic, low protein-binding membrane made of regenerated cellulose).

6.9 Reversed phase solid phase extract (RP SPE)

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

6.10 Ion chromatograph (pump, autosampler) with suppressed conductivity detection

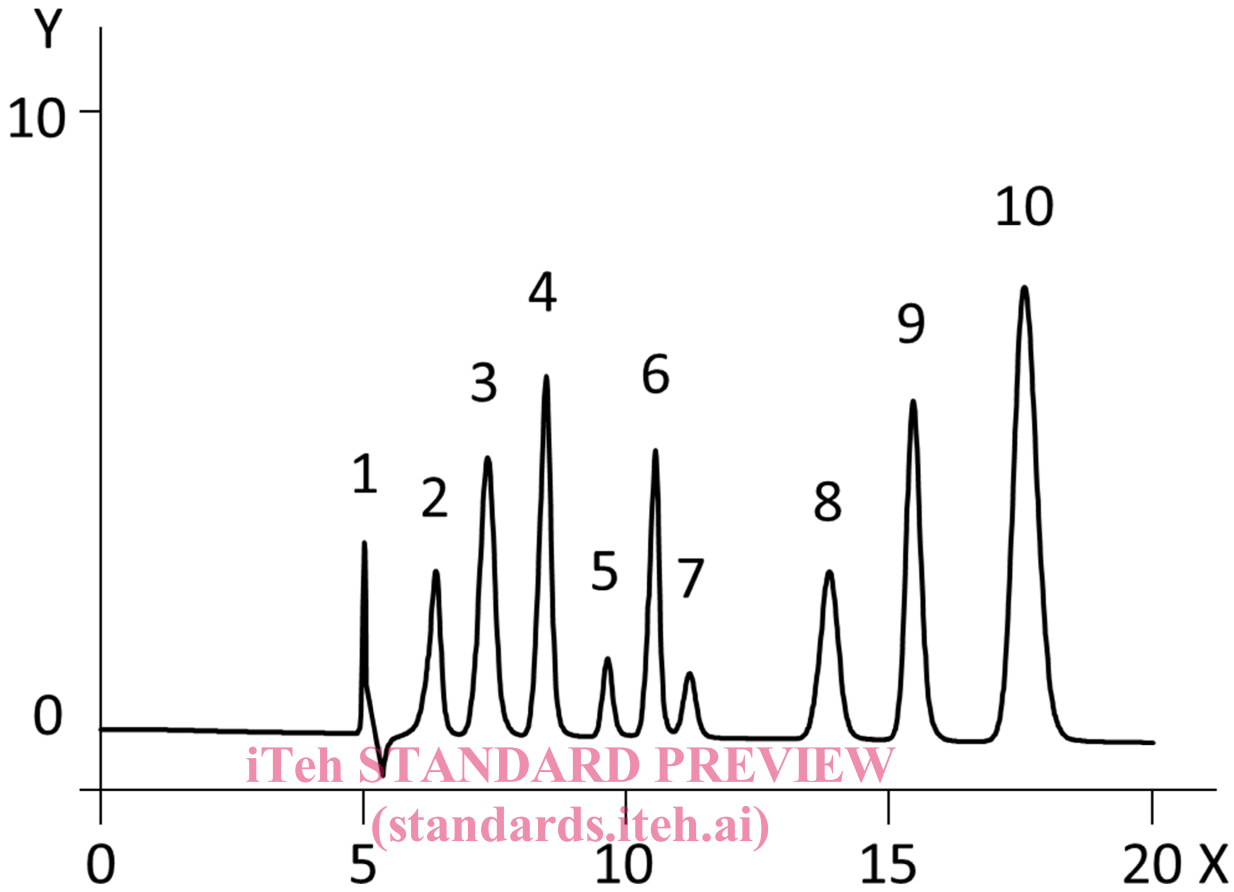
6.10.1 IC column, with specified separation performance (6.11).

6.10.2 Precolumn

6.11 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 (2) or Formula (3) and Figure 2].

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

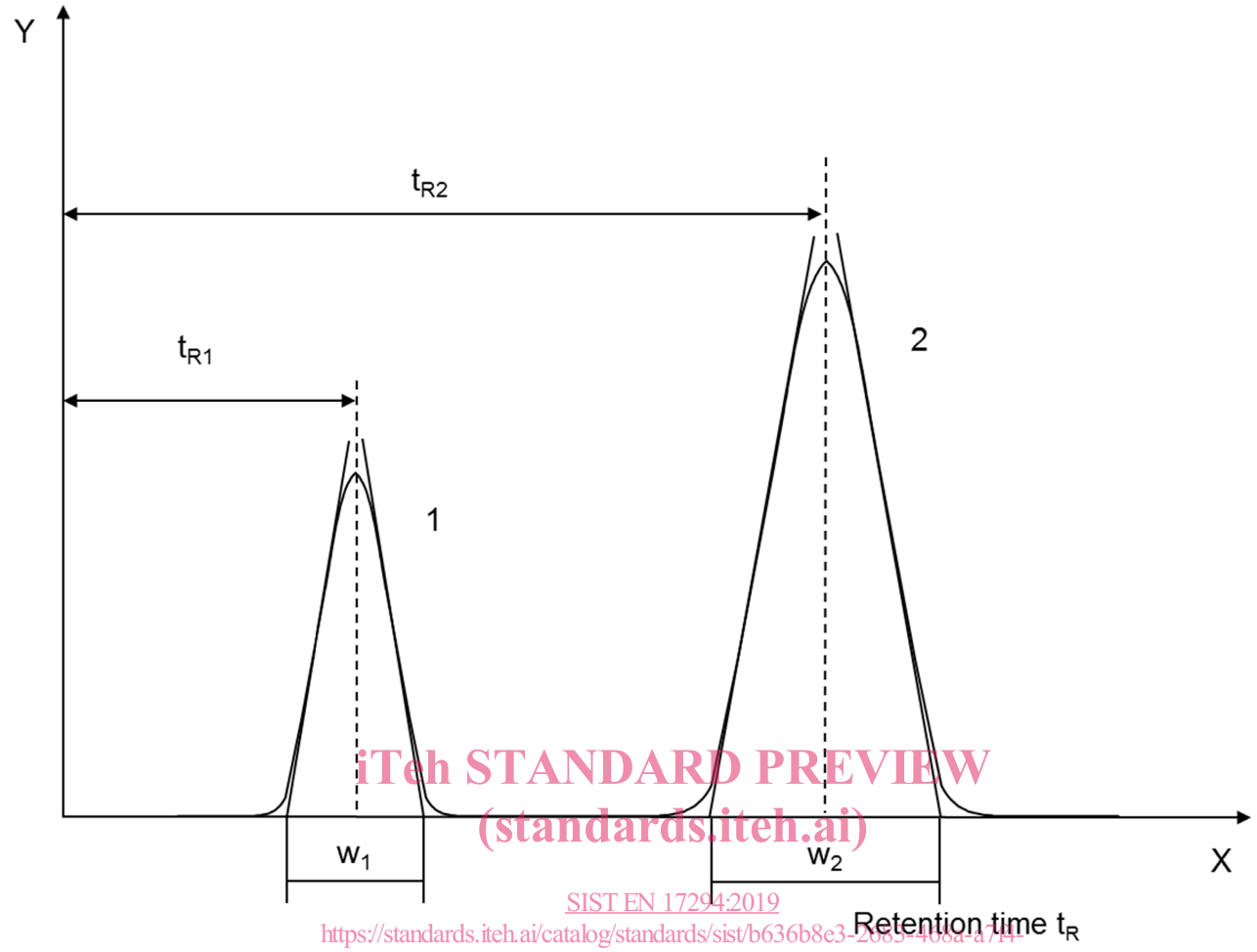


Key

X = retention time t_R (s) 1 to 10 = peak 1 to 10

Y = signal

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



Key

X	retention time t_R (s)	1	peak 1
Y	signal	2	peak 2
W	peak width (s)		

Figure 2 — Graphical representation of the parameters to calculate the peak resolution *R*

Base peak widths, w_1 and w_2, are obtained by constructing isosceles triangles over the Gaussian peaks.

Calculate the peak resolution R using Formula (2).

$$R_{2,1} = \frac{2 \times (t_{R2} - t_{R1})}{w_2 + w_1} \quad (2)$$

where

$R_{2,1}$	is the peak resolution;
t_{R1}	is the retention time of the first peak, in s;
t_{R2}	is the retention time of the second peak, in s;
W_1	is the peak width on the time axis of the first peak, in s;
W_2	is the peak width on the time axis of the second peak, in s.

NOTE Mostly the chromatography software can calculated R by itself.