SLOVENSKI STANDARD
oSIST prEN 17298:2018

01-oktober-2018

Krma: metode vzorčenja in analize - Določevanje benzojske in sorbinske kisline s tekočinsko kromatografijo visoke ločljivosti (HPLC)

Animal feeding stuffs: Methods of sampling and analysis - Determination of benzoic and sorbic acid by High Pressure Liquid Chromatography (HPLC)

Futtermittel - Probenahme- und Untersuchungsverfahren - Bestimmung von Benzoesäure und Sorbinsäure mittels Hochleistungs-Flüssigchromatographie (HPLC)

Aliments des animaux: Méthodes d'échantillonnage et d'analyse - Dosage de l'acide benzoïque et de l'acide sorbique par chromatographie liquide à haute pression (CLHP)

Ta slovenski standard je istoveten z: **prEN 17298**

ICS:

65.120	Krmila	Animal feeding stuffs
71.040.40	Kemijska analiza	Chemical analysis

oSIST prEN 17298:2018 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

DRAFT
prEN 17298

August 2018

ICS 65.120; 71.040.40

English Version

Animal feeding stuffs: Methods of sampling and analysis - Determination of benzoic and sorbic acid by High Pressure Liquid Chromatography (HPLC)

This draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 327.

If this draft becomes a European Standard, CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration.

This draft European Standard was established by CEN in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.

Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

Warning : This document is not a European Standard. It is distributed for review and comments. It is subject to change without notice and shall not be referred to as a European Standard.

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

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

Contents

European foreword

This document (prEN 17298:2018) 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 benzoic acid and sorbic acid in animal feeding stuffs by high-performance liquid chromatography method with ultraviolet detection (HPLC-UV).

The method is intended to be used for the determination of benzoic acid and sorbic acid as active substances in feed additives, premixtures and compound feed and for benzoic acid in water. This method determines the total extractable concentration of these organic acids and their salts.

It is advisable that the working range of the method is determined for each organic acid by the user of this standard. The lower limit of the working range depends on the matrix and the interferences encountered. It is advisable that a working range between 5 mg/l and 100 mg/l is accessible.

The method was successfully tested in an inter-laboratory study in concentrations between 0,02 % up to 9,0 %.

On the basis of the referred working range, sample weigh and extraction volume, limits of quantification (LOQ), as calculated (Table 1) on the basis of a wavelength of 230 nm, can be achievable.

Table 1 — Limits of quantification (LOQ) at 230 nm

Organic acid	LOQ (mg/kg)
Benzoic acid	200
Sorbic 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)*

3 Term 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 a mixture of sodium acetate buffer and methanol. The extract is filtrated or centrifuged and – if necessary – diluted. The amount of organic acids extracted from the sample is determined with high-performance liquid chromatography (HPLC), applying a RP 18 column, in conjunction with diode array detection (DAD) or ultraviolet detection (UV). The quantification is carried out by an external calibration.

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 Water, complying with grade 2 in accordance with EN ISO 3696:1995

5.2 Benzoic acid,p.a., minimum 99 % purity

5.3 Sorbic acid, p.a., minimum 99 % purity

5.4 Acetic acid, p.a., ω = 100 %

5.5 Methanol, gradient grade, ω ≥ 99,8 %

5.6 Sodium acetate trihydrate, p.a

5.7 Sodium acetate solution, c = 1 mol/l

Dissolve 13,6 g sodium acetate (5.6) with approximately 80 ml water (5.1) in a 100 ml volumetric flask, mix and fill up to the mark with water (5.1).

The maximum storage time is 3 months at room temperature.

5.8 Acetic acid solution, c = 1 mol/l

Dissolve 6,00 g acetic acid (5.4) with approximately 80 ml Water (5.1) in a 100 ml volumetric flask, mix and fill up to the mark with water (5.1).

The maximum storage time is 3 months at room temperature.

5.9 Acetate buffer, c = 0,1 mol/l

By means of pipette add 50,0 ml sodium acetate solution (5.7) and 50,0 ml acetic acid solution (5.8) in a 1000 ml volumetric flask and add approximately 700 ml water (5.1). Adjust the pH with acetic acid solution (5.8) to 4,6. Fill up to the mark with water (5.1).

The maximum storage time is 6 months at room temperature.

NOTE A pH of 4,6 is the best range for an optimal HPLC separation regarding the referred conditions.

5.10 HPLC Mobile phase

Mix acetate buffer (5.9) and methanol (5.5) in proportion of 75:25 (v/v) by means of graduated cylinder. Filter for HPLC use.

The maximum storage time is 6 months at room temperature.

5.11 Extraction solution

Mix acetate buffer (5.9) and methanol (5.5) in proportion of 60:40 (v/v) by means of graduated cylinder.

The maximum storage time is 6 months at room temperature.

5.12 Standard stock solution, app. 500 mg/l

Weigh 125 mg benzoic acid (5.2) and 125 mg sorbic acid (5.3) into the same 250 ml volumetric flask. Dissolve with approximately 150 ml extraction solution (5.11), dissolve 5 min in an ultrasonic bath and fill to the mark with extraction solution (5.11).

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

NOTE 1 The shelf life of the stock solution is limited because of esterification reactions.

NOTE 2 If available, single acid standard solutions with adequate and required specifications can be used.

EXAMPLE Example of dilution:

Determine the actual concentration of the stock solution using the reference standard purity value provided by the supplier according to Fomula (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 divided by 100, in %;

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.

6 Apparatus

Usual laboratory equipment and, 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 weigh to an accurancy of 0,1 mg

6.3 Ultrasonic bath

6.4 pH meter capable of being read to at least one place of decimals

6.5 Pipettes (electronic or manual) in the range 100 µl to 5000 µl

6.6 Centrifuge, approx. 5000 × g

6.7 Membrane filter, for HPLC use

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

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 HPLC system (pump, autosampler, column oven) with DAD/UV detector

6.10 HPLC column, RP 18 column, e.g. Nucleosil 120 C18, 5 µm, 250x4,0 mm

6.11 Precolumn (optional)

The use of a precolumn is recommended for protecting the analytical HPLC column.

7 Sampling

It is important that the laboratory receive a sample which is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this European Standard. A recommended sampling method is given in EN ISO 6497 [3].

Store the sample in such a way that deterioration and change in its composition are prevented.

8 Preparation of test sample

Prepare the test sample in accordance with EN ISO 6498.

Grinding (≤1 mm) shall be done in conditions such that the substance is not appreciably heated.

The whole ground product is placed in a flask made of e.g. polypropylene, which can be stoppered and stored in such way to prevent any change in composition.

Before any weighing is carried out for the analysis, the whole test sample shall be thoroughly mixed for reasons of homogeneity.

9 Procedure

9.1 Extraction

Accurately weigh 5,00 g ± 10 mg of prepared sample into a 250 ml conical flask. Add 100 ml extraction solution (5.11), mix well and dissolve the sample for 30 min in an ultrasonic bath (6.3).

NOTE 1 Avoid temperature above 50°C because of evaporation of extraction solution.

NOTE 2 For high concentrated homogenous samples (premixtures) sample weigh can be reduced because of the limited solubility of sorbic acid.

Let the particles settle down. Filter through folded filter (6.8), discarding the first 3 ml of the filtrate, and collect the rest. Alternatively centrifuge the sample extract for 3 min (appr. 5000 × g).

Dilute the filtered/centrifuged solution with extraction solution (5.11) to a final concentration according to the working range of the calibration (5 - 100 µg/ml) – see below for a dilution example.

Before HPLC analysis filter through a 0,45 µm membrane filter (6.7) discarding the first drops of the filtrate and collect the rest.