SLOVENSKI STANDARD
SIST-TS CEN/TS 15754:2008

01-december-2008

**?fa U!˙8c`c Yj Ub^Y˙j gYVbcgh]˙g`UX_cf^U!˙?fca Uhc[fUZg_Ua YhcXUn˙j]gc_c
`c ˙^]j cgh^c˙fk D5 97!D58Ł**

Animal feeding stuffs - Determination of sugar content - High performance exchange chromatographic method (HPAEC-PAD)

Futtermittel - Bestimmung des Zuckergehalts - Hochleistungs-Anionenaustausch-Chromatographieverfahren (HPAEC-PAD)

iTeh STANDARD PREVIEW
(standards.iteh.ai)

Aliments des animaux - Détermination de la teneur en sucre - Chromatographie d'échange d'anions haute performance couplée à la détection par ampérométrie pulsée (HPAEC-PAD)

SIST-TS CEN/TS 15754:2008
https://standards.iteh.ai/catalog/standards/sist/101286d0-cb35-443e-b508-288fa96915d9/sist-ts-cen-ts-15754-2008

Ta slovenski standard je istoveten z: **CEN/TS 15754:2008**

ICS:

65.120	Krmila	Animal feeding stuffs

SIST-TS CEN/TS 15754:2008 **en,fr,de**

2003-01.Slovenski inštitut za standardizacijo. Razmnoževanje celote ali delov tega standarda ni dovoljeno.

SIST-TS CEN/TS 15754:2008

iTeh STANDARD PREVIEW
(standards.iteh.ai)

SIST-TS CEN/TS 15754:2008
https://standards.iteh.ai/catalog/standards/sist/101286d0-cb35-443e-b508-288fa96915d9/sist-ts-cen-ts-15754-2008

SIST-TS CEN/TS 15754:2008

TECHNICAL SPECIFICATION

SPÉCIFICATION TECHNIQUE

TECHNISCHE SPEZIFIKATION

CEN/TS 15754

September 2008

ICS 65.120

English Version

Animal feeding stuffs - Determination of sugar content - High performance exchange chromatographic method (HPAEC-PAD)

Aliments des animaux - Détermination de la teneur en sucre - Chromatographie d'échange d'anions haute performance couplée à la détection par ampérométrie pulsée (HPAEC-PAD)

Futtermittel - Bestimmung des Zuckergehalts - Hochleistungs-Anionenaustausch-Chromatographieverfahren (HPAEC-PAD)

This Technical Specification (CEN/TS) was approved by CEN on 17 May 2008 for provisional application.

The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

CEN members are required to announce the existence of this CEN/TS in the same way as for an EN and to make the CEN/TS available promptly at national level in an appropriate form. It is permissible to keep conflicting national standards in force (in parallel to the CEN/TS) until the final decision about the possible conversion of the CEN/TS into an EN is reached.

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

iTeh STANDARD PREVIEW
(standards.iteh.ai)
SIST-TS CEN/TS 15754:2008
https://standards.iteh.ai/catalog/standards/sist/101286d0-cb35-443e-b508-288fa96915d9/sist-ts-cen-ts-15754-2008

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

Management Centre: rue de Stassart, 36 B-1050 Brussels

© 2008 CEN All rights of exploitation in any form and by any means reserved worldwide for CEN national Members.

Ref. No. CEN/TS 15754:2008: E

SIST-TS CEN/TS 15754:2008

Contents

Page

Foreword ... 3
Introduction ... 4
1 Scope ... 5
2 Normative references ... 5
3 Terms and definitions ... 5
4 Principle ... 5
5 Reagents ... 5
6 Apparatus ... 7
7 Sampling ... 8
8 Preparation of the test sample ... 8
9 Procedure ... 8
10 Calculation and expression of the results ... 11
11 Precision ... 12
12 Test report ... 12
Annex A (informative) Results of an interlaboratory test for each individual sugar ... 13
Bibliography ... 20

iTeh STANDARD PREVIEW
(standards.iteh.ai)
SIST-TS CEN/TS 15754:2008
https://standards.iteh.ai/catalog/standards/sist/101286d0-cb35-443e-b508-288fa96915d9/sist-ts-cen-ts-15754-2008

SIST-TS CEN/TS 15754:2008

Foreword

This document (CEN/TS 15754:2008) 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.

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

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association, and supports essential requirements of EU Directive(s).

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to announce this CEN Technical Specification: Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

SIST-TS CEN/TS 15754:2008
https://standards.iteh.ai/catalog/standards/sist/101286d0-cb35-443e-b508-288fa96915d9/sist-ts-cen-ts-15754-2008

SIST-TS CEN/TS 15754:2008

Introduction

This Technical Specification describes an application of the High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) for the determination of different individual sugars, both mono- and disaccharides, in dry animal feeding stuffs.

HPAEC-PAD is a modern liquid chromatographic technique with high selectivity and sensitivity for especially carbohydrates. Due to this high selectivity and sensitivity of HPAEC-PAD for carbohydrates just a minimum of sample clean-up is required in most carbohydrate applications in food and feed matrices.

This technical specification is meant as an introduction of a new method for the determination of the content of the individual sugars present in animal feeding stuffs. The document should be used to get experienced with this new powerful technique and with the described method for the quantification of the individual sugars in animal feeding stuffs.

Currently the HPAEC-PAD equipment is mainly used in research environments and not wide spread in use in the feed and control laboratories. Therefore, the method is at this time not yet suitable for official control applications of the sugar content in feeding stuffs. It is however expected that within several years more and more laboratories in the field will have this instrumentation at their disposal.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

SIST-TS CEN/TS 15754:2008
https://standards.iteh.ai/catalog/standards/sist/101286d0-cb35-443e-b508-288fa96915d9/sist-ts-cen-ts-15754-2008

SIST-TS CEN/TS 15754:2008

1 Scope

This Technical Specification describes the quantitative determination of specific sugars (glucose, fructose, galactose, sucrose, maltose, and lactose) in dry animal feeding stuffs at the g/kg level by a sophisticated high performance anion exchange chromatography in combination with pulsed amperometric detection (HPAEC-PAD).

2 Normative references

The following referenced documents are indispensable for the application 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 3696:1987)*

ISO 6498:1998, *Animal feeding stuffs — Preparation of test samples*

3 Terms and definitions

iTeh STANDARD PREVIEW
(standards.iteh.ai)

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

SIST-TS CEN/TS 15754:2008
https://standards.iteh.ai/catalog/standards/sist/101286d0-cb35-443e-b508-288fa9b9f5d9/sist-ts-cen-ts-15754-2008

3.1
total sugar content
sum of the content of the individual sugars, glucose, fructose, galactose, maltose, sucrose and lactose, expressed as g/kg in the sample as such

4 Principle

The sugars present in the test potion of the animal feeding stuff sample are extracted with water. The clean-up of the aqueous extract is done by either a C-18 SPE procedure, or by a deproteination with acetonitrile. After the clean-up, the sample solution is diluted and the sugars present are separated by high performance anion exchange chromatography and detected by pulsed amperometric detection. Quantitation of the sugars present is done by comparison of the peak areas and/or heights with those of an external calibration graph prepared with standard solutions.

5 Reagents

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

SIST-TS CEN/TS 15754:2008

5.1 Water, complying with EN ISO 3696, grade 3.

5.2 Acetonitrile

5.3 Dimethyl Sulfoxide (DMSO)

5.4 Sodium hydroxide solution, mass fraction, *w*(NaOH) = 50 % in water

The reagent should contain the minimum amount of carbonate and mercury. Do not shake or stir the solution before use.

5.5 Sodium acetate trihydrate ($CH_3COONa{\cdot}3H_2O$)

5.6 Eluent 1 (E1), aqueous solution of 500 mmol/l sodium hydroxide (NaOH)

Add to a 1 000 ml volumetric flask about 800 ml degassed water (eluent 3, 5.8) followed by 40,0 g of sodium hydroxide 50 % (m/m) (5.4). Then dilute the aqueous solution to the mark with degassed demineralised water (5.8).

5.7 Eluent 2 (E2), aqueous solution of 500 mmol/l sodium acetate

Add to a 1 000 ml volumetric flask about 800 ml degassed demineralised water (eluent 3, 5.8) followed by 68,0 g sodium acetate trihydrate ($CH_3COONa{\cdot}3H_2O$) (5.5). Then dilute the aqueous solution to the mark with degassed demineralised water (5.8).

iTeh STANDARD PREVIEW
(standards.iteh.ai)

5.8 Eluent 3 (E3), degassed demineralized water

Filter the demineralized water through a 0,2 membrane filter. Degas by sparging with helium for at least 30 min.

SIST-TS CEN/TS 15754:2008
https://standards.iteh.ai/catalog/standards/sist/101286d0-cb35-443e-b508-288fa96915d9/sist-ts-cen-ts-15754-2008

5.9 Sugar standard solutions

5.9.1 Preparation stock solution

Prepare daily fresh aqueous solutions of glucose, fructose, galactose, maltose, sucrose, and lactose. Weigh approximately 40 mg of each sugar to the nearest 0,1 mg into separate 100 ml volumetric flasks (6.6) and dilute to the mark with water (stock standard solutions of 400 mg/l).

NOTE 1 Mixed standard solutions can also be prepared once the retention time of the individual sugars is known under the prevailing chromatographic conditions.

NOTE 2 The standard solutions can be further diluted to reach sugar concentrations similar to those found in the sample solutions.

5.9.2 Working standard solutions for calibration

Prepare dilutions of the sugar standard solutions as specified in Table 1. Dilute 0,25 ml of each obtained standard solution with 1,25 ml DMSO (5.3) in an HPLC vial. Close the vial and mix well.

SIST-TS CEN/TS 15754:2008

Table 1 — Preparation aqueous DMSO sugar standard solutions

Standard solution calibration graph	Volume sugar standard stock solution ml	Volume water ml	Sugar concentration after dilution with DMSO µg/ml[a]
Standard 1	0,00	10,00	0
Standard 2	0,10	9,90	0,67
Standard 3	0,30	9,70	2,00
Standard 4	0,70	9,30	4,67
Standard 5	1,40	8,60	9,33
Standard 6	2,00	8,00	13,33

[a] Based upon a sugar content of exactly 400 mg/l in the aqueous stock solution.

These aqueous DMSO sugar standard solutions will be used for preparing the calibration graphs.

iTeh STANDARD PREVIEW

NOTE Aqueous DMSO is a good solvent for carbohydrates and the use of DMSO prevent microbial deterioration of the carbohydrates in the solutions.

(standards.iteh.ai)

6 Apparatus

SIST-TS CEN/TS 15754:2008
https://standards.iteh.ai/catalog/standards/sist/101286d0-cb35-443e-b508-288fa96915d9/sist-ts-cen-ts-15754-2008

Usual laboratory equipment and, in particular the following:

6.1 Analytical balance, capable of weighting to an accuracy of ± 0,1 mg.

6.2 Glass centrifugation tubes, of a capacity of 50 ml.

6.3 Homogenizer[1]

6.4 Swing-out centrifuge, adjusted to a centrifugation force of about 2 800 g.

6.5 Screw cap closed centrifugation tubes, with screw caps with a polytetrafluoroethylene (PTFE) inlay, of a capacity of about 10 ml.

6.6 One mark volumetric flasks, of a capacity of 100 ml and 1 000 ml.

6.7 Dispensors, resistant to organic solvents and adjusted to 2,5 ml and 20 ml.

6.8 Disposable C-18 SPE cartridges, to be used according to the manufacturer’s recommendations.

[1] An IKA Ultra turrax with apprropriate probe is an example of a suitable commercially available homogenizer.

SIST-TS CEN/TS 15754:2008

6.9 Metal free liquid chromatographic system[2], applicable for a ternair gradient elution profile.

6.10 Column oven, adjusted at 30 °C.

6.11 High performance anion exchange column[3], filled with pellicularpolystyrene-divinylbenzene resin and pre- column[4] (guard column), mounted in the column oven (6.10).

6.12 Pulsed amperometric detector[5], with a gold electrode, situated together with the columns in the column oven at 30 °C (6.10).

Detector settings are as follows:

- 0 s – 0,40 s potential working electrode + 0,1 V;
- 0,41 s – 0,60 s potential working electrode + 0,7 V;
- 0,61 s – 1,00 s potential working electrode – 0,1 V;
- integration window 0,20 s – 0,40 s.

6.13 Integrator chromatography data system

7 Sampling

iTeh STANDARD PREVIEW
(standards.iteh.ai)

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

SIST-TS CEN/TS 15754:2008
https://standards.iteh.ai/catalog/standards/sist/101286d0-cb35-443e-b508-288fa96915d9/sist-ts-cen-ts-15754-2008

Sampling is not a part of the method specified in this Technical Specification. A recommended sampling method is given in EN-ISO 6497 [1].

8 Preparation of the test sample

Prepare the test sample in accordance with ISO 6498.

9 Procedure

9.1 Extraction sugars

Weigh approximately 600 mg of the test sample to the nearest 0,1 mg in a 100 ml glass centrifugation tube (6.2). Add with the dispensor (6.7) 20,0 ml water and homogenize for at least 1 min with the homogenizer (6.3). Transfer quantitatively to a 100 ml volumetric flask, fill up with water to the mark and homogenize. Centrifuge an aliquot of the homogenized aqueous sample extract for 10 min at about 2 800 g with the swing out centrifuge (6.4).

---

2 The Dionex ICS-3000 module is an example of a suitable commercially available metal free chromatographic system with a quaternair gradient elution pump.

3 The Dionex Carbopac PA1 analytical column (4 x 250 mm) is an example of a suitable commercially available high performance anion exchange column.

4 The Dionex Carbopac PA1 Guard column (4 x 50 mm) is an example of a suitable commercially available guard column.

5 The Dionex model PAD-II is an example of a suitable commercially available pulsed amperometric detector.

SIST-TS CEN/TS 15754:2008

9.2 Extract clean-up

9.2.1 Two extract clean-up methods

For the extract clean-up, continue with either 9.2.2 or 9.2.3. After one of these clean-up procedures, continue with 9.3.

9.2.2 Extract clean-up, method 1, C-18 SPE procedure

Dilute 2,0 ml of the clear solution of the centrifuged extract (9.1) with 2,5 ml water. Prepare and activate the C-18 SPE cartridge according to manufacturer's instruction[6].

Elute about 5 ml of the clear solution of the centrifuged extract over the activated C-18 SPE cartridge.

Discard the first 3 ml and collect 1 ml in a glass tube.

Dilute an aliquot of 0,25 ml with 1,25 ml DMSO, mix well and transfer an aliquot in an HPLC sample vial and close the vial.

9.2.3 Extract clean-up, method 2, deproteinate with acetonitrile

Add to the 10 ml glass screw cap closable centrifugation tube (6.5) 2,0 ml of the clear solution of the centrifuged extract (9.1) followed by 2,5 ml acetonitrile (5.1). Close the tube with the screw cap and mix well. Deproteination occurs by standing overnight at ambient temperature. The next morning, centrifuge the suspension for 10 min at about 2 800 g with the swing out centrifuge (6.4). Dilute an aliquot of 0,25 ml of the clear solution with 1,25 ml DMSO, mix well and transfer an aliquot into a HPLC sample vial and close the vial.

iTeh STANDARD PREVIEW
(standards.iteh.ai)

9.3 Chromatographic analysis

SIST-TS CEN/TS 15754:2008
https://standards.iteh.ai/catalog/standards/sist/101286d0-cb35-443e-b908-
288fa96915d9/sist-ts-cen-ts-15754-2008

Set up the chromatographic system (6.9) with the column oven (6.10), the high performance anion exchange column and guard column (6.11), detector (6.12) and integrator (6.13). Program the following gradient elution profile:

6 Usually these C-18 SPE cartridges are activated by first eluting 5 ml – 10 ml methanol through the cartridge, followed by an equal amount of distilled water. Do not allow the cartridges to dry out.