SLOVENSKI STANDARD
SIST EN 16160:2012

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Krma - Določevanje cianovodikove kisline s HPLC

Animal feeding stuffs - Determination of Hydrocyanic acid by HPLC

Futtermittel - Bestimmung von Blausäure mittels HPLC

Aliments des animaux - Dosage de l'acide cyanhydrique par CLHP

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Animal feeding stuffs - Determination of Hydrocyanic acid by HPLC

Aliments pour animaux - Dosage de l'acide cyanhydrique par CLHP

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Contents

Page

Foreword

This document (EN 16160:2012) has been prepared by Technical Committee CEN/TC 327 “Animal feeding stuffs”, 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of an identical text or by endorsement, at the latest by September 2012, and conflicting national standards shall be withdrawn at the latest by September 2012.

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1 Scope

This European Standard is applicable to the quantitative analysis of (bound and free) hydrocyanic acid (HCN) in feed materials of plant origin and compound feed by High Performance Liquid Chromatography (HPLC).

The method is validated from 10 mg HCN/kg to 350 mg HCN/kg. When the method is used outside this range it should be validated at least within the laboratory. A limit of quantification of 2 mg HCN/kg should normally be obtained.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

prEN ISO 6498, *Animal feeding stuffs – Guidelines for sample preparation (ISO/DIS 6498)*

3 Principle

Hydrocyanic acid occurs in feed as cyanoglycosides.

Cyanoglycosides are extracted from feed with an acid solution. After incubation with acid, the pH is adjusted to a value between 5,9 and 6,0 and cyanoglycosides are treated by ß-glucosidase at 38 °C to release hydrocyanic acid. Hydrocyanic acid is collected in a potassium hydroxide solution by steam distillation. Subsequently, cyanide is derivatized with taurine and 2,3 napthylene dicarboxy aldehyde (NDA) to form a fluorescent complex. The cyanide complex is analyzed by HPLC with fluorescence detection.

4 Reagents

Use only reagents of recognized analytical grade and distilled or demineralised water or water of equivalent quality, unless otherwise specified.

WARNING — Use all solvents and solutions in a fume hood. Wear safety glasses, protective clothing, and avoid skin contact. Take special care of the waste containing HCN or CN^-.

4.1 β-glucosidase from almonds, EC 3.2.1.21, minimum 2 units/mg (e.g. Sigma G-0395)[1)]

4.2 Potassium cyanide, KCN

4.3 Amygdalin (e.g. Sigma A-6005)[2)]

[1)] Sigma G-0395 is an example of a suitable product available commercially. This information is given for the convenience of the users of this European Standard and does not constitute an endorsement by CEN of this product.

[2)] Sigma A-6005 is an example of a suitable product available commercially. This information is given for the convenience of the users of this European Standard and does not constitute an endorsement by CEN of this product.

4.4 Potassium dihydrogen phosphate, KH_2PO_4

4.5 di-Potassium hydrogen phosphate, K_2HPO_4

4.6 Sodium hydroxide, NaOH

4.7 Methanol, CH_3OH, HPLC grade

4.8 Sodium acetate trihydrate, $CH_3COONa.3H_2O$

4.9 Acetic acid, CH_3COOH

4.10 Orthophosphoric acid, H_3PO_4, 85 % (15 mol/l)

4.11 Sodium meta borate tetrahydrate, $BNaO_2.4\ H_2O$

4.12 NDA, 2,3 Naphthalene dicarboxy aldehyde, $C_{12}H_8O_2$

4.13 Taurine, $C_2H_7NO_3S$

4.14 EDTA, Titriplex III - $C_{10}H_{14}N_2Na_2O_8.2H_2O$

NOTE Merck art. No. 1.08418.0250 is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.

4.15 Orthophosphoric acid solutions, concentrations $c(H_3PO_4)$ = 0,1 mol/l and 0,02 mol/l:

— 0,1 mol/l: Add 6,9 ml H_3PO_4 85 % (4.10) to a volumetric flask of 1 000 ml containing 500 ml water, fill to mark with water and mix;

— 0,02 mol/l: Transfer 200 ml 0,1 mol/l H_3PO_4 solution to a volumetric flask of 1 000 ml, fill to mark with water and mix.

Prepare these solutions for every series.

4.16 Sodium hydroxide solutions, concentrations *c*(NaOH) = 1,0 mol/l, 0,1 mol/l and 0,01 mol/l:

— 1,0 mol/l: Add 4,0 g sodium hydroxide (4.6) to a volumetric flask of 100 ml containing 50 ml water, dissolve, cool to room temperature and fill to mark with water and mix;

— 0,1 mol/l: Transfer 100 ml 1,0 mol/l sodium hydroxide solution to a volumetric flask of 1 000 ml, fill to mark with water and mix;

— 0,01 mol/l: Transfer 100 ml 0,1 mol/l sodium hydroxide solution to a volumetric flask of 1 000 ml, fill to mark with water and mix.

These solutions are stable for three months.

4.17 EDTA solution, concentration = 0,20 mol/l

Weigh 37,2 g EDTA (4.14) in a 500 ml beaker, add 300 ml water, dissolve and then adjust the pH to 7,0 - 8,0 with a 1,0 mol/l and 0,1 mol/l sodium hydroxide solution (4.16) (use a pH meter (5.12). Transfer to a

volumetric flask of 500 ml; fill to mark with water and mix. The solution remains stable for 1 month when stored at room temperature.

4.18 KCN standard stock solution

Weigh, to the nearest 0,1 mg, 100 mg KCN (4.2) in a 100 ml volumetric flask. Add 50 ml 0,01 mol/l sodium hydroxide solution (4.16) and dissolve the KCN, fill to mark with 0,01 mol/l sodium hydroxide (4.16) and mix. This is the stock solution of 400 µg cyanide ions (CN^-) per ml.

The solution is stable for 3 months when stored in the refrigerator.

4.19 KCN working standard solutions

Add, using a mechanic pipette (5.4), 1,00 ml KCN standard stock solution (4.18) in a 100 ml volumetric flask and fill to the mark with 0,01 mol/l sodium hydroxide solution (4.16) and mix; this is the work solution of 4 µg CN^-/ml. Prepare calibration standard solutions by following schedule (Table 1) in 100 ml volumetric flasks and using volume pipettes (5 ml and 10 ml) (5.2) and/or a mechanic pipette (5.4):

Table 1 — calibration standard solutions

	Amount work solution ml	**Concentration** µg CN^-/ ml
Standard 1	25,00	1,00
Standard 2	10,00	0,40
Standard 3	5,00	0,20
Standard 4	2,50	0,10
Standard 5	1,25	0,05
Standard 6	0,00	0,00

Use 0,01 mol/l sodium hydroxide solution (4.16) to fill the volumetric flasks to the mark.

For every series of analysis, fresh standards are prepared.

4.20 ß-glucosidase solution

Weigh such an amount of ß-glucosidase (4.1) to obtain a final concentration of 200 IU per ml and dissolve in water.

NOTE The amount of ß-glucosidase weighed depends on the activity of the enzyme, given by the manufacturer of the enzyme. E.g. when the activity is 2 IU/mg enzyme, 100 mg enzyme/ml water is weighed. A new batch of ß-glucosidase can be tested by analyzing a sample with a known amount of hydrocyanic acid e.g. a reference sample. If the enzyme is active enough, the expected amount of hydrocyanic acid should be measured.

For every series of analysis, a fresh solution is prepared.

4.21 **Amygdalin spike solution**, concentration *c*(amygdalin) 0,019 mol/l

Dissolve 85,0 mg amygdalin (4.3) in 10 ml water.

For every series of analysis, a fresh solution is prepared.

4.22 Sodium acetate solution, concentration $c(CH_3COONa.3H_2O)$ 0,75 mol/l

Dissolve 100 g sodium acetate trihydrate (4.8) in 800 ml water. Adjust pH to 7,9 with diluted (20x) acetic acid (4.9). Use a pH meter to control (5.12). Transfer to a volumetric flask of 1 000 ml. Fill to the mark with water and mix.

The solution remains stable for 3 months at room temperature.

4.23 Phosphate buffer for HPLC mobile phase, concentration $c(PO_4^{3-})$ 0,05 mol/l

Dissolve 3,40 g potassium dihydrogen phosphate (4.4) and 4,35 g di-potassium hydrogen phosphate (4.5) in 100 ml water in a beaker. Transfer to a volumetric flask of 1 000 ml, fill to mark with water and mix.

The solution remains stable for 3 months at room temperature.

4.24 Phosphate buffer for NDA/taurine solution, concentration $c(PO_4^{3-})$ 0,1 mol/l, concentration $c(BNaO_2)$ 0,025 mol/l

Dissolve 3,40 g potassium dihydrogen phosphate (4.4), 4,35 g di-potassium hydrogen phosphate (4.5) and 3,45 g sodium meta borate tetrahydrate (4.11) in 100 ml water. Transfer to a volumetric flask of 500 ml. Fill to the mark with water and mix.

This solution remains stable for 3 months when stored in the refrigerator.

4.25 NDA solution; concentration c(NDA) 0,002 mol/l

Weigh 36,8 mg NDA (4.12) in a 100 ml volumetric flask. Add 40 ml methanol (4.7) and dissolve the NDA. Fill to the mark with phosphate buffer for NDA/taurine solution (4.24) and mix.

This solution remains stable 3 months when stored in the refrigerator.

4.26 Taurine solution, concentration c(taurine) 0,05 mol/l

Weigh 0,626 g taurine (4.13) in a 100 ml volumetric flask. Add 40 ml phosphate buffer for NDA/taurine solution (4.24) and dissolve taurine. Fill to the mark with phosphate buffer for NDA/taurine solution (4.24) and mix.

This solution remains stable for 3 months when stored in the refrigerator.

4.27 HPLC mobile phase

Weigh 675 g methanol (4.7) in a flask of 2 000 ml and add 1 000 ml phosphate buffer for HPLC mobile phase (4.23), mix and cool to room temperature. Filter the mobile phase using a filtrate system (5.6).

The solution remains stable for 3 months when stored at room temperature.

4.28 Liquid nitrogen.

5 Apparatus

5.1 Common laboratory glassware, such as graduated cylinders, volumetric flasks and screw cap glass bottles.

5.2 Volume pipettes, 5 ml and 10 ml