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Animal feeding stuffs — Determination of Hydrocyanic acid by HPLC

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National foreword

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A list of organizations represented on this committee can be obtained on request to its secretary.

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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of an identical text or by endorsement, at the latest by September 2012, and conflicting national standards shall be withdrawn at the latest by September 2012.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

5.3 One or two neck round bottom flask of 1 000 ml or a sample tube of 500 ml

5.4 Mechanic pipette, 100 µl to 1 000 µl, 1 000 µl to 5 000 µl and 5 000 µl to 10 000 µl

5.5 Oven, at 38 °C ± 1 °C

5.6 Filtration system for HPLC mobile phase with a membrane filter 0,45 µm

5.7 Steam distillation unit

NOTE Landgraaf no. AS136721111 and Buchi K-350 are examples of suitable products 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.

5.8 HPLC system:

— Analytical pump;

— column oven;

— autosampler with a 10 µl sample loop; optional: with cooled tray;

— HPLC Fluorescence detector;

— analytical reversed phase HPLC column: C18 RP-column preferably 125 mm × 4 mm, 5 µm;

— precolumn: C18 RP-column preferably 7,5 mm × 4 mm, 5 µm.

5.9 Analytical balance, able to measure with an accuracy of 0,1 mg

5.10 Balance, able to measure with an accuracy of 0,01 g

5.11 Mill, capacity to grind to a particle size of ≤ 1 mm

5.12 pH meter.

6 Sampling

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

Sampling is not part of the method specified in this European Standard. A recommended sampling method is given in EN ISO 6497 [1]. It is recommended that samples be stored frozen to prevent changes in hydrocyanic acid levels due to endogenous enzyme activity.

7 Preparation of test sample

Prepare the test sample in accordance with prEN ISO 6498.

Grind the laboratory sample (at least 500 g) so it passes completely through a sieve with mesh size of 1 mm. Mix thoroughly.

Optional: When samples with high oil content are milled at room temperature, an emulsion will be formed when adding phosphoric acid. The emulsion can have a negative effect on the reproducibility. To prevent the emulsion formation, liquid nitrogen (4.28) is used with grinding.

8 Procedure

8.1 Precautions

Hydrocyanic acid is very toxic. Protecting gloves should be used during the preparation of the samples and standards. Waste should be disposed separately from normal waste.

If there is a risk of escape of hydrocyanic acid (e.g. distillation), operations should be arranged in a fume cupboard.

In case of methanol, one should use a fume cupboard.

NOTE Hydrocyanic acid is volatile above 26 °C. It is recommended to avoid loss of hydrocyanic acid during sample handling; in particular during manual operations at 8.3.3 (opening and closing of bottle) and 8.3.4 (transfer of sample solution). It is also important to carry out the laboratory experiments at a temperature below 26 °C.

8.2 General

In order to establish the recovery and control the potential losses of hydrocyanic acid, analyze for each series the following control samples:

— blank sample of animal feed; prepare this as a test sample;

— blank animal feed spiked to 10,0 mg HCN/kg; prepare this adding 100 µl amygdalin spike solution (4.21) to 5,0 g feed;

— blank animal feed spiked to 50,0 mg HCN/kg; prepare this adding 500 µl amygdalin spike solution (4.21) to 5,0 g feed.

It is recommended to analyze a reference sample with a known hydrocyanic acid content in every analysis series, e.g. linseed. Preparation of this sample is the same as for the test samples.

Calculate an average recovery with 2 points (10 mg/kg; 50 mg/kg). If the recovery falls outside the range of 90 % to 110 %, all the results of this series can't be validated without correction for recovery (see 9.3).

8.3 Sample preparation

8.3.1 General

Follow for each test portion the following instructions.

8.3.2 Extraction

Weigh, to the nearest 0,01 g, 5,00 ± 0,3 g feed and blank feed in a 100 ml or 150 ml (250 ml) screw cap glass bottle. Add 80 ml of a cold solution (≤ 10 °C) of 0,02 mol/l orthophosphoric acid (4.15) to the sample, close the bottle, and mix very well on a stirring device.

Incubate for 1 h at low temperature (≤ 26°C), under continuous stirring. Add slowly, while maintaining stirring, 15 ml of cold solution (≤ 10 °C) of 0,20 mol/l EDTA solution (4.17), close the screw cap glass bottle - mix for 10 min. Then adjust the pH between 5,9 and 6,0 using 0,1 mol/l sodium hydroxide (4.16) while controlling the pH with a pH meter (5.12). (It may take some time to adjust the pH because of the buffering capacity of the feed).

8.3.3 Enzymatic breakdown

Add 0,5 ml ß-glucosidase solution (4.20) and immediately close the screw cap glass bottle. Place the screw cap glass bottle in an oven at 38 °C (5.5) and incubate overnight under continuous shaking. After incubation, the screw cap glass bottle is cooled on ice to a temperature lower than 10 °C.

8.3.4 Steam distillation

Transfer the sample to a one or two neck round bottom flask or a sample tube (5.3). Use no more than 50 ml cold water (≤ 10 °C) for the transfer of the sample. Add 10 ml of a cold sodium acetate solution (4.22) (< 10 °C) to the sample and connect the one or two neck round bottom flask (5.3) to the steam distillation unit (5.7). Collect distillate [3)] in a 250 ml or 500 ml volumetric flask that contains 25 ml or 50 ml of a cold solution (≤ 10 °C) of 0,1 mol/l sodium hydroxide (4.16) and 25 ml or 80 ml cold water (≤ 10 °C). Fill the flask up to the mark with cold water (≤ 10 °C). The final concentration of sodium hydroxide in the volumetric flask should not exceed 0,01 mol/l. If necessary dilute the sample with 0,01 mol/l sodium hydroxide (4.16).

NOTE It is recommended to rinse/clean the capillary of the steam distillation equipment with water after each distillation run to avoid cross contamination.

8.4 HPLC analysis

8.4.1 Derivatization

Pipette 0,5 ml sample (8.3) into a HPLC vial. Subsequently, add 100 µl taurine solution (4.26) and 100 µl NDA solution (4.25) to the sample. Close the vial, mix and leave for 30 min at room temperature. Place the vial in the tray of the autosampler. Protect the vials from UV/Visible light.

The derivatization procedure is also applied to the standards (4.19).

Optional: When a cooled HPLC autosampler tray has been used, the derivates are stable for at least 24 h.

8.4.2 HPLC conditions

Prior to each series of analysis, the column is first flushed with water and methanol (4.7) and then with mobile phase until pressure and base line are stabilized.

- Pump:
  - flow : 1,0 ml/min;
  - column temp : 30 °C;
  - time of analysis : 15 min, (peak of CN^--complex should occur between 4 min and 10 min).
- Auto sampler:
  - injection volume : 10 µl.
- Fluorescence detector:
  - excitation : 418 nm;
  - emission : 460 nm;
  - gain : 10;
  - response : slow.

3) Approximately 200 ml to 300 ml when using conventional steam distillation equipment or less if using modern equipment.

8.4.3 Analysis

Prior to analysis: inject 10 µl of Standard 1 (1,00 µg CN^-/ml) and Standard 5 (0,05 µg CN^-/ml) (4.19). A single peak should be observed and the peak area ratio of the two standards should be correct.

HPLC analysis sequence: Inject successively the standard samples, the spiked samples, the test samples and again the standard samples.

9 Calculation of results

9.1 Calculation of hydrocyanic acid content

Determine the area of the CN-peak (if available use data acquisition software). Use the standards for calculation of a 6-points calibration line (peak area vs. concentration) using linear regression.

$$C\ (\mu g/ml) = a \times \text{Signal (units)} + b \quad (1)$$

NOTE Calculate the correlation coefficient R^2. Criterion for correlation coefficient: >0,995. Use the above mentioned function to calculate the concentration of CN^- in the measured test solution. Calculate the mass fraction (W_{HCN}) of hydrocyanic acid, in the original material expressed in mg/kg, using the following formula:

$$W\ (\text{mg HCN/kg}) = (C \times V \times D \times 1{,}04) / m \quad (2)$$

where

C is the concentration of CN^- in the injected solution calculated from linear regression (µg/ml);

V is the final volume of the volumetric flask at 8.3.4 (250 ml or 500 ml);

D is the dilution factor (if applicable);

m is the test portion mass (g);

W is the HCN mass fraction of the sample (mg/kg).

9.2 Calculation of recovery

From the results obtained for the blank feed and the spiked feeds (10 mg HCN/kg and 50 mg HCN/kg), calculate a calibration curve ($y = ax + b$) obtained by linear regression between the concentration y and the added concentration x. The slope obtained, multiplied by a factor of 100, is the recovery (%).

9.3 Criteria for acceptance of results

The analysis series is validated if the recovery obtained in 9.2 falls inside the range 90 % to 110 %. If it falls outside the range 90 % to 110 %, the series is invalid. In this case the results for the test samples shall be corrected for recovery.

Correction of recovery can be performed in one of the following ways:

— by means of spiking of a blank sample. Preferably a blank sample similar in type to that of the analyzed sample should be selected. The spiked level should be similar to the concentration of the analyzed sample;

— by means of the standard addition procedure. The concentration added to the analyzed sample should be similar to the concentration found in the analyzed sample.

10 Precision

10.1 Collaborative study

A collaborative study was carried out in 2009 by RIKILT (NL). Details of the tests on precision of the method are summarised in Annex A. The values derived from these tests may not be applicable to concentration ranges and matrices other than those given.

Further details regarding the outcome of the collaborative study can be found in the final report [6].

10.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval time, will in not more than 5 % of the cases be greater than the repeatability limit *r* given in Table 2 – precision data.

10.3 Reproducibility

The absolute difference between two single test results obtained using the same method on identical test material in the same laboratory with different operators using different equipment, will in not more than 5 % of the cases be greater than the reproducibility limit *R* given in Table 2.

Table 2 — Precision data (results statistical analysis corrected for recovery)

	Chicken feed	Pig feed	Horse feed	Tapioca	Linseed	Almonds
Mean, mg HCN/kg	12,5	55,1	88,6	119,4	313,2	59,5
r, mg/kg	4,0	7,8	10,4	8,6	45,6	13,1
R, mg/kg	8,0	12,1	21,8	28,0	105,3	14,6

11 Test report

The test report shall specify the following information:

a) information necessary for complete identification of the sample;

b) the method with which sampling was carried out, if known;

c) the method used;

d) the test result obtained;

e) if the repeatability has been checked, the final quoted result obtained;

f) operating details not specified in this European Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s).

Annex A
(informative)

Results of the collaborative study

A collaborative study was carried out in 2009 with 17 participating laboratories and 6 different animal feedingstuffs, including complete feeds for chicken, pigs and horses, tapioca, linseed and almonds [6]. The samples were homogenised centrally and distributed to the participants. Statistical evaluation was performed according to ISO 5725-2 [2].

The following data were obtained.

Table A.1 — Statistical results of collaborative study (corrected for recovery)

	Chicken feed	Pig feed	Horse feed	Tapioca	Linseed	Almonds
Number of laboratories	17	16	16	16	16	16
Number of non-compliant laboratories	2	2	2	3	2	2
Number of outliers	0	1	2	0	2	1
Number of accepted results	15	13	12	13	12	13
Mean, mg HCN/kg	12,5	55,1	88,6	119,4	313,2	59,5
s_r, mg/kg	1,4	2,8	3,7	3,1	16,3	4,7
s_R, mg/kg	2,9	4,3	7,8	10,0	37,6	5,2
RSD_r, %	11,4	5,1	4,2	2,6	5,2	7,9
RSD_R, %	22,8	7,8	8,8	8,4	12,0	8,8
r, mg/kg	4,0	7,8	10,4	8,6	45,6	13,1
R, mg/kg	8,0	12,1	21,8	28,0	105,3	14,6
$HORRAT_R$	2,1	0,9	1,1	1,1	1,8	1,0

Key
s_r = repeatability standard deviation
s_R = reproducibility standard deviation
RSD_r = repeatability relative standard deviation
RSD_R = reproducibility relative standard deviation
r = repeatability limit
R = reproducibility limit
$HORRAT_R$ = HORRAT value for reproducibility

Annex B
(informative)

Results of the collaborative study without recovery correction

The following table is shown purely for informative purposes. It lists the statistical results of the same collaborative study as reported in Annex A but without recovery correction. The results show that, in order to reach optimal results, recovery correction is necessary.

Table B.1 — Statistical results of collaborative study (not corrected for recovery)

	Chicken feed	Pig feed	Horse feed	Tapioca	Linseed	Almonds	Blank feed spiked to	
							10 mg/kg	50 mg/kg
Number of laboratories	17	17	17	17	17	17	17	16
Number of non-compliant laboratories	1	1	1	2	1	1	2	2
Number of outliers	1	2	1	2	3	1	1	1
Number of accepted results	15	14	15	13	13	15	14	13
Mean, mg HCN/kg	7,7	38,5	58,4	84,7	227,4	39,9	6,3	35,0
s_r, mg/kg	0,8	1,8	2,3	1,2	14,6	2,9	0,5	1,6
s_R, mg/kg	3,0	4,2	9,9	6,1	26,5	5,4	1,5	3,7
RSD_r, %	10,4	4,7	3,9	1,4	6,6	7,2	7,6	4,6
RSD_R, %	38,8	10,9	17,0	7,2	11,9	13,4	24,2	10,5
r, mg/kg	2,3	5,1	6,4	3,4	40,9	8,0	1,3	4,5
R, mg/kg	8,4	11,8	27,7	17,2	74,1	15,0	4,3	10,2
$HORRAT_R$	3,3	1,2	2,0	0,9	1,7	1,5	2,0	1,1

Key
s_r = repeatability standard deviation
s_R = reproducibility standard deviation
RSD_r = repeatability relative standard deviation
RSD_R = reproducibility relative standard deviation
r = repeatability limit
R = reproducibility limit
$HORRAT_R$ = HORRAT value for reproducibility

Annex C
(informative)

Alternative procedure for enzymatic breakdown (8.3.3) and steam distillation (8.3.4)

The following alternative procedure may yield satisfactory results but has not been fully validated through the collaborative study:

The sample is weighed (at 8.3.2 of the procedure) directly into steam-distillation bottles which might be of an automated system too instead of weighing in a screw cap glass bottle.

During the enzymatic breakdown (8.3.3) step the steam-distillation bottle is tightly closed with stoppers and additionally sealed with Parafilm. After enzymatic breakdown, the steam-distillation (8.3.4) can be done directly in this steam-distillation bottle.

NOTE 1 Parafilm is the trade name of a product supplied by Fisher Scientific. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of the product named. Equivalent Products may be used if they can be shown to lead to the same results.

NOTE 2 In the collaborative study one laboratory applied this alternative procedure. The results obtained were as follows.

Table C.1 — Laboratory results (mg HCN/kg)

Chicken feed		**Pig feed**		**Horse feed**		**Tapioca**		**Linseed**		**Almonds**		**Blank feed spiked to**			
												10 mg/kg		**50 mg/kg**	
11,5	11,3	55,7	53,1	87,3	86,0	108,2	105,1	298,1	271,8	54,5	50,5	8,8	8,2	46,9	43,1
13,5	*13,3*	*61,9*	*59,0*	*97,0*	*95,6*	*120,2*	*116,8*	*331,2*	*302,0*	*60,6*	*56,1*				
All samples are analyzed in duplicate. The Italics results are corrected for recovery.															

The recovery corrected results fitted well into the range of results obtained by other laboratories in the collaborative study (see Annex A).

Bibliography

[1] EN ISO 6497, *Animal feeding stuffs – Sampling (ISO 6497)*

[2] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results – Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

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