

BS EN 16160:2012



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Animal feeding stuffs — Determination of Hydrocyanic acid by HPLC

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National foreword

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English Version

**Animal feeding stuffs - Determination of Hydrocyanic acid by
HPLC**

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

Futtermittel - Bestimmung von Blausäure mittels HPLC

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Contents

Page

Foreword.....	3
1 Scope	4
2 Normative references	4
3 Principle	4
4 Reagents	4
5 Apparatus	7
6 Sampling	8
7 Preparation of test sample.....	8
8 Procedure	9
9 Calculation of results	11
10 Precision	12
11 Test report	12
Annex A (informative) Results of the collaborative study	13
Annex B (informative) Results of the collaborative study without recovery correction.....	14
Annex C (informative) Alternative procedure for enzymatic breakdown (8.3.3) and steam distillation (8.3.4).....	15
Bibliography	16

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of an identical text or by endorsement, at the latest by September 2012, and conflicting national standards shall be withdrawn at the latest by September 2012.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

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