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Fertilizers — Determination of dicyandiamide — Method using high-performance liquid chromatography (HPLC)

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ICS 65.080

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National foreword

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Engrais - Détermination de la teneur en dicyandiamide - Méthode par chromatographie liquide à haute performance (HPLC)

Düngemittel - Bestimmung von Dicyandiamid - Verfahren mit Hochleistungs-Flüssigchromatographie (HPLC)

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Foreword

This document (EN 15360:2007) has been prepared by Technical Committee CEN/TC 260 “Fertilizers and liming materials”, the secretariat of which is held by DIN.

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of an identical text or by endorsement, at the latest by November 2007, and conflicting national standards shall be withdrawn at the latest by November 2007.

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1 Scope

This document specifies a method for the selective determination of dicyandiamide (DCD) in addition to all the other forms of nitrogen fixations, particularly in fertilizers to which DCD has been added as a nitrification inhibiting agent.

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 1482-2, *Fertilizers and liming materials — Sampling and sample preparation — Part 2: Sample preparation*

EN ISO 3696, *Water for analytical laboratory use — Specification and test methods (ISO 3696:1987)*

3 Principle

The sample is dissolved or suspended in water using an ultrasonic bath. Methyl dicyandiamide is added to the filtered solution as the internal standard. The solution is then transferred onto a C18 reversed-phase column using a bypass injector and then separated. For detection, a UV-detector is used at a wavelength of 220 nm.

4 Reagents

Use only reagents of recognized analytical grade and water conforming to grade 2 of EN ISO 3696.

4.1 Dicyandiamide standard solution

Weigh 50 mg of dicyandiamide of known purity into a 1 000 ml volumetric flask and dissolve in water; make up the volume to the mark. Pipette 10 ml of this solution into a 100 ml volumetric flask and, after having added 10 ml of internal standard solution (4.2), make up the volume to the mark with water.

4.2 Internal standard solution

Weigh 50 mg of methyl dicyandiamide of known purity into a 1 000 ml volumetric flask, dissolve in water and make up the volume to the mark.

4.3 Methanol

HPLC-grade purity

5 Apparatus

5.1 Ultrasonic bath

5.2 Membrane filter

0,45 μm, with the usual filtration equipment.

5.3 HPLC apparatus

UV-detector for variable wavelengths and an electronic integrator, sample injection valve equipped with a 20 µl bypass injector.

6 Sampling and sample preparation

Sampling is not part of the method specified in this document; however, a recommended sampling method is given in EN 1482-1. Sample preparation shall be carried out in accordance with EN 1482-2.

7 Procedure

7.1 Preparing the analytical solution

Weigh to the nearest 0,001 g, between 0,8 g and 1,5 g of the ground and thoroughly homogenized test sample (corresponding to approximately 50 mg of DCD) and mix with 750 ml of water into a 1 000 ml volumetric flask and dissolve using an ultrasonic bath (5.1). Those portions that have not dissolved after 5 min are disregarded. Make up the volume to the mark with water. Filter one part of the homogenized sample solution (approximately 50 ml) through the membrane filter (5.2) into a dry vessel.

Pipette 10 ml of this filtrate into a 100 ml volumetric flask and, after having added 10 ml of the internal standard solution (4.2), make up the volume to the mark with water.

7.2 HPLC conditions

Eluent:	Mixture of water and methanol (4.3), (99 + 1) parts by volume
Separation column and packing:	250 mm x 4,6 mm C18 reversed-phase column
Column temperature:	Room temperature
Flow rate:	1,0 ml/min
Wavelength:	220 nm

7.3 HPLC determination

Alternately, transfer the standard solution (4.1) and the test solution (7.1) onto the separation column three times, applying the standard solution before the test solution. Measure the peak areas for DCD and methyl dicyandiamide.

8 Calculation

Calculate the proportion in the standard solution (4.1), $P_{x'}$ according to the following equation:

$$P_{x'} = \frac{A_1}{A_2} \quad (1)$$

where

A_1 is the peak area for DCD (4.1);

A_2 is the peak area of the internal standard.

Calculate the proportion in the test solution (7.1), P_x according to the following equation:

$$P_x = \frac{A_3}{A_2} \quad (2)$$

where

A_2 is the peak area of the internal standard;

A_3 is the peak area for DCD (of 7.1).

Take from each of the groups three values for P_x and $P_{x'}$, the mean values Px'_M and Px_M, and calculate the DCD content of the sample, expressed in g/100 g using the following equation:

$$w_{DCD} = \frac{Px_M \times m'}{Px'_M \times m} \quad (3)$$

where

Px_M is the mean value of the proportions for the sample;

Px'_M is the mean value of the proportions for the standard;

m' is the mass of DCD in the standard solution (4.1) (50 mg), in milligrams;

m is the mass of the sample in the aliquot part of the test solution (7.1) used, in milligrams.

If necessary, correct the final result depending on the purity of the DCD used for the standard solution.

9 Precision

9.1 General

The precision of the method has been determined in an inter-laboratory trial, carried out and evaluated in 1997 according to ISO 5725-1. A summary of the results is given in Annex A. The values derived from this inter-laboratory trial might not be applicable to concentration ranges and matrices other than those given.

9.2 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 short time intervals, will exceed the values for the repeatability limit r, given in Table 1, on average in no more than 5 % of cases.

9.3 Reproducibility

The absolute difference between two independent single test results obtained using the same method on identical test material in different laboratories by different operators using different equipment will exceed the values for the reproducibility limit R, given in Table 1, on average in no more than 5 % of cases.

Table 1 — Precision data

Sample	Level g/100 g	r g/100 g	R g/100 g
No 1 (Alzon 27/1)	2,41	0,089 98	0,466 7
No 2 (Alzon 27/2)	2,18	0,073 7	0,329

10 Notes on procedure

10.1 As described, the method covers a range between 2 g and 10 g of DCD per 100 g.

By variation of the weighed portion and the extent of dilution, DCD contents of between (0,1 g and 100 g) per 100 g can be determined.

10.2 The method may also be carried out using an external standard. In this case the equation for the calculation has to be modified accordingly.

11 Test report

The test report shall include at least the following information:

a) information necessary for complete identification of the sample;

b) test method used, making reference to this document, i.e. EN 15360;

c) test results together with the units used to express them;

d) date the test was finished;

e) statement as to whether the requirement for the repeatability limit has been fulfilled;

f) procedural steps not specified in this document or carried out optionally, as well as details of any circumstances that occurred while carrying out the method that might have influenced the result(s).

Annex A
(informative)

Results of the inter-laboratory trial

The precision of the method was determined in 1997 during an inter-laboratory trial with respectively 13 and 12 laboratories participating and carried out on 2 samples of fertilizer. The statistical results are given in Table A.1.

Table A.1 — Statistical results of the inter-laboratory trial

Parameter	Sample No 1	Sample No 2
Year of the test	1997	
Number of participating laboratories	13	12
Number of laboratories after eliminating outliers	13	12
Level mean value, (g/100 g)	2,41	2,18
Repeatability standard deviation s_r, (g/100 g)	0,03	0,03
Coefficient of variation CV_r (%)	1,348	1,218
Repeatability limit r (2,83 s_r) (g/100 g)	0,089 98	0,073 7
Reproducibility standard deviation, s_R (g/100 g)	0,168 4	0,118 7
Coefficient of variation CV_R (%)	6,99	5,44
Reproducibility limit R (2,83 s_R) (g/100 g)	0,466 7	0,329

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[1] Vilsmeier, K., 1984: *Bestimmung von Dicyandiamid, Nitrit und Nitrat in Bodenextrakten mit Hochdruckflüssigkeitschromatographie*. Zeitschrift für Pflanzenernährung, Düngung und Bodenkunde 147, page 264 to 268

[2] ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*

[3] EN 1482-1, *Fertilizers and liming materials — Sampling and sample preparation — Part 1: Sampling*

[4] *Regulation (EC) No 2003/2003 of the European Parliament and of the Council of 13 October 2003 relating to fertilisers*, Official Journal L 304, 21/11/2003, P. 0001-0194

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