SLOVENSKI STANDARD
SIST-TP CEN/TR 15106:2005

01-december-2005

Gnojila ï Določ evanje sredstev za kelatiziranje EDDHA in EDDHMA z ionsko kromatografijo ï Primerjava nestandardizirane metode po Luceni z EN 13368-2:2001

Fertilizers - Determination of chelating agents EDDHA and EDDHMA by ion pair chromatography - Comparison of non-standardized Lucena method with EN 13368-2:2001

Düngemittel - Bestimmung der Chelatbildner EDDHA und EDDHMA mit Ionenpaarchromatographie - Vergleich der nicht standardisierten Lucena-Methode mit EN 13368-2:2001

Engrais - Détermination des agents chélatants EDDHA et EDDHMA par chromatographie ionique - Comparaison entre la méthode Lucena non normalisée et la norme EN 13368-2:2001

Ta slovenski standard je istoveten z: **CEN/TR 15106:2005**

ICS:

65.080 Gnojila Fertilizers

SIST-TP CEN/TR 15106:2005 **en**

TECHNICAL REPORT

RAPPORT TECHNIQUE

TECHNISCHER BERICHT

CEN/TR 15106

May 2005

ICS 65.080

English version

Fertilizers - Determination of chelating agents EDDHA and EDDHMA by ion pair chromatography - Comparison of non-standardized Lucena method with EN 13368-2:2001

Engrais - Détermination des agents chélatants EDDHA et EDDHMA par chromatographie ionique - Comparaison entre la méthode Lucena non normalisée et la norme EN 13368-2:2001

Düngemittel - Bestimmung der Chelatbildner EDDHA und EDDHMA mit Ionenpaarchromatographie - Vergleich der nicht standardisierten Lucena-Methode mit EN 13368-2:2001

This Technical Report was approved by CEN on 7 April 2005. It has been drawn up by the Technical Committee CEN/TC 260.

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

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

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

Ref. No. CEN/TR 15106:2005: E

Contents

page

Foreword

This document (CEN/TR 15106:2005) has been prepared by Technical Committee CEN/TC 260 “Fertilizers and liming materials”, the secretariat of which is held by DIN.

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

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

Introduction

European Regulation (EC) No 2003/2003 of the European parliament and of the Council of 13th October 2003 relating to fertilizers states that the synthetic commercial iron chelates shall be water soluble products obtained by the chemical combination of iron with a chelating agent. The iron (Fe) chelated by each chelating agent shall be declared on the label.

EN 13368-2:2001 specifies a method for the determination of EDDHA and EDDHMA by ion chromatography. This method determines the amount of chelated iron in Fe-EDDHA and Fe-EDDHMA commercial products. A ring test performed by CEN/TC 260, showed that reproducibility data were poor, possibly due to the low number of participating laboratories.

Some laboratories used a non-standardized method [1], [2], based on ion-pair chromatography. This can be performed using common chromatography equipment and some experiences [3] suggested that it can give adequate reproducibility and repeatability data.

CEN/TC 260/WG 5 agreed to launch a new work item in order to compare the reproducibility, repeatability and accuracy of the two methods (standardized and non-standardized). The aim being to develope a European Standard for the non-standardized method if better results were obtained. The results of the first ring test (ring test A) were not significant enough to confirm that the non-standardized method is better than the method standardized in EN 13368-2:2001.

The comments of the participating laboratories (see Annex B) suggested that the non-standardized method could be improved with some modifications which could then result in significant differences with respect to EN 13368-2:2001.

For this reason, WG 5 decided to carry out a second ring test (ring test B) using the method with the suggested modifications.

This Technical Report includes the results and conclusions from the two ring tests.

1 Scope

This Technical Report covers the results from two ring tests (A and B, see Introduction) to compare the repeatability and reproducibility of the standardized method for the determination of EDDHA and EDDHMA by ion chromatography (EN 13368-2:2001) with a non-standardized alternative method using ion-pair chromatography ("Lucena-method", given in Annex A). This Technical Report also provides the results of an univariate multifactorial ANOVA test and F-test for ring test A and F-test for ring test B to verify the significance of the reproducibility and the repeatability data.

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 13368-2:2001, *Fertilizers - Determination of chelating agents in fertilizers by ion chromatography - Part 2: EDDHA and EDDHMA.*

3 Test procedure for ring test A

3.1 Methods used in ring test A for the determination of chelating agents EDDHA and EDDHMA by chromatography.

Both methods tested for the determination of EDDHA and EDDHMA are based on high performance liquid chromatography.

The standardized method, based on ion chromatographyis is given in EN 13368-2:2001.

The non-standardized method, based on ion-pair chromatography is given in Annex A (without the modifications presented in italics, which were only used for ring test B).

3.2 Test Samples

Four different samples were provided to all the participants. Two samples were commercial iron chelates in solid form, one containing Fe-EDDHA and the other containing Fe-EDDHMA. The other two samples were solutions prepared from commercially available standards.

3.3 Ring test A procedure

The test samples were sent to 15 private or official laboratories from seven countries of which only 14 provided results.

The participating laboratories were requested to carry out 2 replicate analyses of each sample according to each method.

Table 1 shows the samples analysed by each participating laboratory.

Table 1 — Samples analyzed by each participant laboratory

LABORATORY	STANDARDIZED METHOD				NON-STANDARDIZED METHOD			
	Commercial samples (solid form)		Solutions from standards		Commercial samples (solid form)		Solutions from standards	
	EDDHA	EDDHMA	EDDHA	EDDHMA	EDDHA	EDDHMA	EDDHA	EDDHMA
1	x	x	x	x	x	x	x	x
2	x	x	x	x	x	x	x	x
3	x	x	x	x	x	x	x	x
4	-	-	-	-	x	x	x	x
5	-	-	-	-	x	-	x	-
6	-	-	-	-	x	x	x	x
7	x	x	x	x	x	x	x	x
8	x	x	x	x	x	x	x	x
9	x	x	x	x	x	x	x	x
10	-	-	-	-	x	x	x	x
11	x	x	x	x	-	-	-	-
12	x	x	x	x	x	x	x[a]	x
13	-	-	-	-	x	x	x	x
14	x	x	x	x	x	x	x	x
15	-	-	-	-	-	-	-	-
TOTAL	9	9	9	9	13	12	13	12

[a] only one replicate was reported

NOTE Most of the laboratories which did not analyse samples by the standardized method did not have the necessary equipment for the determination given in EN 13368-2:2001.

Test results, observations and remarks have been reported on the appropriate sheets.

3.4 Results and statistical interpretation of ring test A

3.4.1 General

Statistical calculations have been carried out using all the results, according to ISO 5725-2 [4].

Parameters of repeatability and reproducibility have been evaluated for each sample and method (mean value, standard deviation of repeatability (s_r), standard deviation of reproducibility (s_R), repeatability limit (r), reproducibility limit (R), relative standard deviation of repeatability (RSD_r) and relative standard deviation of reproducibility (RSD_R).

3.4.2 Statistical results of ring test A

3.4.2.1 Repeatability and reproducibility

Table 2 shows the statistical results of the ring test for repeatability and reproducibility.

Table 2 — Statistical results for ring test A

Parameter	STANDARDIZED METHOD				NON-STANDARDIZED METHOD			
	Commercial samples (solid form)		Solutions from standards		Commercial samples (solid form)		Solutions from standards	
SAMPLE	EDDHA	EDDHMA	EDDHA	EDDHMA	EDDHA	EDDHMA	EDDHA	EDDHMA
Number of laboratories	9	9	9	9	13	12	13	12
Number of outliers	0	0	2	1	1	0	1	1
Number of laboratories after elimination of outliers	9	9	7	8	12	12	12	11
Mean value	3,04 gFe/100 g	4,58 gFe/100 g	24,47 mg/l	25,31 mg/l	3,09 g Fe/100 g	4,28 g Fe/100 g	24,04 mg/l	26,49 mg/l
Repeatability limit, *r*	0,21 gFe/100 g	0,36 gFe/100 g	0,47 mg/l	1,38 mg/l	0,04 g Fe/100 g	0,12 g Fe/100 g	0,20 mg/l	0,32 mg/l
RSD_r, %	2,5	2,8	0,7	2,0	0,5	1,0	0,3	0,4
Repeatability standard deviation s_r	0,07 gFe/100 g	0,13 gFe/100 g	0,17 mg/l	0,49 mg/l	0,02 g Fe/100 g	0,04 g Fe/100 g	0,07 mg/l	0,11 mg/l
Reproducibility limit, *R*	0,41 gFe/100 g	1,31 gFe/100 g	2,97 mg/l	7,21 mg/l	0,33 gFe/100 g	1,11 g Fe/100 g	2,58 mg/l	4,85 mg/l
RSD_R, %	4,8	10,2	4,3	10,2	3,8	9,2	3,8	6,5
Reproducibility standard deviation s_R	0,15 gFe/100 g	0,47 gFe/100 g	1,27 mg/l	2,57 mg/l	0,12 gFe/100 g	0,37 g Fe/100 g	0,92 mg/l	1,73 mg/l
Horwitz value *R*	3,4	3,2	9,9	9,8	3,4	3,2	9,9	9,8
Horrat *R* index	1,43	3,22	0,44	1,03	1,12	2,88	0,38	0,67

3.4.2.2 Reproducibility and repeatability significance

The comparison of the reproducibility and repeatability between the two methods for each sample is given in Table 3.

Table 3 — ANOVA test results for mean values by types of samples

PARAMETER	Commercial samples (solid form)		Solutions from standards	
SAMPLE	EDDHA	EDDHMA	EDDHA	EDDHMA
Calculated F (*r*)	21,83[a]	9,04[a]	5,57[a]	19,15[a]
Calculated F (*R*)	1,58	1,40	1,32	2,21
Tabulated F	2,95	2,95	3,09	3,14
[a] significant at $\alpha = 0{,}05$				

3.4.2.3 Accuracy comparison

A univariant multifactorial ANOVA test has been developed for the comparison of the means to examine the significance of the differences between the two test methods.

Table 4 — *R*- an *r*- values and their significance by types of samples

PARAMETER	Commercial samples (solid form)		Solutions from standards	
SAMPLE	EDDHA	EDDHMA	EDDHA	EDDHMA
Calculated F (Fischer)	139,24	8,82	95,44	274,42
Significance α	0,054	0,207	0,068	0,038

3.5 Conclusions for ring test A

3.5.1 General

Laboratories have provided more results by non-standardized method than by EN 13368-2.

In this ring test more results have been obtained for the standardized method than were available in the ring test carried out by CEN/TC 260 in the past. This gives a better estimate of the repeatability and reproducibility data.

3.5.2 Repeatability and reproducibility

The non-standardized method shows better repeatability for all samples, even for the solid samples where the complete procedure was applied (i.e. sample preparation and analytical determination). s_r is significantly lower for the non-standardized method.

There are no significant differences regarding s_R between the two methods, but the tendency is that the non-standardized method shows lower reproducibility values for all samples, even for the solid samples where the complete procedure shall be applied (i.e. sample preparation and analytical determination).

3.5.3 Accuracy comparison

There are no significant differences between the methods at $\alpha = 0{,}05$ as shown by the ANOVA test results (see Table 4) except for Fe-EDDHMA in solutions.

3.5.4 Final conclusions of the ring test A:

The results show good correlation between the two methods.

Data are not significant enough to confirm that the non-standardized method is better than the standardized method. Based on the comments of the participating laboratories (see Annex B), the non-standardized method could be improved with some modifications, and then it might result in significant differences with respect to the standardized method.

For this reason, ring test on the non-standardized method should be repeated with the following modifications (see modifications given in italics in Annex A):

- Preparation of the standards:
  - Iron nitrate is deliquescent and therefore it shall be added as a solution of a known concentration.
  - To determine the titrimetric purity of the chelating agent standards if there is any doubt on the actual purity.
  - For the dissolution of the standards it is important to use CO_2 free fresh alkali solution.
- Sample preparation
  - To remove the filtration with cellulose filters.
- Calibration curves:
  - To use six standards and two replicates for each standard.
  - Significant statistical negative intercept could be caused due to the contamination of the column.
- Integration:
  - If overlapping occurs, baseline corrections in the integration should be made (see Figure A.4).

These modifications are included in Annex A in italics in the relevant positions.

4 Test procedure for ring test B

4.1 Method used in ring test B for the determination of chelating agents EDDHA and EDDHMA by chromatography.

The samples were tested only by the non-standardized method including the modifications suggested by CEN/TC 260/WG 5 and included in Annex A in italics .

4.2 Test Samples

Seven different samples have been provided to all the participants. Five samples were commercial iron chelates in solid form, three containing Fe-EDDHA and two containing Fe-EDDHMA. The other two samples were solutions prepared from commercially available standards, one containing Fe-EDDHA and the other containing Fe-EDDHMA.

4.3 Ring test B procedure

The test samples were sent to 16 private and official laboratories from 8 countries, of which only 14 provided results.

The participating laboratories were requested to carry out 2 replicate analyses on each sample using the non-standardized method including the modifications in italics. The results of the two replicates of each sample should be reported to two decimal places.

Table 5 shows the samples that were tested by each participating laboratory.