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Fertilizers, soil conditioners and beneficial substances — Simultaneous determination of N-(n-Butyl) thiophosphoric triamide and dicyandiamide by high-performance liquid chromatography

Engrais, Amendements et Substances Bénéfiques — Détermination Simultanée du N-buthylthiophosphore Triamide (NBPT) et du Dicyandiamide (DCD) par Chromatographie Liquide à Haute Performance (HPLC)

Reference number
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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 134, *Fertilizers, soil conditioners and beneficial substances*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

The stability of urea today is receiving greater attention due to a major increase in no-tillage or minimum-tillage crop production. N-(n-butyl) thiophosphoric triamide (NBPT) can slow urea breakdown by controlling the activity of the urease enzyme. Dicyandiamide (DCD) temporarily inhibits nitrification by deactivating the enzyme of ammonia monooxygenase (AMO) in ammonia-oxidizing microbes. This document provides a method to determine the content of NBPT and DCD in nitrogen fertilizers simultaneously, which will help governmental authorities, fertilizer producers and consumers around the world. Also, it can save time and protect the environment by reducing experimental waste.

Fertilizers, soil conditioners and beneficial substances — Simultaneous determination of N-(n-Butyl) thiophosphoric triamide and dicyandiamide by high-performance liquid chromatography

1 Scope

This document specifies the analytical method for the simultaneous determination of N-(n-butyl) thiophosphoric triamide (NBPT) and dicyandiamide (DCD) in fertilizers by high-performance liquid chromatography (HPLC) method.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 8157, *Fertilizers, soil conditioners and beneficial substances — Vocabulary*

ISO 8358, *Solid fertilizers — Preparation of samples for chemical and physical analysis*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 8157 and the following apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
N-(n-butyl) thiophosphoric triamide
NBPT
white crystalline solid major urease inhibitor of commercial and practical importance in agriculture

Note 1 to entry: CAS Registry Number®[1] 94317-64-3.

3.2
dicyandiamide
DCD
2-cyanoguanidine
nitrification inhibitor and slow-release nitrogen source that has 4 % to 5 % solubility in water

Note 1 to entry: DCD, $C_2H_4N_4$ (CAS 461-58-5).

1 Chemical Abstracts Service (CAS) Registry Number® is a trademark of the American Chemical Society (ACS). This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

4 Principle

This analytical method is based on the principles of liquid chromatography, with the absorption in the ultraviolet region is used for detection of separated compounds.

5 Reagents

During the analysis unless otherwise stated, use only reagents of recognized analytical grade (AR) and only water in accordance with ISO 3696 with electrical resistivity ≥18,2 MΩ·cm.

5.1 Acetonitrile (ACN), HPLC grade.

5.2 N-(n-butyl) thiophosphoric triamide (NBPT), assay, of a mass fraction ≥98 %.

5.3 Dicyandiamide (DCD) assay, of a mass fraction ≥99 %.

5.4 Stock solutions.

5.4.1 NBPT stock solution (1 000 mg/l).

Weigh to the nearest 0,1 mg, approximately 250 mg of NBPT into a 250 ml volumetric flask and dissolve to volume with water.

5.4.2 DCD stock solution (1 000 mg/l).

Weigh to the nearest 0,1 mg, approximately of 250 mg DCD into a 250 ml volumetric flask and dissolve to volume with water.

5.5 Standard solutions.

Prepare standard solutions (including both NBPT and DCD) in volumetric flasks in concentrations ranges listed in Table 1.

The concentration ranges for standard solutions may be adjusted according to the levels expected in the samples.

Table 1 — Preparation of standard solutions

Standard solution	NBPT mg/l	DCD mg/l
Blank	0	0
Standard 1	5,0	1,0
Standard 2	10,0	2,5
Standard 3	25,0	10,0
Standard 4	50,0	25,0
Standard 5	100,0	100,0

6 Apparatus and materials

The usual laboratory apparatus and, in particular, the following shall be used.

6.1 HPLC apparatus with photo-diode array (PDA) detector (dual or multiple wavelengths, recommended) or UV detector.

6.2 Analytical balance, to the nearest of 0,1 mg.

6.3 Ultrasonic bath.

6.4 Membrane filter, 0,45 µm, with the usual filtration equipment.

7 Test procedure

7.1 General

Two replicate experiments shall be done for the determination.

7.2 Preparation of test sample

For solid fertilizers, prepare a test portion by reducing the fertilizer sample to 100 g in accordance with ISO 8358. Grind the sample until it passes through a sieve of aperture size 0,5 mm and mix until homogenous. Place in a clean and dry bottle with a lid. For liquid fertilizers, shake the fertilizer sample up until homogenous and pour out 100 ml. Place in a clean and dry bottle with a lid.

7.3 Preparation of the test solution

Accurately weigh to the nearest 0,1 mg, an appropriate amount (0,1 g to 3 g) of the ground test portion and mix with 100 ml ultra-pure water into a 250 ml volumetric flask and dissolve using an ultrasonic bath for 20 min. Make up the volume to the mark with water. Filter one part of the homogenized sample solution through the membrane filter (6.4).

7.4 HPLC conditions

7.4.1 Column: 250 mm × 4,6 mm C18 reversed-phase column.

7.4.2 Injection volume: 10 µl.

7.4.3 Detector: PDA detector (214 nm for DCD, 205 nm for NBPT, recommended) or UV-detector with absorbance set to 214 nm only.

7.4.4 Eluent: Mixture of acetonitrile (5.1) and water, gradient shown in Table 2.

Table 2 — Gradient elution schedule

Time min	Acetonitrile %	Water %
0	5	95
3	5	95
10	25	75
16	25	75
17	5	95
31	5	95

7.3.5 Flow rate: 1,0 ml/min.

7.5 Determination of standard working solutions and sample test solutions

7.5.1 Determination of standard working solutions

Use NBPT and DCD standard solutions (5.5) to prepare the standard working solutions given in Table 1. Plot the standard curves using the concentration of NBPT and DCD, corresponding to the peak areas obtained in the test.

7.5.2 Determination of sample test solutions

Test a blank solution and the sample test solutions under the same conditions as the standards. Use the retention times to identify NBPT and DCD, and derive the concentrations of NBPT and DCD, in the test solutions from the standard curves, as shown in Annex A.

It is highly recommended that the DCD shall be determined under the wavelength of 214 nm, while the NBPT shall be determined under the wavelength of 205 nm.

Only if the HPLC equipped with a single-wavelength UV detector, consider testing the DCD and NBPT both under the wavelength of 214 nm.

The blank solution is prepared in the same manner as the test solutions, except for adding any test samples.

If the response value (peak area) of any compound in a test solution exceeds the linear calibration range of a standard solution, appropriate dilutions should be prepared.

8 Calculation and expression of results

8.1 General

The mass fraction of NBPT and DCD, w, in the unit of %, is calculated as follows:

$$w = \frac{\rho \times V \times f}{m \times 10\,000}$$

where

ρ is the mass concentration of NBPT and DCD, expressed in milligrams per litre of the test solutions;

V is the volume of the test solutions, expressed in millilitres;

f is the dilution factor of the test solutions;

m is the mass of the test portion, expressed in grams.

The determination result is the arithmetic average of two parallel determination results and shall be rounded off to three significant figures.

8.2 Precision

8.2.1 Ring test

Details of ring test on the precision of the method are summarized in Annex B.

8.2.2 Repeatability, r

For DCD content of all levels, the repeatability limit, r, is $0{,}067w - 0{,}014$, in mass fraction percentage.

For NBPT content of all levels, the repeatability limit, *r,* is 0,074*w* − 0,011, in mass fraction percentage.

8.2.3 Reproducibility, *R*

For DCD content of all levels, the reproducibility limit, *R,* is 0,160*w* − 0,021, in mass fraction percentage.

For NBPT content of all levels, the reproducibility limit, *R,* is 0,137*w* + 0,034, in mass fraction percentage.

9 Test report

The test report shall contain at least the following information:

a) all information necessary for the complete identification of the sample;

b) the International Standard used (including its year of publication);

c) test method used with reference to this document;

d) test results obtained (signal-to-noise ratio plots per unknown, instrument detection limits and percent recoveries are suggested to be presented, if necessary;

e) date of sampling and sampling procedure (if known);

f) date of analysis completion;

g) whether or not the requirement of the repeatability limit is fulfilled;

h) any deviations from the procedure;

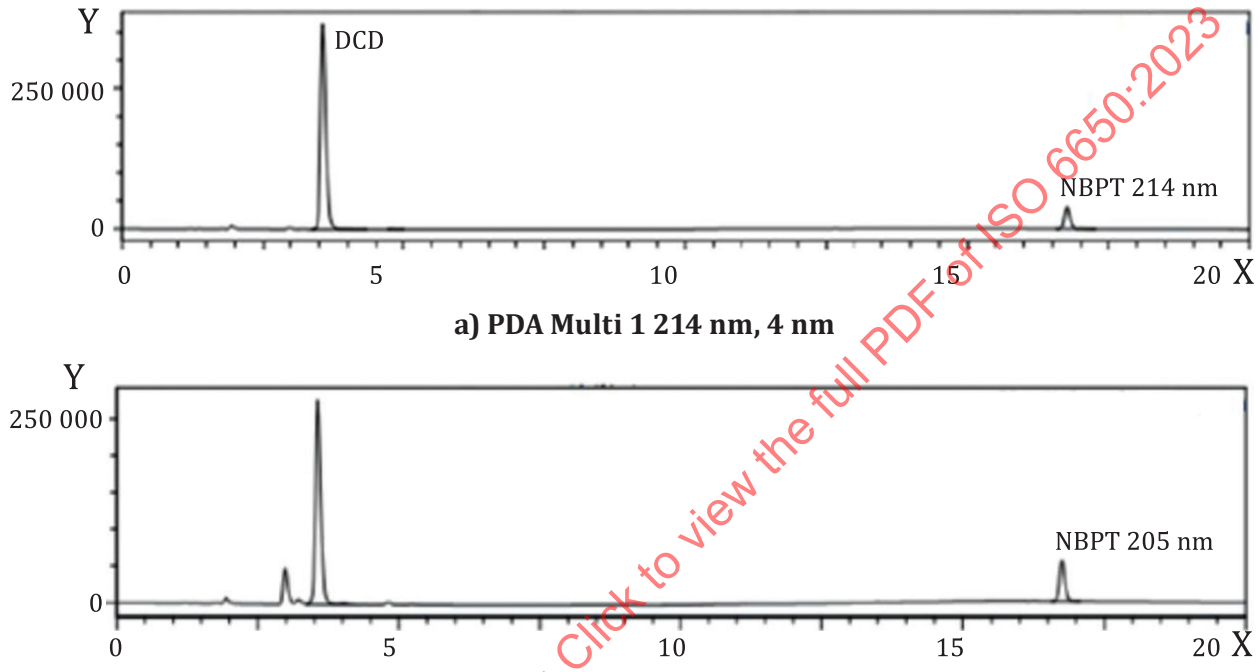
i) any unusual features observed;

j) all operating details not specified in this document, or regarded as optional, together with details of any incidents occurred when performing the method, which can have influenced the test results.

Annex A
(normative)

Typical HPLC chromatogram of DCD and NBPT

A typical HPLC chromatogram of DCD and NBPT of urea-based fertilizer is shown in Figure A.1.



a) PDA Multi 1 214 nm, 4 nm

b) PDA Multi 2 205 nm, 4 nm

Figure A.1 — Typical HPLC chromatogram of DCD and NBPT of a urea-based fertilizer

Annex B
(informative)

Interlaboratory test report of the International Laboratories Ring Test

B.1 Overview

The International Laboratories Ring Test for this document was done between December 2022 to March 2023. Eighteen laboratories participated in the two parallel tests on four test samples. This international interlaboratory test and the statistician analysis/final report was accomplished by Shanghai Institute of Chemical Industry Testing, Co., Ltd., P. R. China and Shanghai Research Institute of Chemical Industry, Co., Ltd., P. R. China. Eighteen participating laboratories around the world which have successfully participated in the interlaboratory test are listed as follows:

— Center for Soil Testing and Fertilizer Analysis, Agricultural Resources and Regional Planning Institute, Chinese Academy of Agricultural Sciences, P. R. China

— Fujian Inspection and Research Institute for Product Quality, P. R. China

— Guizhou Institute of Products Quality Inspection and Testing, P. R. China

— Hebei Research Institution for Product Quality Supervision and Inspection, P. R. China

— Hunan Provincial Institute of Product and Goods Quality Inspection, P. R. China

— Jiangsu product quality testing and inspection institute, P. R. China

— Linyi Testing and Inspection Centre, P. R. China

— Ministry of Agriculture, Food and Forestry - Dept of ICQRF - Laboratory of Catania, Italy

— National Testing and Certification International Group Jingcheng Testing Co., Ltd, P. R. China

— Office of Indiana State Chemist, USA

— Shandong Institute for Product Quality Inspection, P. R. China

— Shandong Zhongsheng Huajian Certification and Testing Co., Ltd, P. R. China

— Shanghai Institute of Chemical Industry Testing Co., Ltd., P. R. China

— Sichuan Research Institute of Chemical Quality and Safety Testing, P. R. China

— Stanley Agriculture Group Co., Ltd., P. R. China

— University of Kentucky - Division of Regulatory Services, USA

— Xinjiang Uygur Autonomous Region Product Quality Supervision and Inspection Institute, P. R. China

— Yunnan Supervision and Inspection Institute for Product Quality, P. R. China

NOTE Participating laboratories are listed in the alphabetic order, which has no relation to the sequences listed in Table B.1 to B.12.

The test method described in this document was adopted for this ring test to determine the DCD and NBPT content in fertilizer samples.

Four different types of fertilizer samples were used during the ring test, each with its well-designed mean level of DCD and NBPT content. The test samples were: sample 6650-A (urea), sample 6650-B (urea), sample 6650-C (complex fertilizer), sample 6650-D (urea ammonium nitrate solution).

The DCD and NBPT content (mass fraction %) to be determined and involved in the statistics in the four fertilizer samples generally lie in the range of 0,2 % to 5 %.

The precision of the test results is evaluated according to ISO 5725-2.

B.2 Statistical analysis of the test results of DCD contents

B.2.1 Original test results

Eighteen laboratories have participated in the determination of DCD contents in the four fertilizer samples (A, B, C and D). The results are listed in Table B.1, as mass fraction %. The test results of all four samples reported by laboratory 2 have too large a systematic error in the level of its test results. Laboratory 2 was considered as an outlying laboratory. The data of DCD contents results from laboratory 2 were therefore discarded.

Table B.1 — Original test results of the determination of DCD contents

Laboratory	DCD at level *j* mass fraction %							
	A		B		C		D	
1	0,885	0,915	4,593	3,870	1,802	1,843	0,198	0,201
2	—	—	—	—	—	—	—	—
3	0,882	0,894	4,068	4,231	1,665	1,591	0,178	0,168
4	0,998	0,999	4,299	4,255	1,832	1,828	0,199	0,199
5	0,980	0,984	4,665	4,717	1,896	1,891	0,199	0,198
6	0,990	0,990	4,274	4,258	1,810	1,811	0,193	0,196
7	1,039	1,095	4,810	4,732	1,968	1,982	0,196	0,200
8	1,112	1,126	5,039	4,863	1,969	1,932	0,212	0,211
9	1,054	1,027	4,403	4,261	1,869	1,943	0,203	0,203
10	0,995	0,992	4,558	4,712	1,993	1,946	0,203	0,199
11	0,954	0,972	4,137	4,268	1,811	1,785	0,200	0,197
12	1,020	0,935	4,560	4,410	2,020	1,940	0,206	0,207
13	1,000	1,013	4,779	5,050	1,920	1,884	0,204	0,204
14	0,947	0,993	4,666	4,499	1,932	1,906	0,170	0,165
15	1,090	1,085	4,533	4,625	2,032	1,951	0,193	0,195
16	1,083	1,135	4,700	4,608	2,022	2,081	0,206	0,210
17	1,030	1,020	4,665	4,782	2,001	1,959	0,199	0,199
18	1,032	1,052	4,851	4,576	1,870	1,829	0,202	0,201

B.2.2 Cell means by each laboratory

The cell means (means of the analyses) by each laboratory for the determination of DCD contents are listed in Table B.2, as mass fraction %.

Table B.2 — Cell means of the determination of DCD contents

Laboratory	DCD at level *j* mass fraction %			
	A	B	C	D
1	0,900 0	4,231 5	1,822 5	0,199 5
2	—	—	—	—
3	0,888 0	4,149 5	1,628 0	0,173 0
4	0,998 5	4,277 0	1,830 0	0,199 0
5	0,982 0	4,691 0	1,893 5	0,198 5
6	0,990 0	4,266 0	1,810 5	0,194 5
7	1,067 0	4,771 0	1,975 0	0,198 0
8	1,119 0	4,951 0	1,950 5	0,211 5
9	1,040 5	4,332 0	1,906 0	0,203 0
10	0,993 5	4,635 0	1,969 5	0,201 0
11	0,963 0	4,202 5	1,798 0	0,198 5
12	0,977 5	4,485 0	1,980 0	0,206 5
13	1,006 5	4,914 5	1,902 0	0,204 0
14	0,970 0	4,582 5	1,919 0	0,167 5
15	1,087 5	4,579 0	1,991 5	0,194 0
16	1,109 0	4,654 0	2,051 5	0,208 0
17	1,025 0	4,723 5	1,980 0	0,199 0
18	1,042 0	4,713 5	1,849 5	0,201 5

B.2.3 Cell absolute differences of the analyses by each laboratory

The cell absolute differences of the analyses by each laboratory for the determination of DCD contents are listed in Table B.3, as mass fraction %.

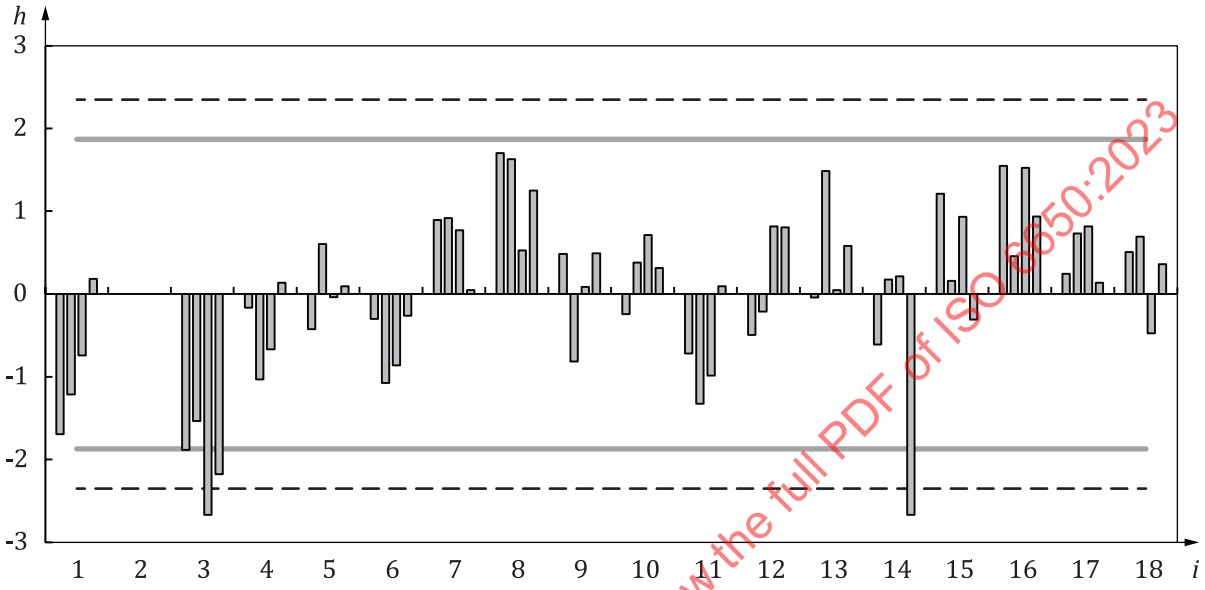
Table B.3 — Cell absolute differences of the determination of DCD contents

Laboratory	DCD at level *j* mass fraction %			
	A	B	C	D
1	0,030	0,723	0,041	0,003
2	—	—	—	—
3	0,012	0,163	0,074	0,010
4	0,001	0,044	0,004	0,000
5	0,004	0,052	0,005	0,001
6	0,000	0,016	0,001	0,003
7	0,056	0,078	0,014	0,004
8	0,014	0,176	0,037	0,001
9	0,027	0,142	0,074	0,000
10	0,003	0,154	0,047	0,004
11	0,018	0,131	0,026	0,003
12	0,085	0,150	0,080	0,001
13	0,013	0,271	0,036	0,000
14	0,046	0,167	0,026	0,005
15	0,005	0,092	0,081	0,002
16	0,052	0,092	0,059	0,004
17	0,010	0,117	0,042	0,000
18	0,020	0,275	0,041	0,001

B.2.4 Evaluation of the results for consistency and outliers

B.2.4.1 General

Graphical evaluation of the analytical results for consistency by Mandel's *h* and *k* statistics were studied; the interlaboratory consistency Mandel statistic *h* and the intralaboratory consistency Mandel statistic *k*, for each level of each laboratory were calculated. The *h* and *k* values for each cell for the respective laboratories were plotted to obtain the Mandel's *h* and *k* graphs (see Figures B.1 and B.2).

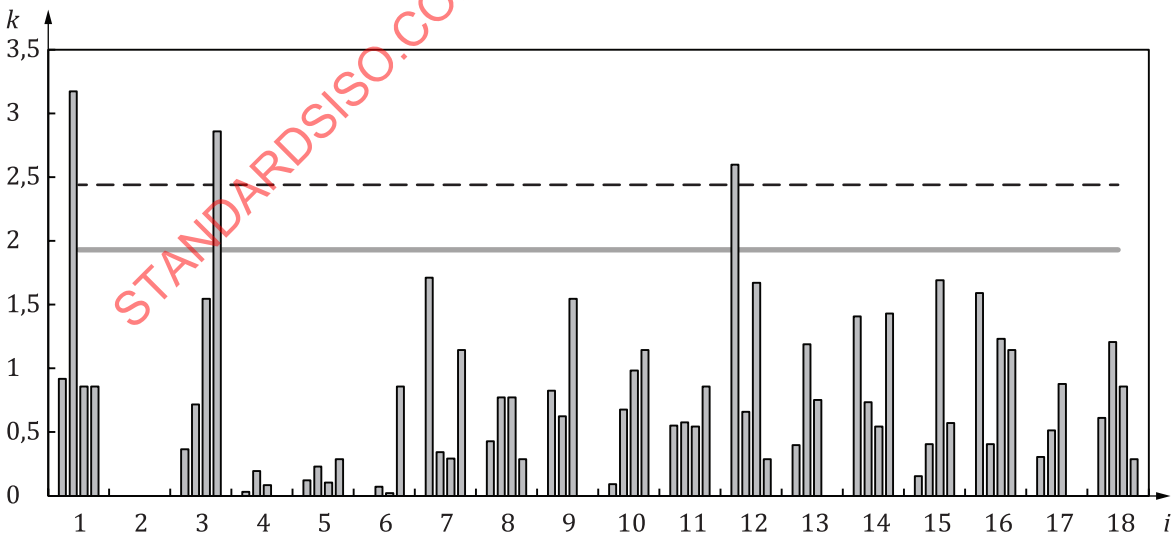


Key

h Mandel's statistic

i laboratory

Figure B.1 — Mandel's interlaboratory consistency statistic, *h*, grouped by laboratories



Key

k Mandel's statistic

i laboratory

Figure B.2 — Mandel's intralaboratory consistency statistic, *k*, grouped by laboratories

The Mandel's interlaboratory consistency statistic *h* graph indicated that laboratory 3 can have one outlier on level C and laboratory 14 can have one outlier on level D.

The Mandel's intralaboratory consistency statistic *k* graph did exhibit some variability between replicate test results for laboratory 1 on level B (outlier), laboratory 3 on level D (outlier) and laboratory 12 on level A (outlier).

B.2.4.2 Cochran's test

Cochran's test is the test of the intralaboratory variability and should be applied first, then any necessary action should be taken, and also with repeated tests if necessary.

The application of Cochran's test led to the values of the test statistic *C* given in Table B.4.

Table B.4 — Values of Cochran test statistic, *C*

Before scrutiny	**Level** *j*				**Type of test**
	A	B	C	D	
C (Cochran)	0,397 1	0,592 4[a]	0,168 3	0,480 8[b]	Cochran's test statistics
Stragglers (5 %)	0,434 (p = 17, *n* = 2)	0,434 (p = 17, *n* = 2)	0,434 (p = 17, *n* = 2)	0,434 (p = 17, *n* = 2)	Cochran's critical values
Outliers (1 %)	0,532 (p = 17, *n* = 2)	0,532 (p = 17, *n* = 2)	0,532 (p = 17, *n* = 2)	0,532 (p = 17, *n* = 2)	Cochran's critical values
After scrutiny	**Level** *j*				**Type of test**
	A	B[a]	C	D[b]	
C (Cochran)	0,397 1	0,210 3	0,168 3	0,231 5	Cochran's test statistics
Stragglers (5 %)	0,434 (p = 17, *n* = 2)	0,452 (p = 16, *n* = 2)	0,434 (p = 17, *n* = 2)	0,452 (p = 16, *n* = 2)	Cochran's critical values
Outliers (1 %)	0,532 (p = 17, *n* = 2)	0,553 (p = 16, *n* = 2)	0,532 (p = 17, *n* = 2)	0,553 (p = 16, *n* = 2)	Cochran's critical values

[a] This data is an outlier.

[b] This data is a straggler.

If the test statistic is greater than its 5 % critical value and less than or equal to its mass fraction of 1 % critical value, the item tested is regarded as a straggler.

If the test statistic is greater than its 1 % critical value, the item tested is regarded as an outlier.

Cochran's test showed that the test statistic reached 0,592 4, calculated by the maximum cell absolute difference from laboratory 1 on level B.

The Cochran's critical value at the 1 % significance level was 0,532, for p = 17 and *n* = 2, therefore the test results from laboratory 1 on level B is an outlier, which should be discarded.

Cochran's test showed that the test statistic reached 0,480 8, calculated by the maximum cell absolute difference from laboratory 3 on level D.

The Cochran's critical value at the 1 % significance level was 0,532, at the 5 % significance level was 0, 434, for p = 17 and *n* = 2, therefore the test results from laboratory 3 on level D is a straggler, which should be discarded.

Cochran's tests (p = 16, n = 2) were repeated on the remaining tests values from the remain 16 laboratories on level B. The test statistic obtained this time was 0,210 3. The values was less than the Cochran's critical value at the 5 % significance level (0,452, p = 16, n = 2). This confirmed that no outlier or straggler existed in level B by Cochran's test anymore.

Cochran's tests (p = 16, n = 2) were repeated on the remaining tests values from the remain 16 laboratories on level D. The test statistic obtained was 0,231 5. The value was less than the Cochran's critical value at the 5 % significance level (0,452, p = 16, n = 2). This confirmed that no outlier or straggler existed in level D by Cochran's test anymore.

B.2.4.3 Grubbs' test

The Grubbs' test is primarily a test of interlaboratory variability. The test data used herein are those which have passed the Cochran's test.

The application of Grubbs' test to cell means led to the values of the test statistic G shown in Table B.5.

Table B.5 — Application of Grubbs' test to cell means

Level j;p	Single low	Single high	Double low	Double high	Type of test
A;17	1,882	1,700	0,626 3	0,545 8	Grubbs' test statistics
B;16	1,644	1,582	0,652 6	0,638 5	
C;17	2,667[b]	1,525	0,775 5	0,439 2	
D;16	3,279[a]	1,301	0,804 7	0,196 2[a]	
C[b];16	1,535	1,810	0,674 3	0,677 8	
D [a];15	1,472	2,157	0,450 9	0,667 1	
Stragglers (5 %)					Grubbs' critical values
p = 17	2,620	2,620	0,382 2	0,382 2	
p = 16	2,585	2,585	0,360 3	0,360 3	
p = 15	2,549	2,549	0,336 7	0,336 7	
Outliers (1 %)					
p = 17	2,894	2,894	0,299 0	0,299 0	
p = 16	2,852	2,852	0,276 7	0,276 7	
p = 15	2,805	2,805	0,253 0	0,253 0	

[a] This data is an outlier.

[b] This data is a straggler.

For the Grubbs' test for one outlying observation, outliers and stragglers give rise to values which are larger than its 1 % and 5 % critical values respectively.

For the Grubbs' test for two outlying observation, outliers and stragglers give rise to values which are smaller than its 1 % and 5 % critical values respectively.

The application of the Grubbs' test to the cell means indicated that the test results from laboratory 3 on level C is a straggler, and test results from laboratory 14 on level D is an outlier, which should be discarded. After discarding the data, the Grubbs' test was reapplied to the remaining data and confirmed that there were no more outliers or stragglers.

B.2.5 Calculation of the general mean and standard deviations

The calculation of the general mean w, repeatability standard deviation s_r, reproducibility standard deviation s_R of DCD contents in each sample is listed in Table B.6, as mass fraction %.

Table B.6 — Calculation results of the general mean, s_r and s_R of DCD contents

Sample	Level			
	A	B	C	D
Number of laboratories	17	16	16	15
Outliers or stragglers	0	1	1	2
General mean, w, percentage content, mass fraction %	1,009	4,558	1,914	0,201
Repeatability standard deviation, s_r, mass fraction %	0,023 1	0,106 0	0,032 4	0,001 7
Reproducibility standard deviation, s_R, mass fraction %	0,066 5	0,259 6	0,079 2	0,005 0

B.2.6 Dependence of precision on general mean (level), w

As shown in Table B.6, the repeatability standard deviations, s_r, show a linear relationship with the general mean (level), w: $s_r = 0{,}023\ 8w - 0{,}004\ 9$, $R^2 = 0{,}984\ 7$.

The reproducibility standard deviations, s_R, show a linear relationship with the general mean (level), w: $s_R = 0{,}057\ 2w - 0{,}007\ 4$, $R^2 = 0{,}977\ 0$.

For all levels, the repeatability standard deviation, s_r, is $0{,}023\ 8w - 0{,}004\ 9$.

For all levels, the reproducibility standard deviation, s_R, is $0{,}057\ 2w - 0{,}007\ 4$.

B.2.7 Final values of precision

The precision of the DCD contents measurements was discerned from Table B.6.

The conclusion above was determined from a uniform-level experiment involving 18 laboratories, in which laboratory 2 was considered as an outlying laboratory, one test value from laboratory 1 on level B, two test values from laboratory 3 on level C and D, and one test value from laboratory 14 on level D have been discarded as outliers or stragglers.

The precision of the available phosphorus contents measurement method should be quoted as follows:

— repeatability standard deviation: $s_r = 0{,}023\ 8w - 0{,}004\ 9$ (mass fraction %).

— reproducibility standard deviation: $s_R = 0{,}057\ 2w - 0{,}007\ 4$ (mass fraction %).

B.3 Statistical analysis of the test results of NBPT contents

B.3.1 Original test results

Eighteen laboratories have participated in the determination of NBPT contents in the four fertilizer samples (A, B, C and D). The results are listed in Table B.7, as mass fraction %.

Table B.7 — Original test results of the determination of NBPT contents

Laboratory	NBPT at level *j* mass fraction %							
	A		B		C		D	
1	0,845	0,863	4,638	3,725	1,273	1,284	0,165	0,166
2	1,025	1,094	4,309	4,569	1,359	1,360	0,215	0,195
3	0,829	0,836	3,972	4,178	1,665	1,591	0,153	0,133
4	0,951	0,947	4,210	4,135	1,310	1,328	0,179	0,179
5	0,918	0,923	4,484	4,484	1,356	1,357	0,175	0,175
6	0,931	0,928	4,086	4,089	1,268	1,301	0,176	0,173
7	0,931	0,983	4,426	4,359	1,336	1,351	0,177	0,176
8	0,906	0,931	4,430	4,199	1,282	1,276	0,165	0,158
9	0,983	0,970	4,234	4,099	1,320	1,381	0,171	0,170
10	0,876	0,900	4,243	4,400	1,309	1,332	0,200	0,196
11	0,910	0,908	3,969	4,189	1,284	1,287	0,177	0,175
12	1,010	0,936	4,380	4,250	1,480	1,350	0,212	0,213
13	0,945	0,973	4,576	4,761	1,553	1,331	0,168	0,168
14	0,982	0,969	4,274	4,143	1,284	1,245	0,158	0,172
15	0,985	0,998	4,367	4,477	1,349	1,324	0,195	0,195
16	0,962	0,992	4,130	4,074	1,376	1,425	0,177	0,176
17	0,799	0,790	3,753	3,852	1,157	1,131	0,222	0,222
18	1,039	0,982	4,544	4,298	1,201	1,242	0,170	0,163

B.3.2 Cell means by each laboratory

The cell means (means of the analyses) by each laboratory for the determination of NBPT contents are listed in Table B.8, as mass fraction %.

Table B.8 — Cell means of the determination of NBPT contents

Laboratory	NBPT at level *j* mass fraction %			
	A	B	C	D
1	0,854 0	4,181 5	1,278 5	0,165 5
2	1,059 5	4,439 0	1,359 5	0,205 0
3	0,832 5	4,075 0	1,628 0	0,143 0
4	0,949 0	4,172 5	1,319 0	0,179 0
5	0,920 5	4,484 0	1,356 5	0,175 0
6	0,929 5	4,087 5	1,284 5	0,174 5
7	0,957 0	4,392 5	1,343 5	0,176 5
8	0,918 5	4,314 5	1,279 0	0,161 5
9	0,976 5	4,166 5	1,350 5	0,170 5
10	0,888 0	4,321 5	1,320 5	0,198 0
11	0,909 0	4,079 0	1,285 5	0,176 0
12	0,973 0	4,315 0	1,415 0	0,212 5
13	0,959 0	4,668 5	1,442 0	0,168 0
14	0,975 5	4,208 5	1,264 5	0,165 0
15	0,991 5	4,422 0	1,336 5	0,195 0
16	0,977 0	4,102 0	1,400 5	0,176 5
17	0,794 5	3,802 5	1,144 0	0,222 0
18	1,010 5	4,421 0	1,221 5	0,166 5

B.3.3 Cell absolute differences of the analyses by each laboratory

The cell absolute differences of the analyses by each laboratory for the determination of NBPT contents are listed in Table B.9, as mass fraction %.

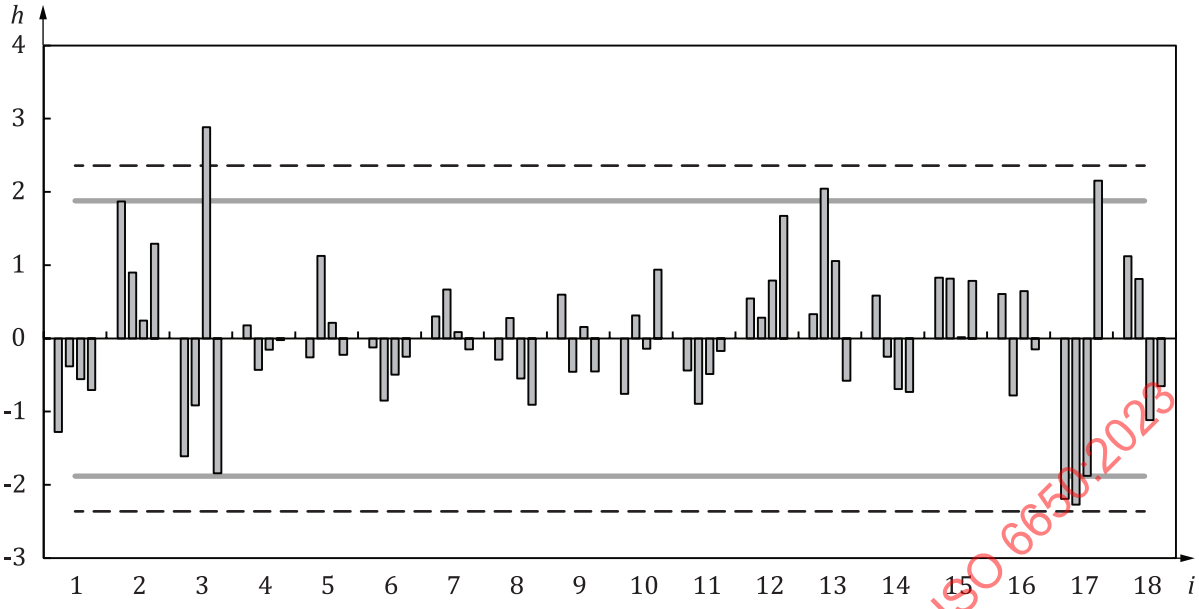
Table B.9 — Cell absolute differences of the determination of NBPT contents

Laboratory	NBPT at level *j* mass fraction %			
	A	B	C	D
1	0,018	0,913	0,011	0,001
2	0,069	0,260	0,001	0,020
3	0,007	0,206	0,074	0,020
4	0,004	0,075	0,018	0,000
5	0,005	0,000	0,001	0,000
6	0,003	0,003	0,033	0,003
7	0,052	0,067	0,015	0,001
8	0,025	0,231	0,006	0,007
9	0,013	0,135	0,061	0,001
10	0,024	0,157	0,023	0,004
11	0,002	0,220	0,003	0,002
12	0,074	0,130	0,130	0,001
13	0,028	0,185	0,222	0,000
14	0,013	0,131	0,039	0,014
15	0,013	0,110	0,025	0,000
16	0,030	0,056	0,049	0,001
17	0,009	0,099	0,026	0,000
18	0,057	0,246	0,041	0,007

B.3.4 Evaluation of the results for consistency and outliers

B.3.4.1 General

Graphical evaluation of the analytical results for consistency by Mandel's *h* and *k* statistics were studied. The interlaboratory consistency Mandel's statistic *h* and the intralaboratory consistency Mandel statistic's *k*, for each level of each laboratory were calculated. The *h* and *k* values for each cell for the respective laboratories were plotted to obtain the Mandel's *h* and *k* graphs (see Figures B.3 and B.4).

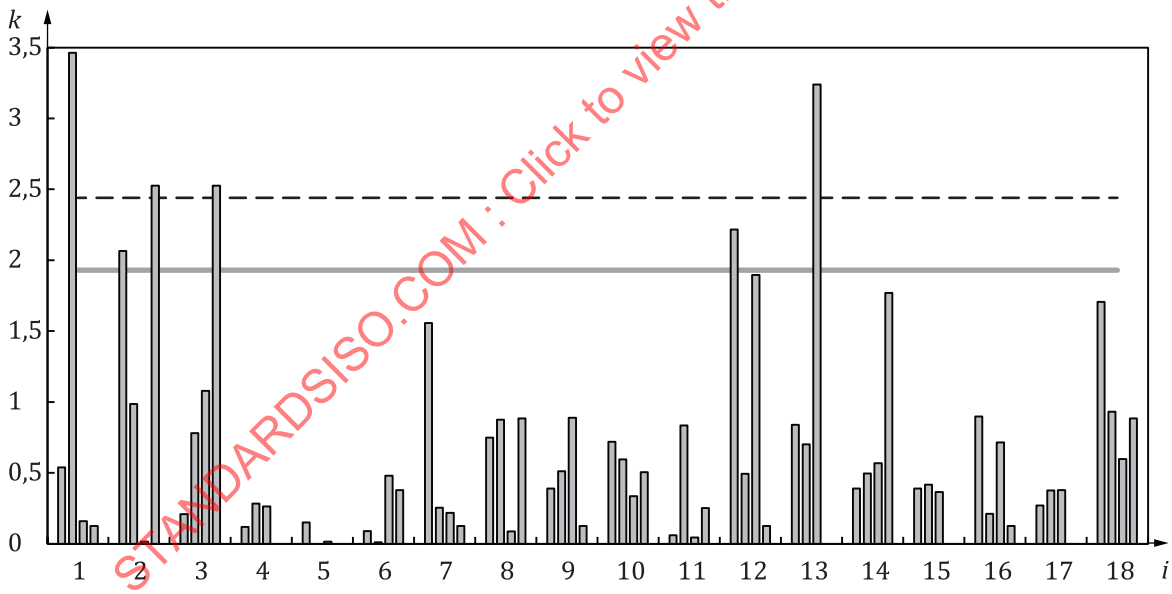


Key

h Mandel's statistic

i laboratory

Figure B.3 — Mandel's interlaboratory consistency statistic, *h*, grouped by laboratories



Key

k Mandel's statistic

i laboratory

Figure B.4 — Mandel's intralaboratory consistency statistic, *k*, grouped by laboratories

The Mandel's interlaboratory consistency statistic *h* graph indicated that laboratory 3 can have one outliers on level C.

The Mandel's intralaboratory consistency statistic *k* graph did exhibit some variability between replicate test results for laboratory 1 on level B (outlier), laboratory 2 and laboratory 3 on level D (outlier), and laboratory 13 on level C (outlier).