
**Fertilizers and soil conditioners —
Determination of biuret content of
urea-based fertilizers — HPLC method**

Engrais et amendements — Détermination de la teneur en biuret des engrais à base d'urée — Méthode HPLC

iTeh STANDARD PREVIEW
(standards.iteh.ai)

[ISO 18643:2016](https://standards.iteh.ai/catalog/standards/sist/17bb1760-9f33-46c1-8cbb-beed97b73f6/iso-18643-2016)

<https://standards.iteh.ai/catalog/standards/sist/17bb1760-9f33-46c1-8cbb-beed97b73f6/iso-18643-2016>



Reference number
ISO 18643:2016(E)

© ISO 2016

iTeh STANDARD PREVIEW
(standards.iteh.ai)

ISO 18643:2016

<https://standards.iteh.ai/catalog/standards/sist/17bb1760-9f33-46c1-8cbb-beedf97b73f6/iso-18643-2016>



COPYRIGHT PROTECTED DOCUMENT

© ISO 2016, Published in Switzerland

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

Page

| | |
|--|-----------|
| Foreword | iv |
| Introduction | v |
| 1 Scope | 1 |
| 2 Normative references | 1 |
| 3 Principle | 1 |
| 4 Reagents | 1 |
| 5 Apparatus | 1 |
| 6 Procedure | 2 |
| 6.1 Preparation of test sample..... | 2 |
| 6.2 Preparation of test solution..... | 2 |
| 6.3 Preparation of biuret working standard solutions..... | 2 |
| 6.4 HPLC conditions..... | 2 |
| 6.5 Preparation of standard curve..... | 3 |
| 6.6 Determination of the biuret content in the test solution..... | 3 |
| 6.7 Calculation and expression of the results..... | 3 |
| 6.8 Precision..... | 3 |
| 6.8.1 Ring test..... | 3 |
| 6.8.2 Repeatability, r | 4 |
| 6.8.3 Reproducibility, R | 4 |
| 7 Test report | 4 |
| Annex A (informative) Report of international laboratories ring test | 5 |
| Annex B (informative) Alternative method for determination of biuret in fertilizers | 11 |
| Bibliography | 12 |

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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 documents 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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information.

The committee responsible for this document is ISO/TC 134, *Fertilizers and soil conditioners*.

[ISO 18643:2016](https://standards.iteh.ai/catalog/standards/sist/17bb1760-9f33-46c1-8cbb-beed97b73f6/iso-18643-2016)

<https://standards.iteh.ai/catalog/standards/sist/17bb1760-9f33-46c1-8cbb-beed97b73f6/iso-18643-2016>

Introduction

Biuret, also known as 2-imidodicarbonic diamide ($\text{NH}_2\text{CONHCONH}_2$), is one of several by-products formed when molten urea is heated near or above its melting point (132 °C) during the manufacturing of urea.[1][2] The exact mechanism of biuret damage to different plants is still under investigation, but the harmful effects of high concentrations have been well documented, and many regulations/standards concerning the maximum allowed concentrations and/or the analytical methods have been published around the world.[1][3][4][5][6][7][8] Nowadays, there are at least three kinds of analytical methods available for the determination of biuret in fertilizers, including traditional spectrophotometric methods,[5][7] the atomic absorption spectrophotometric method,[8] and HPLC methods.[2][5][10][11] Recently, HPLC methods have shown superiority over other types of methods, owing to their ability to quantitatively determine biuret content by completely separating biuret from numerous urea-condensates. ISO/TC 134 is well aware of great efforts made by analysts/scientists around the world on separately seeking a uniform, quick and accurate method for the determination of biuret in fertilizers and attempted to unify the HPLC method to the greatest extent herein, based on the preliminary research by the China, US, and European experts.[2][5][10][11]

iTeh STANDARD PREVIEW (standards.iteh.ai)

ISO 18643:2016

<https://standards.iteh.ai/catalog/standards/sist/17bb1760-9f33-46c1-8cbb-beedf97b73f6/iso-18643-2016>

iTeh STANDARD PREVIEW **(standards.iteh.ai)**

ISO 18643:2016

<https://standards.iteh.ai/catalog/standards/sist/17bb1760-9f33-46c1-8cbb-beedf97b73f6/iso-18643-2016>

Fertilizers and soil conditioners — Determination of biuret content of urea-based fertilizers — HPLC method

1 Scope

This International Standard specifies the test procedure for determination of the biuret content in liquid and solid urea-based fertilizers based on the HPLC method.

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.

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

3 Principle

The biuret content in the fertilizer is extracted by aqueous acetonitrile mobile phase and separated from other contents by reversed liquid chromatography on an amino/aminopropyl column. The peak is detected by UV detector attached to the HPLC.

4 Reagents

ISO 18643:2016

<https://standards.iteh.ai/catalog/standards/sist/17bb1760-9f33-46c1-8cbb->

WARNING — Acetonitrile is flammable and toxic. Refer to applicable Safety Data Sheet (SDS). The related operations shall be performed in the fume hood. This International Standard does not point out all possible safety problems and the user shall bear the responsibility to take proper safety and health measures and ensure the operations are compliant with the conditions stipulated by the related laws and regulations of the state.

Use only water conforming to grade 3 of ISO 3696.

4.1 Acetonitrile, HPLC grade.

4.2 Mobile phase, 150 ml water + 850 ml acetonitrile, filtered by 0,22 µm membrane as pre-treatment, and ultrasonic degas for 10 min before use.

NOTE An alternative method using acetonitrile-free mobile phase is listed in [Annex B](#), with some limitations on the scope of application.

4.3 Biuret stock solutions (0,5 mg/ml = 500 ppm), weigh 0,500 0 g high purity Biuret, dissolved by mobile phase ([4.2](#)), and transferred into a 1 l volumetric flask, dilute to volume with mobile phase ([4.2](#)) and mix.

The biuret of at least 97 % purity and the biuret purity claimed on the label shall be based on the biuret content, not on the N-content. There are methods available for the purification procedure of biuret such as those described in AOAC 960.04A(c) or ISO 17322.

5 Apparatus

5.1 Ordinary laboratory apparatus.

5.2 Ultrasonic bath.

5.3 High-performance liquid chromatography instrument, with UV detector.

5.4 Microsyringe, 5 µl ~ 50 µl.

5.5 Syringe-driven filter, with organic filter membrane of 0,22 µm pores.

5.6 Injection loop, volume of 10 µl.

5.7 Sieve, with the aperture size of 0,50 mm.

6 Procedure

6.1 Preparation of test sample

For urea fertilizers, simply take 500 g of divided sample as the test portion; for compound fertilizers, take a reduced laboratory sample of 100 g, and grind until it passes through a sieve of aperture size 0,5mm. Mix thoroughly to homogenize the sample. Place in a clean dry bottle with lid.

6.2 Preparation of test solution

Duplicate replicate experiments shall be done for the final determination of result.

Weigh 0,1 g ~ 0,5 g test sample (accurately to 0,000 2 g, with biuret content of 1 mg ~ 2 mg ca.) into a 25 ml beaker. Add 10 ml mobile phase (4.2) and dissolve using an ultrasonic bath for 10 min. Transfer to a 25 ml volumetric flask and dilute to volume with mobile phase (4.2). Mix thoroughly and leave standing. Filter with a syringe filter to obtain the test solution.

6.3 Preparation of biuret working standard solutions

According to Table 1, pipette 0,00 ml, 0,50 ml, 1,00 ml, 3,00 ml, 5,00 ml and 10,00 ml biuret stock solution (4.3) into six separate 25 ml volumetric flasks. Dilute with respective volumes of mobile phase (4.2) and make up to the mark and mix thoroughly. Filter with 0,22 µm organic filter membrane.

Table 1 — Biuret working standard solutions

| Volume of biuret stock solution ml | Mass of biuret mg |
|---------------------------------------|----------------------|
| 0,00 ^a | 0,00 |
| 0,50 | 0,25 |
| 1,00 | 0,50 |
| 3,00 | 1,50 |
| 5,00 | 2,50 |
| 10,00 | 5,00 |
| ^a Blank solution. | |

6.4 HPLC conditions

Recommended operating conditions of HPLC are listed in Table 2. Other HPLC conditions that can achieve the same separation effects may be used.

Table 2 — Recommended operating conditions of HPLC

| | |
|--|---|
| Chromatographic column | Amino column or Aminopropyl column, ^a 4,6 mm × 250 mm, with 5 µm packing |
| Flow rate | 1,0 ml/min ~ 1,3 ml/min |
| Inject volume | 10 µl |
| Column temperature | 35 °C |
| Detector wavelength | 195 nm |
| ^a If the column is new or has not been in service for more than a week, condition the column for 4 h at room temperature with LC-grade isopropanol at a flow rate that will maintain at least 200 bars column back pressure. This is typically 1 ml/min. Wash the column again for 4 h with 100 % LC-grade acetonitrile at flow rate of 1 ml/min followed by washing with the mobile phase at flow rate of 1 ml/min until a stable base line is achieved. | |

NOTE 1 The best separation condition can be determined according to different equipment and situations.

NOTE 2 Depending on the type of urea-based fertilizers, other conditions may apply.

NOTE 3 Alternative method using acetonitrile-free mobile phase is listed in [Annex B](#).

6.5 Preparation of standard curve

Ensure that the HPLC apparatus operating conditions are optimized. Successively inject 10 µl working standard solution (6.3) and determine the series of biuret working standard solution. Each working standard solution shall be determined two times. Draw the standard curve or get the linear regression equation by calculating the average peak areas of the biuret and the corresponding mass.

6.6 Determination of the biuret content in the test solution

Determine the test solution (6.2) with the same method of the working standard solution, measure the peak area, and calculate the biuret mass in each test solution according to the standard curve or linear regression equation. After completing the determination, first wash the system with mobile phase (4.2) for 30 min, then wash the system with absolute acetonitrile (4.1) for 30 min. Finally, turn off the apparatus according to the operating procedures.

6.7 Calculation and expression of the results

The mass fraction of biuret (%), w , is calculated as given in Formula (1):

$$w = \frac{m_1 \times 10^{-3}}{m} \times 100 \quad (1)$$

where

m_1 is the mass of biuret, in mg, of the test solution, calculated according to the standard curve or linear regression equation corresponding to the peak areas;

m is the mass, in g, of the test portion.

The determination result is the arithmetic average of the parallel determination results.

6.8 Precision

6.8.1 Ring test

Details of ring test on the precision of the method are summarized in [Annex A](#).

6.8.2 Repeatability, r

For all levels, the repeatability limit r is 0,022, with the unit of mass fraction (%).

6.8.3 Reproducibility, R

For all levels, the reproducibility limit R is 0,102, with the unit of mass fraction (%).

7 Test report

The test report shall contain at least the following information:

- a) all information necessary for the complete identification of the sample;
- b) test method used with reference to this International Standard, i.e. ISO 18643:2016;
- c) test results obtained;
- d) date of sampling and sampling procedure (if known);
- e) date when the analysis was finished;
- f) whether the requirement of the repeatability limit has been fulfilled;
- g) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents occurred when performing the method, which might have influenced the test results.

(standards.iteh.ai)
ISO 18643:2016
<https://standards.iteh.ai/catalog/standards/sist/17bb1760-9f33-46c1-8cbb-beed97b73f6/iso-18643-2016>

Annex A (informative)

Report of international laboratories ring test

A.1 General

- a) International laboratories ring tests based on ISO/CD 18643 were conducted between January 2014 and March 2014. Thirteen laboratories participated in the two parallel tests on seven test samples.
- b) The test method described in ISO/CD 18643 was adopted here for the determination of biuret contents in the fertilizer samples.
- c) Seven different types of fertilizer samples were used during the ring test, each with several mean levels. The test samples were sample A-NPK compound fertilizer, sample B-urea formaldehyde complex fertilizer, sample C-urea, sample D-NPK complex fertilizer, sample E-urea ammonium nitrate (UAN) solution, sample F-polymer sulfur coated urea (PSCU), and sample G-urea formaldehyde slow release liquid fertilizer (Trisert®¹). The biuret contents in all of the seven fertilizer samples lie in the range of 0,09 % ~ 1,01 % (mass fraction).
- d) The precision of the test results was evaluated based on ISO 5725-2.

A.2 Statistical analysis of the test results of biuret contents

A.2.1 Original test results

Thirteen laboratories participated in the determination of biuret contents in fertilizer test samples. The results are listed in [Table A.1](#), with the unit of mass fraction (%).

Table A.1 — Original test results of the determination of biuret contents

Results in mass fraction (%)

| Laboratory <i>i</i> | Level <i>j</i> | | | | | | | | | | | | | |
|---------------------|----------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | A | | B | | C | | D | | E | | F | | G | |
| 1 | 0,615 | 0,602 | 0,505 | 0,509 | 1,006 | 0,995 | 0,300 | 0,310 | 0,231 | 0,225 | 0,946 | 0,945 | 0,093 | 0,091 |
| 2 | 0,580 | 0,610 | 0,580 | 0,580 | 0,960 | 1,050 | 0,340 | 0,350 | 0,290 | 0,280 | 0,980 | 0,970 | 0,130 | 0,120 |
| 3 | 0,653 | 0,619 | 0,485 | 0,516 | 0,970 | 0,938 | 0,276 | 0,271 | 0,211 | 0,185 | 0,788 | 0,784 | 0,067 | 0,070 |
| 4 | 0,651 | 0,654 | 0,552 | 0,547 | 1,047 | 1,057 | 0,326 | 0,336 | 0,250 | 0,249 | 1,061 | 1,015 | 0,104 | 0,106 |
| 5 | 0,625 | 0,614 | 0,509 | 0,510 | 1,013 | 1,015 | 0,277 | 0,279 | 0,219 | 0,212 | 0,975 | 0,985 | 0,070 | 0,068 |
| 6 | 0,635 | 0,622 | 0,523 | 0,532 | 1,035 | 1,038 | 0,319 | 0,314 | 0,248 | 0,249 | 0,982 | 0,985 | 0,106 | 0,106 |
| 7 | 0,644 | 0,644 | 0,547 | 0,548 | 1,053 | 1,050 | 0,323 | 0,325 | 0,244 | 0,238 | 0,961 | 0,965 | 0,094 | 0,093 |
| 8 | 0,610 | 0,620 | 0,500 | 0,500 | 0,980 | 0,980 | 0,310 | 0,310 | 0,220 | 0,220 | 0,950 | 0,940 | 0,100 | 0,100 |
| 9 | 0,600 | 0,590 | 0,500 | 0,490 | 0,890 | 0,900 | 0,290 | 0,300 | 0,210 | 0,210 | 0,870 | 0,870 | 0,090 | 0,090 |
| 10 | 0,590 | 0,590 | 0,600 | 0,610 | 1,110 | 1,090 | 0,350 | 0,350 | 0,300 | 0,300 | 0,940 | 0,980 | 0,150 | 0,150 |
| 11 | 0,609 | 0,613 | 0,505 | 0,504 | 1,010 | 1,006 | 0,300 | 0,301 | 0,233 | 0,231 | 0,937 | 0,939 | 0,091 | 0,093 |
| 12 | 0,614 | 0,615 | 0,503 | 0,500 | 1,010 | 1,007 | 0,314 | 0,313 | 0,231 | 0,234 | 0,934 | 0,930 | 0,101 | 0,100 |
| 13 | 0,618 | 0,620 | 0,515 | 0,498 | 1,001 | 1,010 | 0,309 | 0,318 | 0,215 | 0,217 | 0,976 | 0,911 | 0,086 | 0,091 |

1) Trisert® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.