
**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*

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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).

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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*.

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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]

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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

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.