SLOVENSKI STANDARD
SIST EN 17706:2025

01-februar-2025

Nadomešča:
SIST-TS CEN/TS 17706:2023

Rastlinski biostimulanti - Določanje anorganskega arzena

Plant biostimulants - Determination of inorganic arsenic

Pflanzen-Biostimulanzien - Bestimmung von anorganischem Arsen

Biostimulants des végétaux - Dosage de l'arsenic inorganique

Ta slovenski standard je istoveten z: **EN 17706:2024**

ICS:

65.080	Gnojila	Fertilizers

SIST EN 17706:2025 **en,fr,de**

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 17706

November 2024

ICS 65.080

Supersedes CEN/TS 17706:2022

English Version

Plant biostimulants - Determination of inorganic arsenic

Biostimulants des végétaux - Dosage de l'arsenic inorganique

Pflanzen-Biostimulanzien - Bestimmung von anorganischem Arsen

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

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European foreword

This document (EN 17706:2024) has been prepared by Technical Committee CEN/TC 455 “Plant Biostimulants”, the secretariat of which is held by AFNOR.

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of an identical text or by endorsement, at the latest by May 2025, and conflicting national standards shall be withdrawn at the latest by May 2025.

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.

This document supersedes CEN TS 17706:2022.

In comparison with the previous edition no technical changes were applied in this document. The following main editorial changes have been made:

— Introduction;

— Scope – text regarding blends changed;

— Annex B – results of the interlaboratory study added;

— Annex ZA added;

— Bibliography reordered.

This document has been prepared under a Standardization Request addressed to CEN by the European Commission. The Standing Committee of the EFTA States subsequently approves these requests for its Member States.

For relationship with EU Legislation, see informative Annex ZA, which is an integral part of this document.

Any feedback and questions on this document should be directed to the users’ national standards body. A complete listing of these bodies can be found on the CEN website.

According to the CEN-CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye and the United Kingdom.

Introduction

The European Committee for Standardization (CEN) was requested by the European Commission (EC) to draft European Standards or European Standardization deliverables to support the implementation of Regulation (EU) 2019/1009 of 5 June 2019 [1] laying down rules on the making available on the market of EU fertilising products ("FPR" or "Fertilising Products Regulation").

This standardization request, presented as SR M/564 and relevant amendments, also contributes to the Communication on "Innovating for Sustainable Growth: A Bio economy for Europe". The interest in plant biostimulants has increased significantly in Europe as a valuable tool to use in agriculture. Standardization was identified as having an important role in order to promote the use of biostimulants. The work of CEN/TC 455 seeks to improve the reliability of the supply chain, thereby improving the confidence of farmers, industry, and consumers in biostimulants, and will promote and support commercialisation of the European biostimulant industry.

The standard is based on a mild acid oxidative extraction of the arsenic species followed by liquid chromatography (HPLC or IC) coupled to the element-specific detector ICP-MS for the determination of the mass fraction of inorganic arsenic.

The other standards developed for determination of inorganic arsenic content in animal feedingstuffs and foodstuffs were studied as a basis of the described method [2], [3], [4], [5].

The inter-laboratory study reflects the final statistical characteristics of the method for the determination of inorganic arsenic content in plant biostimulants. The results are given in Annex B (informative).

WARNING — Persons using this document should be familiar with usual laboratory practice. This document does not purport to address all of the safety issues, if any, associated with its use. It is the responsibility of the user to establish appropriate health and safety practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted according to this document are carried out by suitably trained staff.

1 Scope

This document specifies a method for extraction, separation, and determination of inorganic arsenic (iAs) in plant biostimulants using anion-exchange high performance liquid chromatography (HPLC) or ion chromatography (IC) coupled to ICP-MS.

This document is applicable to the blends of fertilizing products where a blend is a mix of at least two of the following component EU fertilising products categories: Fertilizers, Liming Materials, Soil Improvers, Growing Media, Inhibitors, Plant Biostimulants, and where the following category Plant Biostimulants is the highest percentage in the blend by mass or volume, or in the case of liquid form by dry mass. If Plant Biostimulants is not the highest percentage in the blend, the European Standard for the highest percentage of the blend applies. In case a blend of fertilizing products is composed of components in equal quantity or in case the component EU fertilising products used for the blend have identical formulations[1], the user decides which standard to apply.

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.

EN 17702-1:2024, *Plant biostimulants — Sampling and sample preparation — Part 1: Sampling*

EN 17702-2:2024, *Plant biostimulants — Sampling and sample preparation — Part 2: Sample preparation*

EN 17704:2024, *Plant biostimulants — Determination of dry matter*

3 Terms and definitions

For the purposes of this document, the following terms and definitions 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
analyte
parameter to be determined

3.2
blank calibration solution
solution prepared in the same way as the calibration solution but leaving out the analytes

3.3
blank test solution
solution prepared in the same way as the test sample solution but omitting the test portion

[1] An example of such a blend is a product with two claimed functions consisting of a non-microbial plant biostimulant and an organic fertilizer composed of 1kg/kg of plant biostimulant from seaweed.

3.4
calibration solution
solution used to calibrate the instrument, prepared from stock solutions by adding acids, buffer, reference element and salts as needed

3.5
stock solution
solution with accurately known analyte concentration(s), prepared from pure chemicals

4 Principle

This document specifies a method for the determination of inorganic arsenic in plant biostimulants. Inorganic arsenic consists of arsenite As (III) and arsenate As(V). A representative test portion of the sample is treated with a diluted nitric acid and hydrogen peroxide solution in a heated water bath. By this means the sample is solubilised, arsenic species are extracted into solution and As (III) is oxidized to As(V). The inorganic arsenic is selectively separated from other arsenic compounds using anion exchange HPLC (High Performance Liquid Chromatography) coupled online to the element-specific detector ICP-MS (Inductively Coupled Plasma Mass Spectrometer) for the determination of the mass fraction of the inorganic arsenic. External calibration with solvent matrix-matched standards is used for the quantification of the amount of the inorganic arsenic. Alternatively, IC (ion chromatography) coupled to ICP-MS can be used.

A preliminary determination of the total arsenic in aqua regia extracts by ICP-AES (EN 17701-1 [6] and EN 17701-2 [7]) could reduce the number of the samples where the determination of iAs is necessary because if the content of aqua regia (total) extractable arsenic is lower than the legislative limit for iAs then the determination of iAs is not necessary.

5 Reagents

When using a method of high sensitivity like ICP-MS and HPLC the control of the blank levels of water, acid and other reagents is very important. The reagents shall be of adequate purity and of recognized analytical grade. The concentration of arsenic species in the reagents and water used shall be negligible and low enough not to affect the results of the determination. Generally ultra-pure water from a purification system and nitric acid of minimum p.a. quality is recommended.

5.1 Water with an electrical conductivity not higher than 0,1 mS/m at 25 °C, having a resistivity greater than 18,2 MΩ·cm.

5.2 Nitric acid (HNO_3), concentrated, ≥ 65 % (mass fraction), mass concentration of approximately $\rho(HNO_3)$ 1,4 g/ml.

Use only nitric acid available with high purity (minimum p.a. quality) in order to avoid potential contamination.

5.3 Hydrogen peroxide, H_2O_2 not less than 30 % (mass fraction).

High purity is essential to avoid potential contamination. Commercially available hydrogen peroxide for analysis should be tested for contamination of arsenic prior to use. It is necessary to prevent peroxide degradation and ensure the stability of the solution, this is in the discretion of the analyst to use only H_2O_2 of adequate quality.

5.4 Extraction solution, 0,1 mol/l HNO_3 in 3 % (volume fraction) H_2O_2.

Add 6,5 ml of HNO_3 (5.2) and thereafter 100 ml of hydrogen peroxide (5.3) into 800 ml water (5.1) in a 1 000 ml volumetric flask. Fill the flask to the mark with water (5.1.). This solution is prepared on the day of use.

It is recommended that the total volume needed for the analysis is estimated and only this amount is produced in the day of use.

5.5 Ammonium carbonate, $(NH_4)_2CO_3$, mass fraction $w \geq 99{,}999$ %, for preparation of the mobile phase solution.

5.6 Aqueous ammonia, (NH_3(aq).) $w \geq 25$ %, for adjustment of pH in the mobile phase.

5.7 Methanol, (CH_3OH), HPLC grade, for preparation of the mobile phase solution.

5.8 Mobile phase, e.g. 50 mmol/l ammonium carbonate in 3 % (volume fraction) methanol at pH 10,3.

Dissolve 4,80 g of ammonium carbonate (5.5) in approximately 800 ml water (5.1). Adjust the pH to 10,3 with aqueous ammonia (5.6) and add 30 ml of methanol (5.7) and then fill up to 1 000 ml with water (5.1). Prior to use filter the mobile phase solution through a 0,45 µm filter using a filtering device (6.4).

The optimal concentration of ammonium carbonate in the mobile phase depends on the analytical column used (e.g. brand, particle size and dimensions) and should be verified in advance. The appropriate concentration of ammonium carbonate (usually between 10 mmol/l and 50 mmol/l) is highly dependent on the column used and is up to the discretion of the analyst. It should fulfil the criteria for sufficient resolution of the arsenate peak.

Methanol is added to the mobile phase in order to enhance the signal intensity for arsenic. The concentration of methanol to achieve the highest signal to noise ratio depends on the instrument used and should be identified by the analyst.

Different mobile phase may be also used according to the instructions of the manufacturer of the column, but it is necessary to verify optimal separation conditions.

For example, Agilent[2] column G3154-65001 with a guard column G3154-65002 and a mobile phase recommended for this column, as a mixture of potassium dihydrogen phosphate (KH_2PO_4) 2 mmol/l, ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA disodium salt) 0,2 mmol/l and the pH 6,0 adjusted with sodium hydroxide (NaOH) 1 mol/l, were successfully used for the analysis.

5.9 Arsenic (V) standard stock solution, with an arsenic (V) mass concentration of 1 000 mg/l.

The use of commercial standards of arsenic As (V), with a mass concentration of 1 000 mg/l is recommended.

5.10 Arsenic (V) standard solution I, with an arsenic (V) mass concentration of 10 mg/l in 2 % (volume fraction) HNO_3.

Pipette 1 ml of arsenic standard stock solution (5.9) into a 100 ml volumetric flask. Add 2 ml of nitric acid (5.2), fill to the mark with water (5.1) and mix well. This solution is stable in a refrigerator at least one week.

5.11 Arsenic (V) standard solution II, with an arsenic (V) mass concentration of 1 mg/l.

[2]Agilent® is a registered trademark of Agilent Technologies Inc. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of the product named.

Pipette 10 ml of arsenic standard solution I (5.10) into a 100 ml volumetric flask, fill to the mark with water (5.1) and mix well. This solution should be prepared on the same day of use.

5.12 Solution for checking chromatographic separation, containing organic arsenic compounds (e.g. 10 µg/l) monomethylarsenous acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine (AB), as well as arsenate (e.g. 10 µg/l), arsenite (e.g. 10 µg/l) and chloride (e.g. 100 mg/l).

This solution is recommended to demonstrate satisfactory resolution of individual arsenic species, possible interferents and to find out how the chromatographic conditions should be optimized (e.g. by changing the mobile phase concentration or the mobile phase flow rate).

The solution shall be prepared in water (5.1), not in the extraction solution, to check the retention time of the individual arsenic species, their visual presentation in the chromatogram and how the peaks are separated.

6 Apparatus

6.1 Common laboratory glass (plastic) ware.

Plastic volumetric flasks are recommended for the preparation of the mobile phase and calibration solutions. All glassware and plastic ware shall be adequately cleaned and stored to avoid any contamination.

6.2 Laboratory grinder, capable of grinding to a particle size less than 0,5 mm.

6.3 Analytical balance, capable of weighing to an accuracy of 1 mg or better.

6.4 Filtering device, for filtration of mobile phase with a filter, pore size 0,45 µm.

6.5 Shaking heated water bath, capable of maintaining 90 °C.

Some fine materials can form a thin layer on the surface of the extraction solution and the contact of the sample with the extraction solution can be less intensive. Therefore, the shaking water bath is recommended to ensure the efficient extraction of the sample.

6.6 Centrifuge, for minimum 4 000 min^{-1} (approx. 2 000 g).

6.7 Single use syringe filters (0,45 µm) or HPLC vials with filters, compatible with acidic solutions for filtering of test solutions prior to analysis.

6.8 High Pressure Liquid Chromatograph (HPLC).

6.9 Anion exchange chromatographic column, suitable for the selective separation of arsenate from other arsenic compounds present in the sample extracts.

It is highly recommended to use a guard column to prolong the lifetime of the analytical column.

Use of a different column and a different mobile phase is possible, providing the results are comparable. It is necessary and very important to verify optimal separation conditions. The other columns may be used in combination with the suitable type of mobile phase depending on the recommendations of the manufacturer (see 5.8).

6.10 Inductively coupled plasma mass spectrometer (ICP-MS).

6.11 Argon gas, purity ≥ 99,99 %.