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Plant biostimulants - Determination of chromium(VI)

Biostimulants des végétaux - Dosage du chrome(VI)

Biostimulanzien für die pflanzliche Anwendung -
Bestimmung von Chrom (VI)

This Technical Specification (CEN/TS) was approved by CEN on 3 January 2022 for provisional application.

The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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European foreword

This document (CEN/TS 17703:2022) has been prepared by Technical Committee CEN/TC 455 “Plant Biostimulants”, the secretariat of which is held by AFNOR.

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 has been prepared under a Standardization Request given to CEN by the European Commission and the European Free Trade Association.

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 announce this Technical Specification: 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, Turkey and the United Kingdom.

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CEN/TS 17703:2022 (E)**Introduction**

This document was prepared by the experts of CEN/TC 455 “Plant Biostimulants”. 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 laying down rules on the making available on the market of EU fertilizing products (“FPR” or “Fertilising Products Regulation”).

This standardization request, presented as M/564, also contributes to the Communication on “Innovating for Sustainable Growth: A Bio economy for Europe”. The Working Group 4 “Other safety parameters”, was created to develop a work program as part of this request. The technical committee CEN/TC 455 “Plant Biostimulants” was established to carry out the work program that will prepare a series of standards. The interest in 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.

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1 Scope

This document was developed to provide a method for verifying that hexavalent chromium (CrVI) is not present in plant biostimulants in a concentration that exceeds the respective limits outlined in the EU Regulation on Fertilising Products [1].

This document is applicable to all types of plant biostimulants (solid and liquid¹) used in agriculture.

The method described is suitable to quantify the chromium(VI) content in plant biostimulants down to 2 mg/kg.

The results obtained from this method are strictly dependent on the extraction conditions. Results obtained by using other extraction procedures (extraction solution, pH, extraction time, etc.) are not comparable with the results produced by the procedure described in this document.

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.

CEN/TS 17701-1, *Plant biostimulants — Determination of specific elements — Part 1: Digestion by aqua regia for subsequent determination of elements*

CEN/TS 17701-2, *Plant biostimulants — Determination of specific elements — Part 2: Determination of total content of Cd, Pb, Ni, As, Cr, Cu and Zn*

CEN/TS 17702-1, *Plant biostimulants — Sampling and sample preparation — Part 1: Sampling*

CEN/TS 17702-2, *Plant biostimulants — Sampling and sample preparation — Part 2: Sample preparation*

CEN/TS 17704, *Plant biostimulants — Determination of dry matter*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1

chromium(VI) content

amount of chromium(VI) in plant biostimulant determined after extraction with an aqueous salt solution at pH 7,0 to 8,0

Note 1 to entry: The chromium(VI) content is reported as chromium(VI) in milligrams per kilogram (mg/kg), expressed as the dry mass of the sample.

[SOURCE: EN ISO 17075-2:2017, definition 3.1]

¹ According to the definition of “solid form” and “liquid form” to the current EU legislation when it is published.

CEN/TS 17703:2022 (E)**4 Principle**

A preliminary determination of the total chromium in aqua regia extracts by ICP-AES (CEN/TS 17701-1 and CEN/TS 17701-2) could reduce the number of the samples where the determination of chromium(VI) is necessary because if the content of aqua regia (total) extractable chromium is lower than the legislative limit for chromium(VI) then the determination of chromium(VI) is not necessary.

Extractable chromium(VI) is leached from the sample in phosphate buffer at pH 7,0 to 8,0. An aliquot of the filtered extract is analysed for Cr(VI) using ion-exchange chromatography with UV-VIS detection.

5 Chemicals

All reagents used shall have at least analytical grade purity.

5.1 Extraction solution

Dissolve 22,8 g dipotassium hydrogenphosphate $K_2HPO_4 \cdot 3H_2O$ in 1 000 ml water (5.7), adjusted to pH $8,0 \pm 0,1$ with phosphoric acid (5.2). Degas this solution with either argon or nitrogen (5.6) or ultrasonic bath.

Standard practice is to make up a fresh solution each day. However, the solution can be kept for up to one week in a refrigerator at $(4 \pm 3) ^\circ C$ but shall be warmed to room temperature and degassed prior to use.

5.2 Phosphoric acid solution

700 ml *o*-phosphoric acid, $\rho = 1,71$ g/ml, made up to 1 000 ml with water (5.7).

First add approximately 200 ml of deionised water (5.7) to a 1 000 ml volumetric flask, then add the 700 ml of *o*-phosphoric acid and dilute to the mark with deionised water.

5.3 Potassium dichromate ($K_2Cr_2O_7$), dried for (16 ± 2) h at $(105 \pm 5) ^\circ C$.

5.4 Chromium(VI) stock solution

Dissolve 2,829 g potassium dichromate ($K_2Cr_2O_7$) (5.3) in water (5.7) in a volumetric flask and make up to 1 000 ml with water (5.7). One ml of this solution contains 1 mg of chromium.

The solution can be kept for up to 12 months in a refrigerator at $(4 \pm 3) ^\circ C$ but shall be warmed to room temperature prior to use.

It is also possible to use a commercial standard solution with a certified Cr(VI) concentration that can be connected to national standards. Observe the expiry date or recommended shelf life stated by the manufacturer.

5.5 Chromium(VI) standard solution

Pipette 1 ml of solution (5.4) into a 1 000 ml volumetric flask and make up to the mark with extraction solution (5.1). One ml of this solution contains 1 μg of chromium.

The solution can be kept for up to one week in a refrigerator at $(4 \pm 3) ^\circ C$ but shall be warmed to room temperature prior to use.

A stock solution of hexavalent chromium at this concentration level is an alternative available commercially.

5.6 Argon or nitrogen, oxygen-free

Preference should be given to argon as an inert gas instead of nitrogen because argon has a higher specific mass than air.

5.7 Distilled or deionised water, with a specific conductivity not higher than 0,2 mS/m at 25 °C.

5.8 Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$)

Dissolve 85,4 g in a 100 mL volumetric flask, dilute with water (5.7), close and mix thoroughly.

6 Apparatus and materials

Usual laboratory equipment and, in particular, the following.

6.1 Suitable mechanical orbital shaker, $(100 \pm 10) \text{ min}^{-1}$.

6.2 Conical flask, of capacity 250 ml, with stopper.

6.3 Aeration tube and flow meter, suitable for a flow rate of $(50 \pm 10) \text{ ml/min}$.

6.4 pH meter, with glass electrode.

6.5 Membrane filter, 0,45 μm pore size [polytetrafluoroethylene (PTFE) or polyamide 66].

6.6 Common laboratory glassware and pipettes.

6.7 Vacuum device, suitable for filtration of extraction solution, mobile phase, and sample extracts.

6.8 Ion-exchange chromatograph, with UV or visible detector or high performance liquid chromatography (HPLC) with anion-exchange column and UV or visible detector. A photo diode array detector (DAD) is recommended.

6.9 Analytical balance, capable of weighing to 0,1 mg.

6.10 Syringe membrane filters of nylon of 0,45 μm for filtration of standards.

6.11 Suitable vials for HPLC.

7 Procedure

7.1 Sampling and preparation of samples

Sample in accordance with CEN/TS 17702-1. If sampling in accordance with CEN/TS 17702-1 is not possible, details about the sampling shall be given in the test report.

Preparation of samples in accordance with CEN/TS 17702-2. For products that may decompose or react when heated, grinding shall be carried out in order to prevent heating.

7.2 Preparation of analytical solution

Weigh (6.9) approximately $(2 \pm 0,1) \text{ g}$ of sample of plant biostimulant to the nearest 0,001 g. Pipette 100 ml of degassed solution (5.1) into a 250 ml conical flask (6.2). Displace oxygen by passing oxygen-free argon (or nitrogen) (5.6) into the flask for 5 min with a volume flow of $(50 \pm 10) \text{ ml/min}$. Remove the aeration tube (6.3), add the sample of plant biostimulant and close the flask with a stopper. Record the extract volume as V_0 .

Shake the conical flask with the sample of plant biostimulant for $(30,0 \pm 0,5) \text{ min}$ on a mechanical orbital shaker at $(100 \pm 10) \text{ min}^{-1}$ (6.1) at room temperature to extract the chromium(VI).

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Shake the suspension in a smooth circular movement to keep the sample of plant biostimulant from adhering to the wall of the flask and avoid shaking faster than specified.

Immediately after completing the 30 min of extraction, filter the contents of the conical flask through a membrane filter into a glass or plastic vessel with lid. Centrifugation can be used as an alternative to the filtration. Check the pH of the solution. The pH of the solution shall be between 7,0 and 8,0. If the pH of the solution is not within this range, start the complete procedure again.

Consider using a smaller sample mass, if the pH is not between 7,0 and 8,0. In this case, the quantification limit will be increased.

Transfer an aliquot of the filtered extract into a vial (6.11). Instrumental determination of chromium(VI) should be performed as soon as possible and no later than 60 min after the extraction phase.

If the sample is expected to contain a significant amount of chromium(III), 1 ml of magnesium chloride solution (5.8) may be added before displacing oxygen into the 250 ml volumetric flask (6.2) containing the 100 ml of the extraction solution (5.1).

7.3 Chromatographic conditions

Determination of chromium(VI) is performed using the ion chromatographic technique. As the instrumental equipment of the laboratories may vary, no specific applicable instructions can be provided for analysis. However, the operating parameters and examples of the ion chromatographic analysis for chromium(VI) listed in Annexes A and B have been successfully tested and used. Annex A determines chromium(VI) by direct detection of chromate peak at 372 nm. Annex B determines chromium(VI) after a post-column reaction with 1,5-diphenylcarbazide by measuring the absorption peak at 540 nm.

Record the injection volume as V_M and record the area of the chromate peak as A.

7.4 Calibration

The content of chromium(VI) in the sample of plant biostimulant is determined with an external standard calibration.

Prepare calibration solutions from the standard solution (5.5). The chromium(VI) concentration in these solutions should cover the expected range of measurement.

Plot a suitable calibration curve by using at least five standards, diluting a proper volume of standard solution (5.5). Pipette the given volumes of standard solution (5.5) into 10 ml volumetric flasks. Make up to volume with the extraction solution (5.1), mix well, filter (6.10) and transfer a suitable aliquot volume into a vial (6.11).

For example, calibration levels may be prepared as specified in Table 1.

Table 1 — Calibration levels preparation

Volume of the standard solution (5,5) (ml)	0,50	1,00	2,00	3,00	5,00
Final volume (ml)	10 ml in volumetric flask				
Concentration of hexavalent chromium ($\mu\text{g/l}$)	50	100	200	300	500

Transfer an aliquot to a suitable vial (6.11) corresponding for the chromatography system (6.8).