



**SLOVENSKI STANDARD**  
**oSIST prEN 17703:2023**  
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**Rastlinski biostimulanti - Določevanje kroma Cr(VI)**

Plant biostimulants - Determination of chromium(VI)

Pflanzen-Biostimulanzien - Bestimmung von Chrom(VI)

Biostimulants des végétaux - Dosage du chrome(VI)

**Ta slovenski standard je istoveten z: prEN 17703**

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## Plant biostimulants - Determination of chromium(VI)

Biostimulants des végétaux - Dosage du chrome(VI)

Pflanzen-Biostimulanzien - Bestimmung von  
Chrom(VI)

This draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 455.

If this draft becomes a European Standard, CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration.

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Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

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

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<b>Contents</b>	<b>Page</b>
European foreword .....	3
Introduction .....	4
1 Scope.....	5
2 Normative references.....	5
3 Terms and definitions .....	5
4 Principle.....	6
5 Chemicals.....	6
6 Apparatus and materials.....	7
7 Procedure .....	8
7.1 Sampling and preparation of samples.....	8
7.2 Preparation of analytical solution.....	8
7.3 Chromatographic conditions.....	8
7.4 Calibration.....	8
7.5 Determination of the recovery rate .....	9
8 Calculation and expression of results .....	10
8.1 Calculation of chromium(VI) content.....	10
8.2 Recovery rate (according to 7.5).....	10
8.3 Expression of results .....	11
9 Test report.....	11
Annex A (informative) Results of the inter-laboratory study.....	12
A.1 Inter-laboratory tests.....	12
A.2 Statistical results for the determination of Cr(VI).....	12
Annex B (informative) Chromatographic conditions for direct detection method .....	14
B.1 General.....	14
B.2 Example of ion chromatographic conditions.....	14
B.2.1 Mobile phase reagents .....	14
B.2.2 Instrumental conditions.....	14
Annex C (informative) Chromatographic conditions for method with post-column reaction .....	16
C.1 General.....	16
C.2 Chromatographic system and apparatus required .....	16
C.3 Example of analytical conditions .....	17
Annex ZA (informative) Relationship of this European Standard and the essential requirements of Regulation (EU) 2019/1009 making available on the market of EU fertilising products aimed to be covered .....	19
Bibliography .....	20

## European foreword

This document (prEN 17703:2023) has been prepared by Technical Committee CEN/TC 455 “Plant Biostimulants”, the secretariat of which is held by AFNOR.

This document is currently submitted to the CEN Enquiry.

This document will supersede CEN/TS 17703:2022.

This document has been prepared under a Standardization Request given to CEN by the European Commission and the European Free Trade Association, and supports essential requirements of EU Directive(s) / Regulation(s).

For relationship with EU Directive(s) / Regulation(s), see informative Annex ZA, which is an integral part of this document.

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## 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 and M/564 Amd1, also contributes to the Communication on “Innovating for Sustainable Growth: A Bio economy for Europe”. Working Group 4 “Other safety parameters”, was created to develop a work programme as part of this request. Technical committee CEN/TC 455 “Plant Biostimulants” was established to carry out the work programme 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 boosting 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 liquid<sup>1</sup>) 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. Therefore, this document is highly recommended to quantify the chromium(VI) content in blends of fertilizing products containing plant biostimulants, independently of the percentage of the different parts forming the blend.

## 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:—<sup>2</sup>, *Plant biostimulants — Sampling and sample preparation — Part 1: Sampling*

EN 17702-2:—<sup>3</sup>, *Plant biostimulants — Sampling and sample preparation — Part 2: Sample preparation*

EN 17704:—<sup>4</sup>, *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]

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

<sup>2</sup> Under preparation

<sup>3</sup> Under preparation

<sup>4</sup> Under preparation

## 4 Principle

To prove the compliance with the limit value defined in Regulation (EU) 2019/1009 for chromium(VI) in plant biostimulants, there is the possibility to choose between:

- Using a testing method determining the total chromium content, as long as the result of the test shows a content below the limit value for chromium(VI). In such a case, it can be safely assumed that the plant biostimulant complies with the limit value for chromium (VI).
- Using a testing method which determines only chromium(VI) content.

The determination of the total chromium in plant biostimulants can be performed using the following references:

EN 17701-1:—<sup>5</sup>, Plant biostimulants — Determination of specific elements — Part 1: Digestion by aqua regia for subsequent determination of elements

EN 17701-2:—<sup>6</sup>, Plant biostimulants — Determination of specific elements — Part 2: Determination of total content of Cd, Pb, Ni, As, Cr, Cu and Zn

This standard specifies the procedure to determine directly the content of chromium (VI) in plant biostimulants: 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 of 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  $(16 \pm 2)$  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.

<sup>5</sup> Under preparation

<sup>6</sup> Under preparation



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  $(4 \pm 3) ^\circ\text{C}$  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 ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ )

Dissolve 85,4 g of magnesium chloride hexahydrate ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ) 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 EN 17702-1:—<sup>2</sup>. If sampling in accordance with EN 17702-1:—<sup>2</sup> is not possible, details about the sampling shall be given in the test report.

Prepare samples in accordance with EN 17702-2:—<sup>3</sup>. 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  $(2 \pm 0,1)$  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  $(50 \pm 10)$  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  $V_0$  and the weight of the sample as  $m$ .

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

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 extraction, filter the contents of the conical flask through a membrane filter into a glass or plastic vessel with a 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).

Inject the standards in the chromatographic system (6.8). Introduce the same volume for each standard.

It is recommended to inject equal volume for samples. Record the volume injected as  $V_c$  in  $\mu\text{l}$ .

Plot the chromium(VI) concentrations in micrograms of Cr per millilitre ( $\mu\text{g/ml}$ ) against the measured areas of the peaks of chromate. Plot the chromium(VI) concentration on the x-axis and the area on the y-axis.

## 7.5 Determination of the recovery rate

The determination of the recovery rate is important to provide information about possible matrix effects which can influence the results.

Spike an aliquot of the solution obtained in 7.2 with a suitable volume of chromium(VI) solution to increase the chromium(VI) concentration by 10 mg/kg. Inject the same volume of this solution as the volume injected in the calibration (recording the area as  $A_s$ ).

Spike an aliquot of the extraction solution (the same volume as that taken before of the solution obtained in 7.2) with a suitable volume of chromium(VI) solution to increase the chromium(VI) concentration by 10 mg/kg, so that the final volume of this solution is the same as that of the above spiked solution with chromium(VI) solution. Inject the same volume of this solution as the volume injected in the calibration (recording the area as  $A_{st}$ ).

The area of the chromate peak of these solutions shall be within the range of the calibration curve, otherwise repeat the procedure using a smaller aliquot. The recovery rate shall be between 80 % and 120 %.

**NOTE** If the added chromium(VI) is not detected or significantly lower than the expected values, this is an indication that the plant biostimulant contains reducing agents. This leads to the conclusion that this plant biostimulant has no chromium(VI) content (below detection limit).