# SLOVENSKI STANDARD
# kSIST-TS FprCEN/TS 17705:2021

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**Rastlinski biostimulansi - Določevanje fosfonatov**

Plant biostimulants - Determination of phosphonates

Biostimulanzien für die pflanzliche Anwendung - Bestimmung von Phosphonaten

Biostimulants des végétaux - Dosage des phosphonates



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# Plant biostimulants - Determination of phosphonates

Biostimulants des végétaux - Dosage des phosphonates

Biostimulanzien für die pflanzliche Anwendung - Bestimmung von Phosphonaten

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

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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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# Contents

Page

## European foreword

This document (FprCEN/TS 17705:2021) has been prepared by the Technical Committee CEN/TC 455 “Plant biostimulants”, the secretariat of which is held by AFNOR.

This document is currently submitted to the Vote on TS.

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

# 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 fertilising products ("FPR" or "Fertilising Products Regulation").

This request, presented as SR 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.

The preparation of this document is based on the Fertilising Products Standardization Request (M/564) to CEN by the European Commission and the European Free Trade Association concerning a development of the methods for analysis of fertilizers in the framework of Regulation (EC) No 2019/1009.

This document describes the extraction and measurement for the determination of phosphonate (phosphite) in plant biostimulants. It is based on a water extraction of the phosphonate (phosphite) followed by ion chromatography with conductivity detection (IC-CD).



The ion chromatography with a conductivity detector (IC-CD) method can be used in well-equipped analytical laboratories for the determination of different ions. In the field of fertilizing products, the method is used and standardized for the determination of perchlorates in mineral fertilizers. The IC-CD method can determine more ions simultaneously.



The legislative limit for phosphonate content is 0,5 % (mass fraction) and the method described in this document was adapted to achieve this requirement and simultaneously to reduce interferences from other co-extracted anions as much as possible.

The definition of phosphonates is not clearly stated in the Regulation 2019/1009 and to avoid any misunderstanding the results are expressed as a content of phosphorus (P) bound in the form of free water-soluble phosphonates (P-PO3).

This document is also applicable to the blends of fertilizing products where plant biostimulants are the main part of the blend. Otherwise, the Technical Specification for the main part of the blend apply.

# 1 Scope

This document specifies a method for the extraction and determination of phosphonates (P–$PO_3$) in plant biostimulants using ion chromatography and conductivity detection (IC-CD).

This document is also applicable to the blends of fertilizing products where plant biostimulants are the main part of the blend. Otherwise, the Technical Specification for the main part of the blend 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.

FprCEN/TS 17704:2021, *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 terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at https://www.electropedia.org/
- ISO Online browsing platform: available at https://www.iso.org/obp



**3.1**
**phosphonates**
salts derived from phosphonic acid ($H_3PO_3$)



# 4 Principle

A representative test portion of the sample is extracted with water. Phosphonate in the extract is selectively separated from other compounds using ion chromatography (IC) and determined by a conductivity detector (CD). External calibration is used for quantification of the amount of the phosphonate.

# 5 Reagents

All reagents shall be of recognized analytical grade. The concentration of phosphonate in the reagents and deionized water used shall be low enough not to affect the results of the determination.

**5.1 Water with a specific conductivity not higher than 0,2 mS/m at 25 °C**.

**5.2 Phosphonate standard stock solution** r(P-PO3) = 1 000 mg/l is prepared by dissolving commercially available sodium phosphite dibasic pentahydrate salt (Mw = 216,04 mol/l, purity ≥ 98 %). For preparation the phosphonate standard stock solution 0,697 ± 0,001 g is weighed, transferred to 100 ml volumetric flask, fill with deionized water (5.1) to final volume and mix thoroughly.

**5.3 Phosphonate standard solution,** r(P-PO3) = 100 mg/l is prepared from a standard stock solution (5.2) by appropriate dilution with deionized water (5.1). Pipette 10 ml of stock solution (5.2) to a 100ml volumetric flask, fill with deionized water (5.1) to final volume and mix thoroughly.

**5.4 Mobile phase,** KOH cartridge for ion chromatography (commercially supplied). Concentration of the KOH mobile phase is electrolytically generated in externally supplied deionized water (5.1).

Different mobile phase may be used according to the instructions of the manufacturer of the column.

**5.5 Chloride standard** r($Cl^-$) = 1 000 mg/l - commercially available.

**5.6 Nitrate standard** r(NO3-) = 1 000 mg/l – commercially available.

**5.7 Solution for checking chromatographic separation** r(P-PO3) = 10 mg/l, r($Cl^-$ and NO3-) = 50 mg/l is prepared by pipetting 1 ml of phosphonate standard stock solution (5.2), 5 ml of chloride standard (5.5), 5 ml of nitrate standard (5.6) to 100ml volumetric flask and fill to final volume with deionized water (5.1).

## 6 Apparatus

**6.1 Common laboratory glassware and plastic**.

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

**6.3 Analytical balance,** accuracy ≤ 1 mg.

**6.4 Shaker, rotary or horizontal shaker.** Shaking shall prevent any settling of the sample during extraction.

**6.5 Extraction vessels,** capacity 200 – 500 ml.



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

**6.7 Single use syringe filters (0,22 µm) or vials with filters,** for filtering of test solutions prior to analysis.



**6.8 Ion chromatograph,** with conductivity detector (IC-CD).

For lower signal noise a supressed conductivity detector is preferred.

**6.9 Chromatographic column for anion exchange,** suitable for the selective separation of phosphonates from other anions present in the sample.

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

Other columns recommended by a manufacturer may also be used, providing the results are comparable.

## 7 Procedure

### 7.1 Sample preparation

Solid samples are milled using a laboratory grinder (6.2) and homogenized if necessary. Excessive heating during the sample pretreatment should be avoided. Liquid samples are homogenized before weighing the test portion.

### 7.2 Water extraction

Weigh a sample test portion of approximately 10 g to the nearest milligram into an extraction vessel and add (100 ± 1) ml of deionized water (5.1). The tightly closed vessels are then placed in a shaker (6.4) and samples are extracted for (60 ± 5) min. A blank solution is prepared following the same procedure as for samples.

For highly water absorbing and gel forming samples, only 2 g of sample and 100 ml of deionized water (5.1) is preferred for the extraction.

NOTE 1 For highly non-homogenous samples it is possible to use a test portion of 25 g and volume of deionized water (5.1) 250 ml. In this case use only extraction vessels of the capacity 500 ml to ensure an effective extraction.

NOTE 2 For samples that completely dissolve in deionized water (5.1), dissolution is used instead of extraction.

After extraction the complete extract is carefully transferred to a 200 ml volumetric flask and filled to final volume with deionized water (5.1). Aliquot of extract is then transferred from the volumetric flask to a centrifuge tube and centrifuged for 10 min at 4 000 $min^{-1}$. The supernatant is then diluted with deionized water (5.1). The optimal dilution is chosen according to the structure of the biostimulant sample and the dry matter content. One millilitre of diluted extract is finally filtered by a syringe filter (6.7) to a clean vial.

## 7.3 Preparation of the calibration solutions

Prepare a blank calibration solution and at least three calibration standard solutions in the linear range of calibration by diluting the standard solution (5.3) with deionized water (5.1). Concentrations of calibration standards 0, 0,5, 1, 2,5, 5, 10, 20 mg/l are recommended. Calibration solutions are prepared according to Table 1. Transfer an aliquot of the calibration solutions to chromatography vials prior to analysis.

**Table 1 — Example of typical calibration solutions preparation**

| Final concentration of calibration solution r(P-PO3) [mg/l] | Portion of phosphonate standard solution r(P-PO3) = 100 mg/l [ml] | Final volume [ml] |
|---|---|---|
| 0 | 0 | 100 |
| 0,5 | 0,5 | 100 |
| 1 | 1 | 100 |
| 2,5 | 2,5 | 100 |
| 5 | 5 | 100 |
| 10 | 10 | 100 |
| 20 | 10 | 50 |
| NOTE It is possible to calibrate the instrument for higher concentrations of phosphonates if the calibration curve is linear. | | |



## 7.4 Measurement

### 7.4.1 Instrument conditions

Due to differences between various kinds of instruments, no detailed instructions can be given to operate the specific instrument. The instruction provided by the manufacturer should be followed.

The software of the instrument is used to calibrate the instrument and to calculate concentrations of the phosphonate in the individual test solutions. All test solutions, blanks and calibration solutions are measured under the same optimized conditions. An example of the instrument conditions are given in Table 2.

**Table 2 — Example of typical settings IC-CD instrumentation**

| IC-CD settings | |
|---|---|
| IC-CD | Dionex Integrion |
| Column and guard column | Dionex IonPac AS11-HC Analytical (2 × 250 mm)<br>Dionex IonPac AG11-HC Guard (2 × 50 mm) |
| Mobile phase | KOH – eluent generator |
| KOH concentrations (mM) | gradual increase of KOH concentration from 10 to 45 mM during 25 min. + 5 min equilibration (10 mM) - electrolytically |
| Suppressor | Dionex ADRS 600, 2 mm |
| Suppressor current | Constant voltage – current is calculated automatically |
| Flow rate (ml·min$^{-1}$) | 0,3 |
| Column temperature (°C) | 30 |
| Compartment temperature(°C) | 15 |
| Conductivity detector temperature (°C) | 35 |
| Autosampler temperature (°C) | 15 |
| Measurement time (s) | 1 800 |
| Injection volume (µl) | 25 |
| NOTE 1 The trade name of the instruments and a column above is an example of a suitable and commercially available equipment. This information is given for the convenience of users of this Technical Specification and does not constitute an endorsement by CEN of the products. Equivalent products may be used if they can be shown to lead to the same results.<br>NOTE 2 Use of a different column and a different mobile phase is possible, providing the results are comparable. | |



### 7.4.2 IC-CD measurement

The retention time for the analyte should be approximately twice the retention time corresponding to the void volume of the column, and the nearest peak in the chromatogram should be separated from the analyte peak by at least one full peak width at 10 % of the analyte peak height. It is recommended to verify sufficient separation of the analyte peak using a solution (5.7). Resolution criteria: both sides of the phosphonate peak must lie on the baseline without any co-elution with the chloride and nitrate peak.

Inject an appropriate volume of the calibration standards and calibrate the instrument. Then analyse a sequence of blank (the blank value must not exceed the LOQ of the method), test samples and control samples under the same conditions. The test solutions, which give a response outside the calibration range, should be diluted more times appropriately with deionized water (5.1). Check the instrument sensitivity e.g. by analysing one of the calibration standard solutions throughout the sequence (for example after each five or ten samples). As an analytical control, internal reference samples with known phosphonate concentration shall be analysed in all series of samples. The internal reference samples are to be subjected to all the steps in the method starting from water extraction. If reference samples are not available, spike experiments should be performed to calculate the recovery of the method. It is advisable to check for memory effects, e.g. by the analysis of blank solutions after reference materials.