CEN/TC 223/WG 4 "Analytical methods"
WG Secretariat: **ASI**
Convenor: **Baumgarten Andreas Mr**

1st WD_WI00223117_phosphonates_2022-02

Document type	Related content	Document date	Expected action
Project / Draft		2022-02-24	**INFO**

Description

Please find enclosed the 1st working draft for *WI 00223099, Soil improvers and growing media - Determination of organic matter content and ash* for your information.

As agreed during the last meeting of WG 4 on 2022-02-09, the enclosed 1st working draft and the forms for the activation of the work item will be circulated to CEN/TC 223 as soon as the call for tender for the project leaders is completed and theproject leaders are known.

CEN/TC 223

Date: 2022-02

prEN 00223117:2022

Secretariat: NEN

Soil improvers and growing media – Determination of phosphonates content

Einführendes Element – Haupt-Element – Ergänzendes Element

Élément introductif – Élément central – Élément complémentaire

ICS:

Contents

Page

European foreword ... 3
Introduction ... 4
1 Scope ... 5
2 Normative references ... 5
3 Terms and definitions ... 5
4 Principle ... 5
5 Reagents ... 5
6 Apparatus ... 6
7 Procedure ... 6
7.1 Sample preparation ... 6
7.2 Water extraction ... 6
7.3 Preparation of the calibration solutions ... 7
7.4 Measurement ... 7
7.4.1 Instrument conditions ... 7
7.4.2 IC-CD measurement ... 8
8 Calculation and expression of the results ... 9
8.1 Integration of peaks ... 9
8.2 Concentration of phosphonates in test solutions ... 9
9 Test report ... 9
Bibliography ... 11

European foreword

This document (prEN 00223117:2022) has been prepared by Technical Committee CEN/TC 223 “Soil improvers and growing media”, the secretariat of which is held by NEN, in collaboration with CEN/TC 260 “Fertilizers and liming materials” and CEN/TC 455 “Plant Biostimulants”.

This document is a working document.

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

Introduction

This document describes the extraction and measurement for the determination of phosphonate (*phosphite*) in growing media and soil improvers. The standard 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.

1 Scope

This European Standard specifies a method for the extraction and determination of phosphonates (P–PO_3) in growing media and soil improvers using ion chromatography and conductivity detection (IC-CD).

2 Normative references

The following document, in whole or in part, is normatively referenced in this document and is 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.

EN 13040-1:—[1], *Soil improvers and growing media – Sample preparation – Part 1: Sample preparation for chemical and physical tests, determination of dry matter content, moisture content and laboratory bulk density*

3 Terms and definitions

For the purposes of this document, the following terms and definitions and the terms and definitions given in CEN/TS 00223137:—[2] apply.

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

5.1 General

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.

5.2 Phosphonate standard stock solution, r(P-PO_3) = 1000 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 to final volume and mix thoroughly.

5.3 Phosphonate standard solution, r(P-PO_3) = 100 mg/l is prepared from a standard stock solution (5.2) by appropriate dilution with deionized water. Pipette 10 ml of stock solution (5.2) to a 100 ml volumetric flask, fill with deionized water to final volume and mix thoroughly.

[1] Under preparation. Stage at the time of publication: EN 13040-1:20**.

[2] Under preparation. Stage at the time of publication: CEN/TS 00223137:20**.

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.

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

5.5 Chloride standard, r(Cl^-) = 1000 mg/l - commercially available.

5.6 Nitrate standard, r(NO_3^-) = 1000 mg/l – commercially available.

5.7 Solution for checking chromatographic separation, r(P-PO_3) = 10 mg/l, r(Cl^- and NO_3^-) = 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 100 ml volumetric flask and fill to final volume with deionized water.

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 ml to 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) according to EN 13040-1 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. 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 is preferred for the extraction.

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

After extraction the complete extract is carefully transferred to a 200 ml volumetric flask and filled to final volume with deionized water. 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. A minimum dilution 50-times is recommended. 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. Concentrations of calibration standards 0 mg/l, 0,5 mg/l, 1 mg/l, 2,5 mg/l, 5 mg/l, 10 mg/l, 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-PO_3) [mg/l]	Portion of phosphonate standard solution r(P-PO_3) = 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 is given in Table 2.

Table 2 – Example of typical settings IC-CD instrumentation

IC-CD parameter	IC-CD settings
IC-CD	Dionex Integrion
Column and guard column	Dionex IonPac AS11-HC Analytical (2 mm × 250 mm) Dionex IonPac AG11-HC Guard (2 mm × 50 mm)
Mobile phase	KOH – eluent generator
KOH concentrations (mM)	gradual increase of KOH concentration from 10 mM 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)	1800
Injection volume (µl)	25

NOTE 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 European Standard 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.

NOTE 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, 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. 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.

8 Calculation and expression of the results

8.1 Integration of peaks

The retention time of phosphonates is identified by the analysis of the calibration solutions. The phosphonates peak area in the standards, blank and sample extract solutions is determined.

8.2 Concentration of phosphonates in test solutions

Calculate the concentration of phosphonates in the test solutions using the calibration function established by linear regression from the calibration curve.

Calculate the content of phosphonates in the soil improver or growing medium, w_X, as a mass fraction in mg/kg according to Formula (1).

$$w_X = \frac{(\rho - \rho 0) \times V \times F \times 100}{m \times DM} \quad (1)$$

where

V is the final volume of extract solution (usually 200 ml), in ml;

ρ is the mass concentration of P-PO3 in the sample test solution, in mg/l;

ρ0 is the mass concentration of P-PO3 in the blank, in mg/l;

m is the mass of the test sample, in g;

DM is the dry matter content in percent;

F is the dilution factor calculated according to Formula (2)

$$F = \frac{V_1}{V_{p1}} \times \frac{V_2}{V_{p2}} \times \ldots \frac{V_n}{V_{pn}} \quad (2)$$

where

$V_{1,2\ldots n}$ are the volumes of the volumetric flasks, in ml;

$V_{p1,2\ldots n}$ are the volumes of the pipetted solutions used for an individual diluting step, in ml.

9 Test report

The test report shall contain at least the following information:

a) all information necessary for the complete identification of the sample;

b) the test method used with reference to this document;

c) the test results obtained;

d) date of sampling and sampling procedure (if known);

e) date when the analysis was finished;

f) all operating details not specified in this document, or regarded as optional, together with details of any incidents occurred when performing the method, which might have influenced the test result(s).

Bibliography

[1] CEN/TS 00223137:—, *Soil improvers and growing media — Terminology*

[2] *Regulation (EC) No 1009/2019 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003. Official Journal of the EU, L 170.*

[3] *EFSA (European Food Safety Authority), Brancato A, Brocca D, Carrasco Cabrera L, De Lentdecker C, Erdos Z, Ferreira L, Greco L, Jarrah S, Kardassi D, Leuschner R, Lythgo C, Medina P, Miron I, Molnar T, Pedersen R, Reich H, Sacchi A, Santos M, Stanek A, Sturma J, Tarazona J, Theobald A, Vagenende B and Villamar-Bouza L, 2018. 2018. Reasoned opinion on the modification of the existing maximum residue levels for potassium phosphonates in certain berries and small fruits. EFSA Journal 2018; 16(9):5411, 26 pp. https://www.efsa.europa.eu/en/efsajournal/pub/5411. Annex E, p. 26.*

[4] *Thao, H. T. B., Yamakawa, T.: Phosphite (phosphorous acid): Fungicide, fertilizer or bio-stimulator? Soil Sci. Plant Nutr. (2009) 55, 228-234. Doi: 10.1111/1.1747-0765.2009.00365.x.*

[5] *Note for the members of the fertilizer working group. Phosphonates in EC fertilizers. European Commission, Brussels, 13 January 2015.*