Annex D.12

Fertilizers, liming materials and inhibitors — Determination of the phosphonates content

Draft method

1 Scope

This document specifies a method for the extraction and determination of phosphonates ($P\text{-}PO_3$) in organic, organo-mineral, mineral fertilizers, liming materials and inhibitors using ion chromatography and conductivity detection (IC-CD).

This document is applicable to the fertilizing products blends where a blend is a mix of at least two of the following components: Fertilizers, Liming Materials, Soil Improvers, Growing Media, Inhibitors, Plant Biostimulants, and where the following category: organic, organo-mineral, mineral fertilizers and liming materials is the highest percentage in the blend by mass or volume, or in the case of liquid form by dry mass. If organic, organo-mineral, mineral fertilizers and liming materials 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, 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 1482-1, *Fertilizers and liming materials - Sampling and sample preparation - Part 1: Sampling*

EN 1482-1, *Fertilizers and liming materials - Sampling and sample preparation - Part 2: Sample preparation*

EN 12944-1, *Fertilizers and liming materials — Vocabulary — Part 1: General terms*

EN 12944-2, *Fertilizers and liming materials — Vocabulary — Part 2: Terms relating to fertilizers*

EN 12944-3, *Fertilizers and liming materials — Vocabulary — Part 3: Terms relating to liming materials*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 12944-1, EN 12944-2, and EN 12944-3 and the following apply.

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

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

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

3.5
test sample solution
solution prepared after extraction or digestion of the test sample according to appropriate specifications

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 detection (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 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, mass concentration $\rho_{(P\text{-}PO3)}$ = 1 000 mg/l is prepared by dissolving commercially available sodium phosphite dibasic pentahydrate salt (M_w = 216,04 mol/l, purity ≥ 98 %). For preparation the phosphonate standard stock solution 0,697g ± 0,001 g is weighed, transferred to 100 ml volumetric flask, fill with water (5.1) to final volume and mix thoroughly.

5.3 Phosphonate working standard solution, $\rho_{(P\text{-}PO3)}$ = 100 mg/l is prepared from a standard stock solution (5.2) by appropriate dilution with water (5.1). Pipette 10 ml of stock solution (5.2) to a 100 ml volumetric flask, fill with 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 water (5.1).

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

5.5 Chloride standard, $\rho_{(Cl-)}$ = 1 000 mg/l – commercially available.

5.6 Nitrate standard, $\rho_{(NO3-)}$ = 1 000 mg/l – commercially available.

5.7 Solution for checking chromatographic separation, $\rho_{(P\text{-}PO3)}$ = 10 mg/l, $\rho_{(Cl-)}$ = 50 mg/l and $\rho_{(NO3-)}$ = 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 water (5.1).

6 Apparatus

6.1 Common laboratory glassware and plastic.

6.2 Analytical balance, capable of weighing to the nearest ≤ 1 mg.

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

6.4 Extraction vessels, capacity 200 ml to 500 ml.

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

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

6.7 Ion chromatograph, with conductivity detection (IC-CD).

For higher signal/noise a supressed conductivity detector is preferred.

6.8 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 Sampling

Sampling and sample preparation are not part of this document. The sampling method and sample preparation method given in EN 1482-1 and EN 1482-2.

8 Procedure

8.1 Preparation of test solution - water extraction

Weigh a sample test portion of approximately 10 g to the nearest milligram into an extraction vessel (6.4) and add (100 ± 1) ml of water (5.1). The tightly closed vessels are then placed in a shaker (6.3) and samples are extracted for (60 ± 5) min. A blank test solution is prepared following the same procedure as for samples.

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

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

For samples that completely dissolve in 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 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 water (5.1). The optimal dilution is chosen according to the structure of the sample and the dry matter content. One millilitre of diluted extract is finally filtered by a syringe filter (6.6) to a clean vial.

8.2 Preparation of the calibration solutions

Prepare a blank calibration solution and at least three calibration solutions in the linear range of calibration by diluting the phosphonate working standard solution (5.3) with water (5.1). 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 $\rho_{(P\text{-}PO3)}$	Portion of phosphonate working standard solution $\rho_{(P\text{-}PO3)}$ = 100 mg/l	Final volume
mg/l	ml	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.		

8.3 Measurement

8.3.1 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 sample 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 settings	
IC-CD	Dionex™ Integrion™[a]
Column and guard column	Dionex™ IonPac ™AS11-HC Analytical[a] (2 mm × 250 mm) Dionex™ IonPac™ AG11-HC Guard[a] (2 mm × 50 mm)
Mobile phase	KOH – eluent generator
KOH concentrations (mmol/l)	gradual increase of KOH concentration from 10 mmol/l to 45 mmol/l during 25 min + 5 min equilibration (10 mmol/l) - electrolytically
Suppressor	Dionex™ ADRS 600[a], 2 mm
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 Use of a different column and a different mobile phase is possible, providing the results are comparable.	
[a] This is an example of suitable and commercially available equipment. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product. Equivalent products may be used if they can be shown to lead to the same results.	

8.3.2 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 shall lie on the baseline without any co-elution with the chloride and nitrate peak.

Inject an appropriate volume of the calibration solutions and calibrate the instrument. Then analyse a sequence of blank (the blank value shall not exceed the LOQ of the method), test samples and control samples under the same conditions. The test sample solutions, which give a response outside the calibration range, should be diluted more times appropriately with water (5.1). Check the instrument sensitivity e.g. by analysing one of the calibration 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 calibration solutions after reference materials.

An example of the chromatographic separation of phosphonates from other interfering ions in a mixture of standards is given in Annex A (informative), Figure A.1.

9 Calculation and expression of the results

9.1 Integration of peaks

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

9.2 Concentration of phosphonates in test sample solutions

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

Calculate the content of phosphonates in the sample, w_x as a mass fraction of P-PO_3 expressed on dry matter basis, in mg/kg, according to Formula (1).

$$w_x = \frac{(\rho - \rho_0) \times V \times F \times 100}{m \times w_{DM}} \quad (1)$$

where

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

ρ is the mass concentration of P-PO_3 in the test sample solution, in mg/l;

ρ_0 is the mass concentration of P-PO_3 in the blank test solution, in mg/l;

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

w_{DM} is the dry matter content of the test sample expressed as a mass fraction 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.

Calculate the content of phosphonates in the sample, w_y as a mass fraction of P-PO_3, in %; according to Formula (3):

$$w_y = \frac{w_x}{10\,000} \quad (3)$$

where

w_x is a mass fraction of P-PO_3 expressed on dry matter basis, in mg/kg.

10 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 standard used (including its year of publication);

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

d) the test results obtained;

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

f) date when the analysis was performed and finished;

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

Annex A
(informative)

Separation of phosphonates from other interfering ions in a mixture of standards

Key

1 chlorides
2 phosphonates
3 nitrates
4 sulfates
5 phosphates

Figure A.1 — Example of chromatographic record of mixture of standards at concentration 10 mg/l

Annex B
(informative)

Results of the inter-laboratory study

Description and results of ILS will be added later

B.1 Inter-laboratory tests

B.2 Statistical results for determination of phosphonates

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

This European Standard has been prepared under a standardization request M/564 annexed to Commission Implementing Decision C(2020)612 and M/564 Amd1 annexed to Commission Implementing Decision C(2022)47 as regards the EU fertilising products to provide one voluntary means of conforming to essential safety requirements of Regulation (EU) 2019/1009 relating to the making available on the market of EU fertilising products.

Once this standard is cited in the Official Journal of the European Union (OJEU), under Regulation (EU) 2019/1009, compliance with the normative clauses of this standard given in Table ZA.1 confers, within the limits of the scope of this standard, a presumption of conformity with the corresponding essential requirements of that Regulation (EU) 2019/1009, and associated EFTA regulations.

Table ZA.1 — Correspondence between this European Standard and Regulation (EU) 2019/1009

Essential Safety Requirements of Regulation (EU) 2019/1009	Clause(s)/sub-clause(s) of this EN	Remarks/Notes
Annex I, Part II, item 6	9, 10	Requirement covered

WARNING 1 — Presumption of conformity stays valid only as long as a reference to this European Standard is maintained in the list published in the Official Journal of the European Union. Users of this standard should consult frequently the latest list published in the Official Journal of the European Union.

WARNING 2 — Other Union legislation may be applicable to the product(s) falling within the scope of this standard.

Will be updated later

Bibliography

[1] Regulation (EU) 2019/1009 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

[2] EN 17246, *Fertilizers — Determination of perchlorate in mineral fertilizers by ion chromatography and conductivity detection (IC-CD)*

[3] FprEN 17705, *Plant biostimulants – Determination of phosphonates*

Will be updated after ILS