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Project / Draft		2022-02-25	**INFO**

Description

Please find enclosed the 1st working draft for WI 00223113, Soil improvers and growing media — Determination of the inorganic arsenic content 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 ofthe work item will be circulated to CEN/TC 223 as soon as the call for tender for the project leaders is completed and the project leaders are known.

CEN/TC 223

Date: 2022-02

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Soil improvers and growing media — Determination of the inorganic arsenic content

ICS:

CCMC will prepare and attach the official title page.

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European foreword

This European Standard (prEN 00223113:2022) has been prepared by Technical Committee CEN/TC 223 "Soil improvers and growing media", the secretariat of which is held by NEN.

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. The annex A is informative.

Introduction

This document describes a procedure of extraction and measurement for the determination of inorganic arsenic in growing media and soil improvers. The standard is based on a mild acid oxidative extraction of the arsenic species followed by liquid chromatography (High Performance Liquid Chromaography - HPLC or Ion Chromtagography - IC) coupled to the element-specific detector Inuctively Coupled Plasma Mass Spectrometry (ICP-MS) for the determination of the mass fraction of iAs.

1 Scope

This document specifies a method for extraction, separation, and determination of inorganic arsenic (iAs) in growing media and soil improvers using anion-exchange HPLC or IC coupled to ICP-MS.

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 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 terms and definitions given in CEN/TS 00223137:—[2] and EN 13040-1:— apply.

4 Principle

This document describes a method for the determination of inorganic arsenic in growing media and soil improvers. Inorganic arsenic consists of arsenite As (III) and arsenate, As (V). A representative test portion of the sample is treated with a diluted nitric acid and hydrogen peroxide solution in a heated water bath. By this means the sample is solubilised, arsenic species are extracted into solution and As (III) is oxidised to As (V). The inorganic arsenic is selectively separated from other arsenic compounds using anion exchange HPLC (High Performance Liquid Chromatography) coupled on-line to the element-specific detector ICP-MS (Inductively Coupled Plasma Mass Spectrometer) for the determination of the mass fraction of the inorganic arsenic. External calibration with solvent matrix-matched standards is used for the quantification of the amount of the inorganic arsenic. Alternatively, IC (ion chromatography) coupled to ICP-MS can be used.

A preliminary determination of the total arsenic in aqua regia extracts according to EN 13650:—[3] by ICP-AES (WI TBD (EN), 2020-02.27 Soil improvers and growing media - Determination of aqua regia soluble elements) could reduce the number of the samples where the determination of iAs is necessary because if the content of aqua regia (total) extractable arsenic is lower than the legislative limit for iAs then the determination of iAs is not necessary.

5 Reagents

5.1 General

When using a method of high sensitivity like ICP-MS, the control of the blank levels of water, acid and other reagents is very important. The reagents shall be of adequate purity and of recognized analytical grade. The concentration of arsenic species in the reagents and water used shall be negligible and low enough not to affect the results of the determination. Generally, ultra-pure water and nitric acid of minimum p.a. quality is recommended.

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

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

[3] Under preparation. Stage at the time of publication: prEN 13650:20**.

5.2 Nitric acid (HNO_3), concentrated, ≥ 65 % (mass fraction), mass concentration of approximately ρ (HNO_3) 1,4 g/ml.

Use only nitric acid available with high purity (minimum p.a. quality) in order to avoid potential contamination.

5.3 Hydrogen peroxide, H_2O_2 not less than 30 % (mass fraction).

High purity is essential to avoid potential contamination. Commercially available hydrogen peroxide for analysis should be tested for contamination of arsenic prior to use. It is necessary to prevent peroxide degradation and ensure the stability of the solution.

5.4 Extraction solution, 0,1 mol/l HNO_3 in 3 % (volume fraction) H_2O_2.

Add 6,5 ml of HNO_3 (5.2) and thereafter 100 ml of hydrogen peroxide (5.3) into 800 ml water in a 1 000 ml volumetric flask. Fill the flask to the mark with water. This solution is prepared on the day of use.

NOTE It is recommended that the total volume needed for the analysis is estimated and only this amount is produced in the day of use.

5.5 Ammonium carbonate, $(NH_4)_2CO_3$, mass fraction w ≥ 99,999 %, for preparation of the mobile phase solution.

5.6 Aqueous ammonia, (NH_3(aq)), mass fraction w ≥ 25 %, for adjustment of pH in the mobile phase.

5.7 Methanol, (CH_3OH), HPLC grade, for preparation of the mobile phase solution.

5.8 Mobile phase, e.g. 50 mmol/l ammonium carbonate in 3 % methanol at pH 10,3.

Dissolve 4,80 g of ammonium carbonate (5.5) in approximately 800 ml water. Adjust the pH to 10,3 with aqueous ammonia (5.6) and add 30 ml of methanol (5.7) and then fill up to 1 000 ml with water. Prior to use filter the mobile phase solution through a 0,45 µm filter using a filtering device (6.4).

The optimal concentration of ammonium carbonate in the mobile phase depends on the analytical column used (e.g. brand, particle size and dimensions) and should be verified in advance. The appropriate concentration of ammonium carbonate (usually between 10 mmol/l to 50 mmol/l) is highly dependent on the column used and is up to the discretion of the analyst. It should fulfil the criteria for sufficient resolution of the arsenate peak.

Methanol is added to the mobile phase in order to enhance the signal intensity for arsenic. The concentration of methanol to achieve the highest signal to noise ratio depends on the instrument used and should be identified by the analyst.

NOTE Different mobile phase may be also used according to the instructions of the manufacturer of the column but it is necessary to verify optimal separation conditions.

For example, Agilent column G3154-65001 with a guard column G3154-65002[4] and a recommended mobile phase for this column (dihydrogen potassium carbonate 2 mmol, EDTA 0,2 mmol, pH 6,0 adjusted with sodium hydroxide solution 1 mol) were successfully used for the analysis.

[4] Agilent column G3154-65001 with a guard column G3154-65002 is an example of a suitable instruments available commercially. This information is given only as an example and does not constitute an endorsement by CEN of these products.

5.9 Arsenic (V) standard stock solution, with an arsenic (V) mass concentration of 1 000 mg/l.

The use of commercial standards of arsenic As (V), with a mass concentration of 1 000 mg/l is recommended.

5.10 Arsenic (V) standard solution I, with an arsenic (V) mass concentration of 10 mg/l in 2 % (volume fraction) HNO_3.

Pipette 1 ml of arsenic standard stock solution (5.9) into a 100 ml volumetric flask. Add 2 ml of nitric acid (5.2), fill to the mark with water and mix well. This solution is stable in a refrigerator at least one week.

5.11 Arsenic (V) standard solution II, with an arsenic (V) mass concentration of 1 mg/l.

Pipette 10 ml of arsenic standard solution I (5.10) into a 100 ml volumetric flask, fill to the mark with water and mix well. This solution should be prepared on the same day of use.

5.12 Solution for checking chromatographic separation, containing organic arsenic compounds (e.g. 10 µg/l) monomethylarsenous acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine (AB), as well as arsenate (e.g. 10 µg/l) and chloride (e.g. 100 mg/l).

6 Apparatus

6.1 Common laboratory glass (plastic) ware.

Plastic volumetric flasks are recommended for the preparation of the mobile phase and calibration solutions. All glassware and plastic ware shall be adequately cleaned and stored to avoid any contamination, including Arsenic V contamination

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

6.3 Analytical balance, capable of weighing to the nearest mg or better.

6.4 Filtering device, for filtration of mobile phase with a filter, pore size 0,45 µm.

6.5 Heated water bath, capable of maintaining 90 °C.

6.6 Centrifuge, for minimum 4 000 min-1 (approx. 2 000 g).

6.7 Single use syringe filters (0,45 µm) or HPLC vials with filters, compatible with acidic solutions for filtering of test solutions prior to analysis.

6.8 High Pressure Liquid Chromatograph (HPLC).

6.9 Anion exchange column, suitable for the selective separation of arsenate from other arsenic compounds present in the sample extracts.

It is highly recommended to use a guard column to prolong the life-time of the analytical column.

Use of a different column and a different mobile phase is possible. The other columns may be used in combination with the suitable type of mobile phase depending on the recommendations of the manufacturer (see 5.8), e.g. IonPac® AS7, ICSep Ion120® and Hamilton PRP X-100®[5]

6.10 Inductively coupled plasma mass spectrometer (ICP-MS).

6.11 Argon gas, purity ≥ 99,99 %.

7 Procedure

7.1 Sample preparation

Solid samples are milled using a laboratory grinder (6.2) according to EN 13040-1:— and homogenized. Excessive heating during the sample pre-treatment should be avoided. Liquid samples are homogenized by thorough mixing before weighing the test portion.

7.2 Water bath extraction

Weigh a test portion of approximately of at least 1,0 g solid sample or an equivalent of liquid sample to the nearest milligram into an extraction tube and add to 20 ml with the extraction solution (5.4). The tubes shall be securely closed with a tight lid. Shake the tubes thoroughly in order to ensure that the sample is wetted sufficiently in the extraction solution (5.4) prior to placing it in the water bath in order to ensure a satisfactory extraction of the analyte.

The solutions are then placed into a heated water bath (6.5) at 90 °C ± 2 °C and extracted for 60 min ± 5 min.

Include also a reagent blank sample. A blank solution is prepared following the same procedure as for samples.

After extraction and cooling to room temperature the tubes are centrifuged for 10 min at 4 000 min^{-1} (approx. 2 000 *g*). The supernatant is transferred to clean plastic (PE or PP) tubes. Sample extracts should be filtered by a syringe filter (6.7) to clean HPLC vials prior to analysis. The sample extracts can be stored in a refrigerator (at approximately 4 °C) for a maximum of one week.

7.3 Preparation of the calibration solutions

The external calibration is used for quantification. Prepare a blank calibration solution and at least three calibration standard solutions in the linear range of calibration for inorganic arsenic (As V) by diluting the arsenic standard solution II of As (V) concentration 1 mg/l (5.11) with extraction solution (5.4). Concentrations of calibration standards 0, 25, 50 and 100 µg/l are recommended. Transfer an aliquot of the calibration solutions to HPLC vials prior to analysis.

NOTE It is possible to calibrate the instrument for higher concentrations of the element if the calibration curve is linear.

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[5] IonPac® AS7, ICSep Ion120® and Hamilton PRP X-100® are examples of suitable instruments available commercially. This information is given only as an example and does not constitute an endorsement by CEN of these products.

7.4 Measurement

7.4.1 General instrumental conditions

The analytical procedure requires an adequate amount of experience in operating and optimizing the apparatus.

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

Arsenic is a mono-isotopic element and can be evaluated at a mass/charge ratio (m/z) of 75 using ICP MS.

It is advisable to allow the HPLC system (incl. the analytical column) to equilibrate and ensure stable conditions by turning on the HPLC flow of the mobile phase (see 6.8) prior to the start of the analysis. Repeated injections of a calibration blank may be necessary until stable chromatography is achieved and the analytical sequence can be started.

The software of the instrument is used to calibrate it and to calculate concentrations of the inorganic arsenic in the individual test solutions. All test solutions, blanks and calibration solutions are measured under the same optimized conditions. Example of the instrument conditions are given in Table 1.

7.4.2 HPLC-ICP/MS measurement of inorganic arsenic

7.4.2.1 HPLC conditions

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 (5.12). Make sure that the HPLC run is long enough for chloride (m/z 35) and for any arsenic compounds with longer retention times than arsenate, to elute them from the column prior to the injection of the next sample. It is highly advisable to monitor isotope Cl (m/z 35) together with As (m/z 75). It should furthermore be ensured that the arsenate and chloride peaks do not co-elute in order to avoid interference from the polyatomic ion $_{40}Ar_{35}Cl_{+}$ in the mass spectrometer.

The quantitative oxidation of arsenite to arsenate in sample solutions should be verified (e.g. visual inspection of chromatogram by looking for an additional peak or a reduced intensity of the arsenate peak).

7.4.2.2 Calibration

The external calibration is used for the quantification of mass fraction of iAs (As V). Inject an appropriate volume of the calibration standards into an HPLC-ICP-MS system and calibrate the instrument e.g. determine the peak area of each of the calibration points to construct a calibration curve.

7.4.2.3 Samples measurement

Analyse a sequence of blank, test samples and control samples (e.g. internal reference material) under the same conditions. Suitable dilution of the sample extracts (e.g. 1 + 9) with the mobile phase solution (5.8) is recommended for matrix suppression and to give a response within the linear calibration range. Test sample solutions, which still give a response outside the calibration range, should be diluted more times and appropriately with the mobile phase solution (5.8). If a significant blank value occurs, identify the source of this blank. If the blank is constant and not avoidable, it should be subtracted.

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) and if necessary, use the results for recalibration of the system. As an analytical control, internal reference samples with known inorganic arsenic contents 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 bath 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.

Examples of the chromatographic separation both for original (natural) samples as well as for laboratory spiked samples, solid (s) and liquid (l), are given in Annex A, Figures A.1 to A.4.

Table 1 — Example of possible settings of HPLC-ICP-MS instrumentation (used instruments Agilent 7900[a] and Agilent LC system 1200[a]

ICP MS parameter	**ICP MS settings**
RF power (W)	1550
Carrier gas flow (l ·min^{-1})	1,05
Plasma gas flow (l · min^{-1})	15
Auxiliary gas flow (l · min^{-1})	0,9
Integration time (ms)	0,1 per point
Isotopes monitored (m/z)	75 (As), 35 (Cl)
HPLC parameter	**HPLC settings**
Column	Anion exchange G3154-65001 with guard column G3154-65002[a]
Flow rate (ml · min^{-1})	1
Column temperature	25 °C
Operating pressure (bar)	50
Injection volume (µl)	50
Measurement time (s)	720

[a] 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.

8 Calculation and expression of the results

8.1 Integration of peaks

The retention time of arsenate is identified from the analysis of the calibration solutions. The arsenate peak area in the standards, reagent blank and sample extract solutions are determined.

8.2 Calculation of inorganic arsenic in the samples

Calculate the concentration of inorganic arsenic in the sample solutions using the calibration function established by linear regression from the calibration curve. Calculate the content of inorganic arsenic in the fertilizer, w_X, as a mass fraction in mg/kg according to Formula (1).

The concentration of inorganic arsenic in the blank solution shall be as low as possible. If the inorganic arsenic concentration in the blank is constant and not avoidable, it should be subtracted from *X*s.

$$m_x = \frac{V_{ext} \times (X_S - X_b) \times D \times 100}{m \times DM} \quad (1)$$

where

m_X is a mass fraction iAs, in mg/kg; expressed on dry matter basis

V_{ext} is the final volume after extraction, in ml;

X_S is the concentration of the test solution, in mg/l;

X_b is the concentration of the blank solution, in mg/l;

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

DM is the dry matter content in percent in the test sample;

D is the dilution factor calculated according to Formula (2):

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

where

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

$V_{p1,2...n}$ 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).

Annex A
(informative)

Examples of typical chromatographic separation

Figure to be added

Key

X	Retention Time - RT (min)
Y	Count
———	75
———	35
1	Organic forms of As (m/z 75)
2	Cl^- (m/z 35)

Full Time Range EIC(75) : 018SMPL.d

Figure A.1 — Original (natural) sample (solid), concentration of iAs is below LOQ (< 0,1 mg/kg)

Figure to be added

Key

X	Retention Time - RT (min)
Y	Count
———	75
———	35
1	Organic forms of As (m/z 75)
2	As V (m/z 75)
3	Cl^- (m/z 35)

Full Time Range EIC(75) : 014SMPL.d

Figure A.2 — Spiked sample (solid), concentration of iAs is 9,02 mg/kg

Figure to be added

Key

X	Retention Time - RT (min)
Y	Count
———	75
———	35
1	Organic forms of As (m/z 75)

2 As V (m/z 75)

3 Cl^- (m/z 35)

Full Time Range EIC(75) : 007SMP_As_speciace221020:D

Figure A.3 — Original (natural) sample (liquid), concentration of iAs is 0,26 mg/kg

Figure to be added

Key

X Retention Time - RT (min)

Y Count

_____ 75

_____ 35

1 Organic forms of As (m/z 75)

2 As V (m/z 75)

3 Cl^- (m/z 35)

Full Time Range EIC(75) : 012SMPL.d

Figure A.4 — Spiked sample (liquid), concentration of iAs is 17,59 mg/kg

Bibliography

[1] 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.

[2] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

[3] EN 15517, *Foodstuffs — Determination of trace elements — Determination of inorganic arsenic in seaweed by hydride generation atomic absorption spectrometry (HGAAS) after acid extraction*

[4] EN 16278, *Animal feeding stuffs — Determination of inorganic arsenic by hydride generation atomic absorption spectrometry (HG-AAS) after microwave extraction and separation by solid phase extraction (SPE)*

[5] EN 16802, *Foodstuffs — Determination of elements and their chemical species — Determination of inorganic arsenic in foodstuffs of marine and plant origin by anion-exchange HPLC-ICP-MS*

[6] EN 17374, *Animal feeding stuffs: Methods of sampling and analysis — Determination of inorganic arsenic in animal feed by anion-exchange HPLC-ICPMS*

[7] SLOTH J.J., Report on collaborative trial, Animal feeding stuffs – Determination of inorganic arsenic in animal feed by HPLC-ICP-MS, National Food Institute DTU, Denmark, 2018

[8] EN 1482 (all parts), *Fertilizers and liming materials — Sampling and sample preparation*

[9] EN 13650:—, *Soil improvers and growing media — Digestion by aqua regia for subsequent determination of elements*