

**CEN/TC 260**

Date: 2023 - XX

**prEN 17775**

Secretariat: DIN

**Organic and organo-mineral fertilizers - Determination of the inorganic arsenic content**

**ICS:**

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## European foreword

This document (CEN/prEN 17775) has been prepared by Technical Committee CEN/TC 260 "Fertilizers and liming materials", the secretariat of which is held by DIN.

This is a working document.

This document will supersede CEN/TS 17775:2022. In comparison with the previous edition the following main changes have been made:

Introduction revised according to the recommended wording from FprEN 17706.

Analysis of blends added into the Scope

Normative references to dry matter content determination added and deleted from Bibliography

Normative references to sampling added and deleted from Bibliography

Terms and definitions revised and completed

Editorial changes and changes required for FprEN 17706 were included

Test report revised

Bibliography revised

Annex A (informative): the chromatograms will be updated and changed later according to the preliminary experiments

Annex B (informative) Results of the inter-laboratory study, text prepared to be added after the validation

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

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).

## Introduction

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 [1] laying down rules on the making available on the market of EU fertilizing products (“FPR” or “Fertilising Products Regulation”).

The interest in organic and organo-mineral fertilizers has increased significantly in Europe as they are a valuable tool to use in agriculture. Standardization was identified as having an important role in order to promote the use of organic and organo-mineral fertilizers. The work of CEN/TC 260 seeks to improve the reliability of the supply chain, thereby boosting the confidence of farmers, industry, and consumers in organic and organo-mineral fertilizers, and will promote and support commercialisation of the European fertilizer industry.

The preparation of this document is based on a standardization request to CEN by the European Commission and the European Free Trade Association (Mandate M/564 and relevant amendments) concerning the modernization of methods of analysis of fertilizers within the framework of Regulation (EU) 2019/1009 of the European Parliament and of the Council [1]. This standardization request, presented as M/564, also contributes to the Communication on “Innovating for Sustainable Growth: A Bio economy for Europe”.

This document specifies a procedure of extraction and measurement for the determination of inorganic arsenic in organic or organo-mineral fertilizers. The method is based on a mild acid oxidative extraction of the arsenic species followed by liquid chromatography (High Performance Liquid Chromatography [HPLC] or ion chromatography [IC]) coupled to the element-specific detector ICP-MS (Inductively Coupled Plasma Mass Spectrometer) for the determination of the mass fraction of inorganic arsenic (iAs).

The other standards developed for determination of inorganic arsenic content in plant biostimulants, animal feedingstuffs and foodstuffs were studied and considered as a basis of the described method [2], [3] [4], [5], [6].

The inter-laboratory study reflects the final statistical characteristics of the method for the determination of inorganic arsenic content in organic and organo-mineral fertilizers. The results are given in Annex B (informative).

**WARNING** — Persons using this document should be familiar with usual laboratory practice. This document does not purport to address all of the safety issues, if any, associated with its use. It is the responsibility of the user to establish appropriate health and safety practices and to ensure compliance with any national regulatory conditions.

**IMPORTANT** — It is absolutely essential that tests conducted according to this document are carried out by suitably trained staff.

## 1 Scope

This document specifies a method for extraction, separation, and determination of inorganic arsenic (iAs) in organic or organo-mineral fertilizers using anion-exchange high performance liquid chromatography (HPLC) or ion chromatography (IC) coupled to ICP-MS.

This document is applicable to the fertilizing product blends where the EU fertilising product organic and organo-mineral fertilizer contained in the blend represents the highest % by mass in the blend. In case of equal shares, the user can apply either this or the standard(s) applicable to the other component product(s).

## 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 (2007), *Fertilizers and liming materials – Sampling and sample preparation – Part 1: Sampling*

EN 1482-2 (2007), *Fertilizers and liming materials – Sampling and sample preparation – Part 2: Sample preparation*

prEN 17773<sup>1</sup>, *Organic and organo-mineral fertilizers – Determination of the dry matter content*

## 3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

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

### 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

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<sup>1</sup> Under preparation

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### 3.4

#### **calibration solution**

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

### 3.5

#### **stock solution**

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

### 3.6

#### **test sample solution**

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

## 4 Principle

This document specifies a method for the determination of inorganic arsenic in organic or organo-mineral fertilizers. 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 by ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometry) according to prEN 17768<sup>2</sup> [8], prEN 17770<sup>3</sup>[9] 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 **Sampling**

Sampling and preparation of the test sample is not part of this procedure. Sampling and sample preparation method given in EN 1482 (all parts) with appropriate adaptations required account for specificities of organic and organo-mineral fertilizers shall be used.

Commenté [BA1]: Should be discussed in WG8

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<sup>2,3</sup> Under preparation

## 6 Reagents

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.

**6.1 Water** with an electrical conductivity not higher than 0,1 mS/m at 25 °C free from the elements to be determined.

**Commenté [BA2]:** The better quality is needed for this ISO document is provided by Eva

**6.2 Nitric acid (HNO<sub>3</sub>)**, concentrated, ≥ 65 % (mass fraction), mass concentration of approximately ρ (HNO<sub>3</sub>) 1,4 g/ml.

**Commenté [BA3]:** Tbd with WG8 and Olivier -according to decision 2 from TC.260 on 22nd Oct 2021

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

**6.3 Hydrogen peroxide, H<sub>2</sub>O<sub>2</sub>** 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, this is in the discretion of the analyst to use only H<sub>2</sub>O<sub>2</sub> of adequate quality.

**6.4 Extraction solution**, 0,1 mol/l HNO<sub>3</sub> in 3% (volume fraction) H<sub>2</sub>O<sub>2</sub>.

Add 6,5 ml of HNO<sub>3</sub> (6.2) and thereafter 100 ml of hydrogen peroxide (6.3) into 800 ml water (6.1) in a 1 000 ml volumetric flask. Fill the flask to the mark with water (6.1). This solution is prepared on the day of use.

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

**6.5 Ammonium carbonate, (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub>**, mass fraction  $w \geq 99,999$  %, for preparation of the mobile phase solution.

**6.6 Aqueous ammonia, (NH<sub>3</sub>(aq))**, mass fraction  $w \geq 25$  %, for adjustment of pH in the mobile phase.

**6.7 Methanol, (CH<sub>3</sub>OH)**, HPLC grade, for preparation of the mobile phase solution.

**6.8 Mobile phase**, e.g. 50 mmol/l ammonium carbonate in 3% (volume fraction) methanol at pH 10,3.

Dissolve 4,80 g of ammonium carbonate (6.5) in approximately 800 ml water (6.1). Adjust the pH to 10,3 with aqueous ammonia (6.6) and add 30 ml of methanol (6.7) and then fill up to 1 000 ml with water (6.1). Prior to use filter the mobile phase solution through a 0,45 µm filter using a filtering device (7.4).

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.

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

NOTE For example, Agilent<sup>1</sup> column G3154-65001 with a guard column G3154-65002 and a mobile phase recommended for this column, as a mixture of potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) 2 mmol/l, ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA disodium salt) 0,2 mmol/l, pH 6,0 adjusted with sodium hydroxide (NaOH) 1 mol/l, were successfully used for the analysis.

**6.9 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.

**6.10 Arsenic (V) standard solution I**, with an arsenic (V) mass concentration of 10 mg/l in 2 % (volume fraction)  $\text{HNO}_3$ .

Pipette 1 ml of arsenic standard stock solution (6.9) into a 100 ml volumetric flask. Add 2 ml of nitric acid (6.2), fill to the mark with water (6.1) and mix well. This solution is stable in a refrigerator at least one week.

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

Pipette 10 ml of arsenic standard solution I (6.10) into a 100 ml volumetric flask, fill to the mark with water (6.1) and mix well. This solution should be prepared on the same day of use.

**6.12 Solution for checking chromatographic separation**, containing organic arsenic compounds (e.g. 10  $\mu\text{g/l}$ ) monomethylarsenous acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine (AB), as well as arsenate (e.g. 10  $\mu\text{g/l}$ ), arsenite (e.g. 10  $\mu\text{g/l}$ ) and chloride (e.g. 100 mg/l).

This solution is recommended to demonstrate satisfactory resolution of individual arsenic species, possible interferences and to find out how the chromatographic conditions should be optimized (e.g. by changing the mobile phase concentration or the mobile phase flow rate).

The solution shall be prepared in water (6.1), not in the extraction solution, to check the retention time of the individual arsenic species, their visual presentation in the chromatogram and how the peaks are separated.

## 7 Apparatus

**7.1 Common laboratory glass and 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.

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

**7.3 Analytical balance**, capable of weighing to an accuracy of 1 mg or better.

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<sup>1</sup>Agilent® is a registered trademark of Agilent Technologies Inc. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of the product named.



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

**7.5 Heated (shaking) water bath**, capable of maintaining 90 °C.

Some fine materials can form a thin layer on the surface of the extraction solution and the contact of the sample with the extraction solution can be less intensive. Therefore, the shaking water bath is recommended to ensure the efficient extraction of the sample.

The manual shaking of the solutions in a specific time intervals (every 10 minutes) is also possible.

**7.6 Centrifuge**, for minimum 4 000 min<sup>-1</sup> (approx. 2 000 g).

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

**7.8 High Pressure Liquid Chromatograph (HPLC).**

**7.9 Anion exchange chromatographic 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, providing the results are comparable. It is necessary and very important to verify optimal separation conditions. The other columns may be used in combination with the suitable type of mobile phase depending on the recommendations of the manufacturer (see 6.8).

**7.10 Inductively coupled plasma mass spectrometer (ICP-MS).**

**7.11 Argon gas, purity ≥ 99,99 %.**

## **8 Procedure**

### **8.1 Sample preparation**

Solid samples are milled using a laboratory grinder (7.2) according to EN 1482-2 and homogenized. Excessive heating during the sample pre-treatment should be avoided. Liquid samples are homogenized by thorough mixing before weighing the test portion.

### **8.2 Water bath extraction**

Weigh a test portion of approximately 0,5 g to 1,0 g solid sample or an equivalent of liquid sample to the nearest milligram into an extraction tube and fill up to 20 ml with the extraction solution (6.4). The tubes shall be securely closed with a tight lid. Shake the tubes thoroughly in order to ensure that the samples are wetted sufficiently in the extraction solution (6.4) prior to placing them in the water bath in order to ensure a satisfactory extraction of the analyte.

The solutions are then placed into a heated shaking water bath (7.5) at 90 °C ± 2 °C and extracted for 60 min ± 5 min.

Include also a reagent blank sample. A blank test 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<sup>-1</sup> (approx. 2 000 g). The supernatant is transferred to clean plastic (PE or PP) tubes. Sample extracts should

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be filtered by a syringe filter (7.7) 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.

### 8.3 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 (6.11) with extraction solution (6.4). Concentrations of calibration standards 0 µg/l, 25 µg/l, 50 µg/l 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.

### 8.4 Measurement

#### 8.4.1 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 instructions 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.

**NOTE** Instruments with single quadrupole ICP-MS can evaluate As at ( $m/z$ ) 75 using He gas collision mode, H<sub>2</sub> gas reaction mode or no gas mode. It is possible to measure As in O<sub>2</sub> cell gas reaction mode using triple quadrupole ICP-MS/MS instrumentation. As is shifted to its product ion AsO<sup>+</sup> and is evaluated at ( $m/z$ ) 91 in mass shift MS/MS mode.

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.

#### 8.4.2 HPLC-ICP/MS measurement of inorganic arsenic

##### 8.4.2.1 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 (6.12). 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 <sup>40</sup>Ar<sup>35</sup>Cl<sup>+</sup> 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 of arsenite As (III) or a reduced intensity of the arsenate As (V) peak). The solution for checking chromatographic separation (6.12), containing organic as well as inorganic arsenic forms (arsenite and arsenate) can be used for comparison with the chromatograms of the real samples.

The quantitative oxidation of arsenite can be also checked by spiking the sample with arsenite As (III), e.g. add a solution of arsenite 10 µg/l to the sample before the extraction procedure. After the extraction, measure the samples without the spike as well as with the spike of As (III) and evaluate the recovery at arsenate As (V) peak. Visual inspection of chromatogram by looking for an additional peak of arsenite As (III) is necessary. If the oxidation of As (III) to As (V) was quantitative, no As (III) peak should appear on the chromatogram.

If a satisfactory resolution between As (V) and Cl<sup>-</sup> was not achieved, the chromatographic conditions should be optimized, e.g. by changing the mobile phase concentration or the mobile phase flow rate.

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

#### 8.4.2.2 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.

#### 8.4.2.3 Samples measurement

Analyse a sequence of blank test solution, test samples and control samples (e.g. internal reference material) under the same conditions. Suitable dilution of the sample extracts with the mobile phase solution (6.8) is recommended for matrix suppression and to give a response within the linear calibration range. The recommended dilution of the samples is approximately 10 times, take 1 volume of the sample extract and add 9 volumes of the mobile phase solution (6.8), or 20 times, take 1 volume of the sample extract and add 19 volumes of the mobile phase solution (6.8). Test sample solutions, which still give a response outside the calibration range, should be diluted more times and appropriately with the mobile phase solution (6.8). 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 sample as well as for spiked sample are given in Annex A (informative), Figures A.x to A.x. (will be updated after the preliminary experiments)

**Table 1 — Example of possible settings of HPLC-ICP-MS instrumentation**

ICP MS settings	
ICP-MS	Agilent 7900 <sup>a</sup>
RF power (W)	1550
Carrier gas flow (l·min <sup>-1</sup> )	1,05

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Plasma gas flow (l·min <sup>-1</sup> )	15
Auxiliary gas flow (l·min <sup>-1</sup> )	0,9
Integration time (ms)	0,1 per point
Isotopes monitored (m/z)	75 (As), 35 (Cl)
<b>HPLC settings</b>	
HPLC	Agilent LC system 1200 <sup>a</sup>
Column	Anion exchange G3154-65001 with guard column G3154-65002 <sup>a</sup>
Flow rate (ml·min <sup>-1</sup> )	1
Column temperature (°C)	25
Operating pressure (bar)	50
Injection volume (μl)	50
Measurement time (s)	720
<sup>a</sup> 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. <del>Equivalent products may be used if they can be shown to lead to the same results.</del>	

## 9 Calculation and expression of the results

### 9.1 Integration of peaks

The retention time of arsenate is identified from the analysis of the calibration solutions. The arsenate peak area in the standard calibration solutions, blank test solution and sample test solutions are determined.

### 9.2 Calculation of inorganic arsenic in the samples

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

The concentration of inorganic arsenic (iAs) in the blank test 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  $X_S$ .

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

where

- $w_X$  is a mass fraction iAs, in mg/kg; expressed on dry matter basis;  
 $V_{\text{ext}}$  is the final volume after extraction, in ml;  
 $X_S$  is the concentration of the test sample solution, in mg/l;  
 $X_b$  is the concentration of the blank test solution, in mg/l;  
 $m$  is the mass of the test sample, in g;  
 $w_{\text{DM}}$  is the dry matter content of the test sample expressed as a mass fraction in percent determined according to prEN 17773<sup>4</sup>  
 $D$  is the dilution factor calculated according to Formula (2):

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

Where

- $V_{1,2,\dots,n}$  are the volumes of the volumetric flasks, in ml;  
 $V_{p1,2,\dots,n}$  are the volumes of the pipetted solutions used for an individual diluting step, in ml.

## 10 Test report

The test report shall contain at least the following information:

- all information necessary for the complete identification of the sample;
- the test method used with reference to this document (prEN17775<sup>5</sup>);
- the standard used (including its year of publication)
- the test results obtained;
- date of sampling and sampling procedure (if known);
- date when the analysis was performed and finished;
- whether the requirement of the repeatability limit has been fulfilled (optional);
- 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).

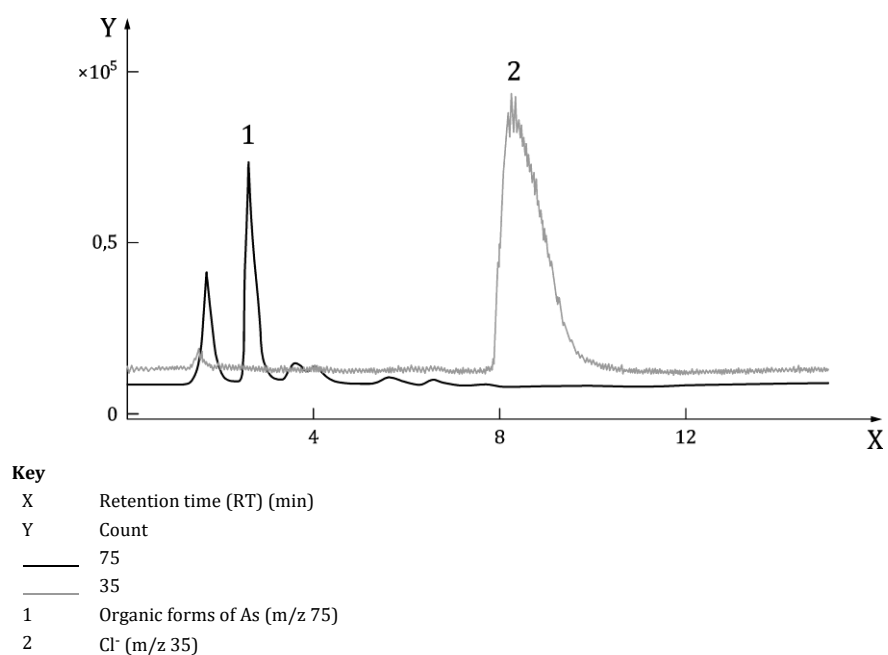
---

<sup>4,5</sup> Under preparation

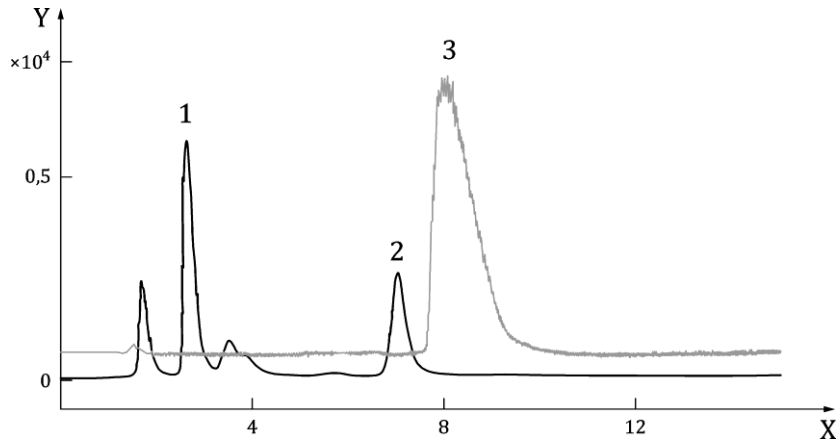
**Annex A**  
(informative)

**Examples of typical chromatographic separation**

The chromatograms will be updated and changed later according to the preliminary experiments



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



- Key**
- X Retention time (min)
  - Y Count
  - 75
  - - - 35
  - 1 Organic forms of As (m/z 75)
  - 2 As(V) (m/z 75)
  - 3 Cl<sup>-</sup> (m/z 35)

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

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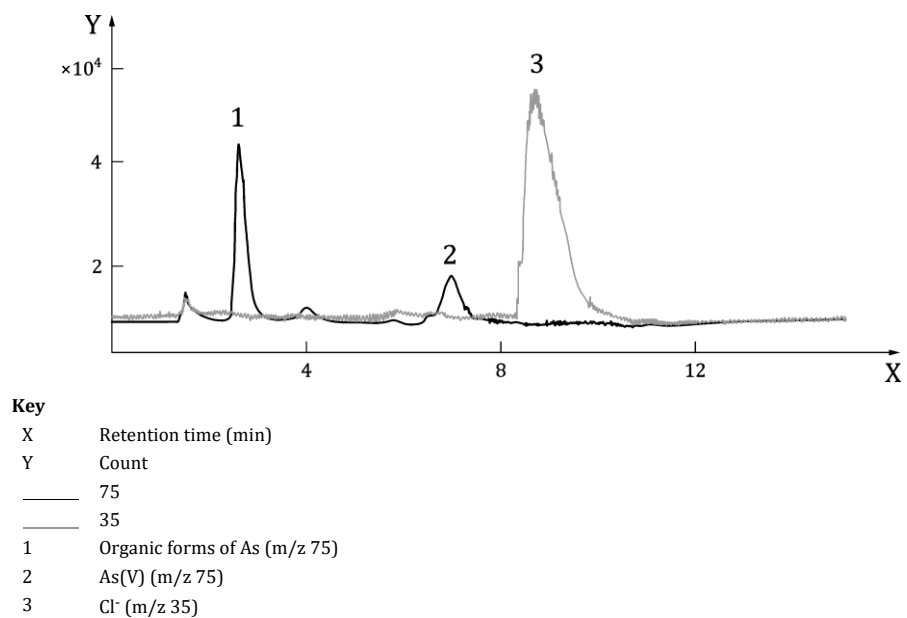
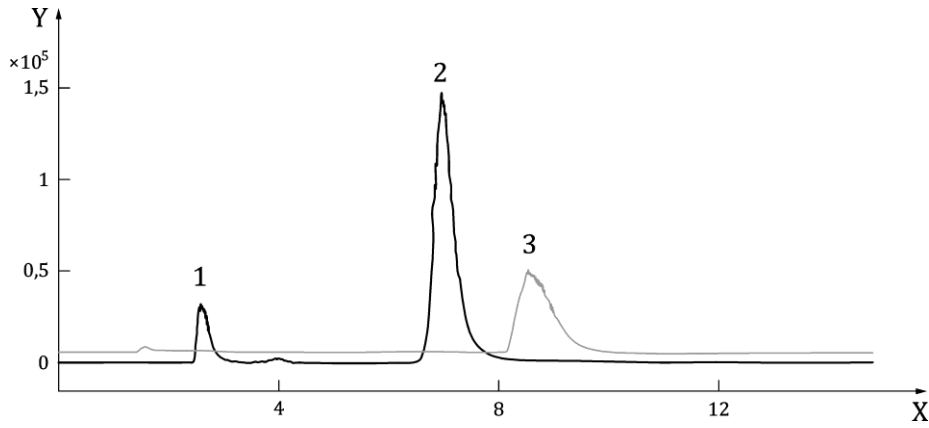


Figure A.3 — Original (natural) sample (liquid), concentration of iAs is 0,26 mg/kg





- Key**
- X Retention time (min)
  - Y Count
  - 75
  - 35
  - 1 Organic forms of As (m/z 75)
  - 2 As(V) (m/z 75)
  - 3 Cl<sup>-</sup> (m/z 35)

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

**Annex B**  
**(informative)**

**Results of the inter-laboratory study,**

**To be included after validation**

**B.1 Inter-laboratory tests**

The experience with an interlaboratory study organized for determination of inorganic arsenic content in different matrices were taken into consideration [7], [11].

The precision of the method has been determined in an inter-laboratory study (ILS) with xx participating laboratories from xx EU countries using xx different samples. Further details regarding the outcome of the inter-laboratory study are available in the final validation report [x].

The samples were chosen to represent all typical organic and organo-mineral fertilizers available on the market. The concentrations of arsenic in the samples were very low (usually below LOQ). Therefore, spiking procedure was necessary to achieve measurable concentrations of inorganic arsenic (iAs).

xx different sample materials (x solid and x liquid, x of them blends) were included in the ILS.

A detailed description of the materials is given in Table B.1.

**Table B.1 — Samples selected for the inter-laboratory study**

<b>Sample ID</b>	<b>Sample type</b>

**B.2 Statistical results for determination of inorganic arsenic**

Statistical evaluation was carried out based on the mathematical algorithms prescribed by ISO 5725-2 [10]. The ILS reflects the final statistical characteristics of the method for the determination of inorganic arsenic including both, the extraction and the measurement steps. The results are summarized in Table B.2.

Based on the statistical evaluation of the results from the inter-laboratory study, it is concluded that the proposed method is suitable for determination of inorganic arsenic after the extraction of the samples using mild acid oxidative mixture of HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> following HPLC (or IC)-ICP-MS quantification in organic and organo-mineral fertilizers and their blends.

Table B.2 — Determination of inorganic arsenic

Sample	iAs-S-1	iAs-S-2	iAs-S-3	iAs-S-4
$L$				
$L_A$				
$N$				
$N_A$				
$O$				
$\bar{x}$				
$s_R$				
$s_r$				
$RSD_R$				
$RSD_r$				
$R$				
$r$				
$HorRat$				
$L$	Number of participating laboratories			
$L_A$	Number of laboratories after elimination of outliers			
$N$	Number of all analytical values			
$N_A$	Number of analytical values after rejection of outliers			
$O$	Percentage of outliers (%)			
$\bar{x}$	Total mean of results (without outliers) (mg/kg)			
$s_R$	Reproducibility standard deviation (mg/kg)			
$s_r$	Repeatability standard deviation (mg/kg)			
$RSD_R$	Relative reproducibility standard deviation (%)			
$RSD_r$	Relative repeatability standard deviation (%)			
$R$	Reproducibility limit ( $2,77 s_R$ ) (mg/kg)			
$r$	Repeatability limit ( $2,77 s_r$ ) (mg/kg)			
$HorRat$	HorRat index			

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<sup>6,7</sup> Under preparation