**CEN/TC 260**

Date: 2023 - XX

**prEN 17778**

Secretariat: DIN

# Organic and organo-mineral fertilizers — Determination of chromium (VI) content by chromatography

**Einführendes Element — Haupt-Element — Ergänzendes Element**

**Élément introductif — Élément central — Élément complémentaire**

**ICS:**

# Contents

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## European foreword

This document (CEN/prEN 17778) 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 17778:2022. In comparison with the previous edition a significant technical change was applied in this document: in clause 7.2.1 it was reduced the timing from extraction from 3 hours to 30 minutes. 3 hours is the timing reported in EN ISO 17075-2:2017 [1] where the samples are pieces of leather, while in this document the samples are represented by fertilisers in a smaller physical form (powder, granules, flakes, pellets, ...). Following preliminary laboratory trials, the reduction of the extraction timing at 30 minutes was considered sufficient.

Furthermore, the following main editorial changes were made:

- In the introduction, standards used as basis for the document and the description of Annexes A, B and C were added;
- In the scope (clause 1), the applicability of the document to blends was added;
- The normative references (clause 2) and the bibliography were reordered. Clause 2 only contains the references that some or all of their content constitute requirements of this document, while the other references were included in the bibliography;
- The principle (clause 4) was better clarified;
- In clause 8, the calculation and the expression of the results were aligned to EN 17773: — [1] for the dry matter expression;
- In the test report (clause 9) further information was added;
- Annex A on the results of the inter-laboratory study was added and consequently Annex A (Chromatographic conditions for direct detection method) and Annex B (Chromatographic conditions for method with post-column reaction) became Annex B and C respectively;
- In Annex B and Annex C, chromatograms were substituted;
- Annex ZA for the relationship with EU Legislation was added.

In the text, if references were superseded, the latest edition has been mentioned.

**Commenté [CM1]:** To maintain whether the chromatograms will be substituted

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 addressed to CEN by the European Commission. The Standing Committee of the EFTA States subsequently approves these requests for its Member States.

For relationship with EU Legislation, see informative Annex ZA, which is an integral part of this document.

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[1] Under preparation

## Introduction

Regulation (EU) 2019/1009 [2] lays down the rules on the making available on the market of EU fertilizing products and the specific safety and quality requirements for the defined product function categories (PFCs). Organic and organo-mineral fertilizers have been classified as PFC 1(A) and PFC 1(B).

This document provides the method for the determination of the chromium (VI) extraction and determination as mentioned in Annex I, Part II, PFC 1(A) and PFC 1(B) of Regulation (EU) 2019/1009 [2]in order to measure the compliance with the related requirement in that Regulation.

Standards for determination of chromium VI in soil and waste [3], fertilizers [4] and for leather [1] were studied and considered as a basis of this document.

This document contains two annexes (B and C) which describe two possible ways for determining chromium VI by ion chromatography. Annex B describes an example of chromatographic conditions for the direct detection method, while Annex C explains the chromatographic conditions for method with post-column reaction. Both methods can be applied to determine Chromium VI. The choice depends on the equipment available in laboratories.

The inter-laboratory study reflects the final statistical characteristics of the method for the determination of chromium VI content in organic and organo-mineral fertilizers. The results are given in Annex A (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 the determination of hexavalent chromium (chromium (VI)) in organic and organo-mineral fertilizers in order to verify that hexavalent chromium (Cr(VI)) is not present in organic and organo-mineral fertilizers in a concentration that exceeds the respective limits outlined in the EU Regulation on Fertilising Products [1].

This document is applicable to fertilizing products (solid and liquid[2]), which are classified as PFC 1(A) and PFC 1(B) or the component of PFC 1(A) and PFC 1(B) in PFC 7 (fertilizing product blend) of Regulation (EU) 2019/1009 [1]..

The method specified is suitable to quantify the chromium (VI) content in organic and organo-mineral fertilizers down to 2 mg/kg dry matter.

The results obtained from this method are strictly dependent on the extraction conditions. Results obtained by using other extraction procedures (extraction solution, pH of the extraction solution, extraction time, extraction temperature, etc.) are not comparable with the results produced by the procedure described in this document. Therefore, this document is highly recommended to quantify the chromium(VI) content in blends of fertilizing products containing organic and organo-mineral fertilizers, independently of the percentage of the different parts forming the blend [*alternatively in according to the agreement reached in the coordination group with CCMC, CEN/TC 223, CEN/TC 260 and CEN/TC 455: this document is applicable to the fertilizing products blends where a blend is a mix of at least two of the following components [select relevant categories1: Fertilizers/Liming Materials/Soil Improvers/Growing Media/Inhibitors/Plant Biostimulants] and where the following category [select relevant category2: organic fertilizers, organo-mineral fertilizers, inorganic fertilizers, liming materials, inhibitors] is the highest % in the blend by mass or volume, or in the case of liquid form by dry mass. If [select relevant category2: organic fertilizers, organo-mineral fertilizers, inorganic fertilizers, liming materials, inhibitors] is not the highest % in the blend, the European Standard for the highest % of the blend applies. In case a fertilizing product blend is composed of components in equal quantity, the user decides which standard to apply.Footnotes for information (not to be included in the standard):*

*1 Select only categories that are relevant and, if necessary, the sub-categories from the FPR, Annex I, part 1 (e.g. Solid organic fertiliser, Liquid organic fertiliser, etc.)*

*2 Select only categories that are relevant for the products in the scope of the TC drafting the standard]*

**Commenté [MC2]:** To be discussed within TG 1 and 2 and on the basis of the ILS

## 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 (all parts), *Fertilizers and liming materials — Sampling and sample preparation*

EN 17773[3]:—, *Organic and organo-mineral fertilizers — Determination of the dry matter content*

**Commenté [MC3]:** Currently the EN 1482's parts under ballot don't include the organic and organo-minerals. To discussed whether the reference "all parts" can be maintained even in case a part 5 will be developed for organic and organo-mineral fertilizers

**Commenté [MC4]:** To be discussed further in according to the draft minutes of WG 8's meeting (N459) relating to point 6 "drafting ENs"

**Commenté [BA5]:** Tbd with WG8

## 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:

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[2] According to the definition of "solid form" and "liquid form" of the EU Regulation on Fertilising Products [1]

[3] Under preparation

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

### 3.1

**Chromium (VI) content**

amount of chromium (VI) in organic and organo-mineral fertilizers determined after extraction with an aqueous salt solution at pH 7,0 to pH 8,0.

Note 1 to entry: The chromium (VI) content is reported as chromium (VI) in milligrams per kilogram (mg/kg), expressed as the dry mass of the sample.

[SOURCE: EN ISO 17075-2:2017[1], 3.1, modified: "leather" is replaced by "organic and organo-mineral fertilizers"]

# 4 Principle

To prove the compliance with the limit value specified in Regulation (EU) 2019/1009 [1] for chromium(VI) in organic and organo-mineral fertilizers, there is the possibility to choose between:

— Using a testing method determining the total chromium content, as long as the result of the test shows a content below the limit value for chromium(VI). In such a case, it can be safely assumed that the organic fertilizer or the organo-mineral fertilizer complies with the limit value for chromium (VI).

— Using a testing method which determines only chromium(VI) content.

The determination of the total chromium in organic and organo-mineral fertilizers can be performed by aqua regia digestion (EN 17768:—[4] [5]) and ICP-AES determination (EN 17770:—[5] [6]).

This standard specifies the procedure to directly determine the content of chromium (VI) in organic and organo-mineral fertilizers: extractable chromium (VI) is leached from the sample in phosphate buffer at pH 7,0 to pH 8,0. An aliquot of the filtered extract is analysed for chromium (VI) using ion-exchange chromatography with UV-VIS detection.

# 5 Reagents

All reagents used shall have at least analytical grade.

## 5.1 Extraction solution

Dissolve 22,8 g dipotassium hydrogen phosphate trihydrate ($K_2HPO_4 \cdot 3H_2O$) in 1 000 ml water (5.7), adjusted to pH 8,0 ± pH 0,1 with phosphoric acid solution (5.2). Degas this solution with either argon or nitrogen (5.6) or ultrasonic bath.

Standard practice is to make up a fresh solution each day. However, the solution may be kept for up to one week in a refrigerator at (4 ± 3) °C but shall be warmed to room temperature and degassed prior to use.

## 5.2 Phosphoric acid solution

700 ml *o*-phosphoric acid, density $\rho$ = 1,71 g/ml, made up to 1 000 ml with water (5.7).

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[4] Under preparation

[5] Under preparation

First add approximately 200 ml of water (5.7) to a 1 000 ml volumetric flask, then add the 700 ml of *o*-phosphoric acid and dilute to the mark with water (5.7).

**5.3 Potassium dichromate** ($K_2Cr_2O_7$), dried for (16 ± 2) h at (105 ± 2) °C.

### 5.4 Chromium(VI) stock solution

Dissolve 2,829 g potassium dichromate ($K_2Cr_2O_7$) (5.3) in water (5.7) in a volumetric flask and make up to 1 000 ml with water (5.7). 1 ml of this solution contains 1 mg of chromium.

The solution can be kept for up to 12 months in a refrigerator at (4 ± 3) °C but shall be warmed to room temperature prior to use.

It is also possible to use a commercial standard solution with a certified Cr(VI) concentration that can be connected to national standards. Observe the expiry date or recommended shelf life stated by the manufacturer.

### 5.5 Chromium(VI) standard solution

Pipette 1 ml of solution (5.4) into a 1 000 ml volumetric flask and make up to the mark with extraction solution (5.1). 1 ml of this solution contains 1 µg of chromium.

The solution can be kept for up to one week in a refrigerator at (4 ± 3) °C but shall be warmed to room temperature prior to use.

A stock solution of hexavalent chromium at this concentration level is an alternative available commercially.

### 5.6 Argon or nitrogen, oxygen-free

Preference should be given to argon as an inert gas instead of nitrogen because argon has a higher specific mass than air.

**5.7 Distilled or deionised water,** with a specific conductivity not higher than 0,2 mS/m at 25 °C, free from the elements to be determined..

## 6 Apparatus

Usual laboratory equipment and, in particular, the following.

**6.1 Suitable mechanical orbital shaker**, (100 ± 10) $\text{min}^{-1}$.

**6.2 Conical flask**, of capacity 250 ml, with stopper.

**6.3 Aeration tube** and **flow meter**, suitable for a flow rate of (50 ± 10) ml/min.

**6.4 pH meter**, with glass electrode.

**6.5 Membrane filter**, 0,45 µm pore size [polytetrafluoroethylene (PTFE) or polyamide 66].

**6.6 Common laboratory glassware and pipettes.**

**6.7 Vacuum device**, suitable for filtration of extraction solution, mobile phase, and sample extracts.

**6.8 Ion-exchange chromatograph, with UV/VIS detector or high-performance liquid chromatography (HPLC) with anion-exchange column and UV/VIS detector.** A photo diode array detector (DAD) is recommended.

**6.9 Analytical balance**, capable of weighing to 0,1 mg.

**6.10 Syringe membrane filters** of polyamide 6.6 of , 0,45 µm pore size, for filtration of standards.

**6.11 Suitable vials for IC or HPLC.**

# 7 Procedure

## 7.1 Sampling and sample preparation

**Commenté [BA6]:** Tbd with WG8

tSampling and sample preparation shall be performed, following the principles described in EN 1482 (all parts) with appropriate adaptations, required to account for specificities of organic and organo-mineral fertilizers. For products that can decompose or react when heated, grinding shall be carried out in order to prevent heating.

**Commenté [MC7]:** Currently the EN 1482’s parts under ballot don’t include the organic and organo-minerals. To discussed whether the reference “all parts” can be maintained even in case a part 5 will be developed for organic and organo-mineral fertilizers

Details about the sampling and sample preparation shall be given in the test report.

## 7.2 Preparation of analytical solution

### 7.2.1 Solid samples

The operator shall weigh approximately 2 ± 0,1 g of sample of organic or organo-mineral fertilizer to the nearest 0,001 g with an analytical balance (6.9).

Following tasks shall be performed:

- Pipette 100 ml ($V_0$) of degassed solution (5.1) into a 250 ml conical flask (6.2). Displace oxygen by passing oxygen-free argon (or nitrogen) (5.6) into the flask for 5 min with a volume flow of (50 ± 10) ml/min. Remove the aeration tube (6.3), add the sample and close the flask with a stopper. Record the extract volume as $V_0$ and the weight of the sample as *m*.
- Shake the conical flask with the sample for (30,0 ± 1) min on a mechanical orbital shaker at (100 ± 10) $\text{min}^{-1}$ (6.1) at room temperature to extract the chromium (VI).
- Shake the suspension in a smooth circular movement to keep the sample from adhering to the wall of the flask and avoid shaking faster than specified.
- Immediately after completing the 30-min of extraction, filter the contents of the conical flask through a membrane filter into a glass or plastic vessel with lid. Check the pH of the solution. The pH of the solution shall be between 7,0 and 8,0. If the pH of the solution is not within this range, start the complete procedure again. Consider using a smaller sample mass, if the pH is not between 7,0 and 8,0. In this case, the quantification limit will be increased.
- Transfer an aliquot of the filtered extract into a vial (6.11). Instrumental determination of chromium (VI) should be performed as soon as possible and no later than 60 min after the extraction phase to avoid risks of reduction.

### 7.2.2 Liquid samples

The operator shall weigh approximately (10 ± 0,1) g of sample of organic or organo-mineral fertilizer to the nearest 0,001 g with an analytical balance (6.9).

The following tasks shall be performed:

- Pipette 100 ml ($V_0$) of degassed solution (5.1) into a 250 ml conical flask (6.2). Displace oxygen by passing oxygen-free argon (or nitrogen) (5.6) into the flask for 5 min with a volume flow of (50 ± 10) ml/min. Remove the aeration tube (6.3), add the sample and close the flask with a stopper. Record the extract volume as $V_0$ (100 ml + sample volume in ml).

- Shake the conical flask with the sample for (10 ± 1) min on a mechanical orbital shaker at (100 ± 10) $\text{min}^{-1}$ (6.1) at room temperature to extract the chromium (VI).

- Shake the suspension in a smooth circular movement to keep the sample from adhering to the wall of the flask and avoid shaking faster than specified.

- Immediately after completing the 10 min of extraction, filter the contents of the conical flask through a membrane filter into a glass or plastic vessel with lid. Check the pH of the solution. The pH of the solution shall be between 7,0 and 8,0. If the pH of the solution is not within this range, start the complete procedure again. Consider using a smaller sample mass, if the pH is not between 7,0 and 8,0. In this case, the quantification limit will be increased.
- Transfer an aliquot of the filtered extract into a vial (6.11). Instrumental determination of chromium (VI) should be performed as soon as possible and no later than 60 min after the extraction phase to avoid risks of reduction.

### 7.3 Chromatographic conditions

Determination of chromium (VI) is performed using the ion chromatography or HPLC equipped with an anion-exchange column. As the instrumental equipment of the laboratories can vary, no specific applicable instructions can be provided for analysis. However, the operating parameters and examples of the ion chromatographic analysis for chromium (VI) listed in Annex B and Annex C have been successfully tested and used. Annex B determines chromium (VI) by direct detection of chromate peak at 372 nm. Annex C determines chromium (VI) after a post-column reaction with 1,5-diphenylcarbazide by measuring the absorption peak at 540 nm.

The method used should be verified using the recovery rate determination (7.5).

In the absence of a proper software for data evaluation, record the injection volume as $V_M$ and record the area of the peak as $A$.

### 7.4 Calibration

The content of chromium (VI) in the sample of organic or organo-mineral fertilizer is determined with an external standard calibration.

The operator shall prepare calibration solutions from the standard solution (5.5). The chromium (VI) concentration in these solutions should cover the expected range of measurement.

The following tasks shall be performed:

- In the absence of a proper software for data evaluation, plot a suitable calibration curve by using at least five standards, within the range 1 ml to 25 ml of standard solution (5.5). Pipette the given volumes of standard solution (5.5) into 25 ml volumetric flasks. Make up to volume with the extraction solution (5.1), mix well, filter (6.10) and transfer a suitable aliquot volume into a vial (6.11)[6].

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[6] In case of more sophisticated instruments, calibration by inline dilution or multiple volume/partial loop injection is also possible, if the operator ensures sufficient validation of the used technique (still to be checked!).

NOTE: Calibration levels may be prepared as specified in Table 1.

Table 1 — Calibration levels preparation

| | | | | | |
|---|---|---|---|---|---|
| Volume of the standard solution (5.5) (ml) | 1,25 | 2,50 | 5,00 | 12,50 | 25,00 |
| Final volume (ml) | 25 in volumetric flask | | | | |
| Concentration of chromium (VI) (µg/l) | 50 | 100 | 200 | 500 | 1 000 |

- Transfer an aliquot to a suitable vial (6.11) corresponding for the chromatographic system (6.8).
- Inject the standards in the chromatographic system (6.8). Introduce the same volume for each standard. It is recommended to inject equal volume for samples. Record the volume injected as $V_c$ in µl.
- In the absence of a proper software for data evaluation, plot the chromium (VI) concentrations in micrograms of chromium per millilitre (µg/ml) against the measured areas of the peaks of chromate. Plot the chromium (VI) concentration on the x-axis and the area on the y-axis.

## 7.5 Determination of the recovery rate

The determination of the recovery rate is important to provide information about possible matrix effects which can influence the results.

The operator shall spike an aliquot of the solution obtained in 7.2 with a suitable volume of chromium (VI) solution to increase the chromium (VI) concentration by 10 mg/kg.

The following tasks shall be performed:

- Inject the same volume of this solution as the volume injected in the calibration (recording the area as $A_s$).
- Spike an aliquot of the extraction solution (the same volume as that taken before of the solution obtained in 7.2) with a suitable volume of chromium (VI) solution to increase the chromium (VI) concentration by 10 mg/kg, so that the final volume of this solution is the same as that of the above spiked solution with chromium (VI) solution. Inject the same volume of this solution as the volume injected in the calibration (recording the area as $A_{st}$).

The area of the chromate peak of these solutions shall be within the range of the calibration curve, otherwise repeat the procedure using a smaller aliquot. The recovery rate shall be between 80 % and 120 %.

NOTE If the added chromium (VI) is not detected or significantly lower than the expected values, this is an indication that the organic or organo-mineral fertilizer contains reducing agents. This leads to the conclusion that this organic or organo-mineral fertilizer has no chromium (VI) content (below detection limit).

# 8 Calculation and expression of results

## 8.1 Calculation of chromium (VI) content

The mass fraction of soluble chromium (VI) in the organic or organo-mineral fertilizer $w_{\text{chromium (VI)}}$ expressed in milligrams per kilogram (mg/kg) shall be calculated according to Formula (1).

$$w_{\text{chromium(VI)}} = \frac{(A - b) \times V_0 \times V_\text{c}}{V_\text{M} \times m \times F} \tag{1}$$

where

$A$ is the area of the peak of chromate in the chromatogram of the extract of the sample;

$F$ is the slope of calibration curve (y/x)[7], expressed in milliliters per microgram in ml/µg;

$b$ is the intercept of calibration curve (y/x);

$m$ is the mass of the sample of organic or organo-mineral fertilizer taken, expressed in grams (g);

$V_0$ is the extract volume of the sample, expressed in millilitres (ml);

$V_C$ is the injection volume in the calibration, expressed in microlitres (µl);

$V_M$ is the injection volume in the sample analysis, expressed in microlitres (µl).

Results are based on dry matter [see Formula (2)].

$$w_{\text{chromium(VI)−dry}} = w_{\text{Cr(VI)}} \times D \tag{2}$$

where

$D$ is the factor for conversion to dry matter [see Formula (3)].

$$D = \frac{100}{w_\text{dr}} \tag{3}$$

where

$W_{dr}$ is the dry residue content of the test sample expressed as mass fraction in percent (%) or in grams per kilogram (g/kg) determined according to EN 17773: —[8]

## 8.2 Recovery rate (according to 7.5)

The recovery rate $\eta$ expressed in percent (%) shall be calculated according to Formula (4).

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[7] Calibration curve (y/x) is intended as the linear interpolation of data plotted in a graph accordingly to paragraph 7.4, where the concentration of Cr(VI) is plotted on the x-axis and the area of the chromatographic peak on the y-axis

[8] Under preparation

$$\eta = \frac{A_s \times (V_1 + V_2) - A \times V_1}{A_{st} \times (V_1 + V_2)} \times 100 \tag{4}$$

where

| | |
|---|---|
| $V_1$ | is the volume of sample solution in the spiked solution, expressed in millilitres (ml); |
| $V_2$ | is the volume of chromate standard in the spiked solution, expressed in millilitres (ml); |
| $A_s$ | is the area of chromate peak of sample solution after adding chromium (VI) as determined in 7.5; |
| $A$ | is the area of chromate peak in the original sample as determined in 7.3; |
| $A_{st}$ | is the area of chromate peak of extraction solution after adding chromium (VI) as determined in 7.5. |

### 8.3 Expression of results

The chromium (VI) content is given in milligrams per kilogram (mg/kg) rounded to the nearest 0,1 mg. The content is based on dry matter. The dry matter determined according to EN 17773:—[9], is given in percent (%) rounded to the nearest 0,1 %.

## 9 Test report

The test report shall include at least the following information:

a) the chromium (VI) content(s) obtained from 8.1 to the nearest 0,1 mg/kg;

b) the standard used (including its year of publication);

c) a description of the sample tested and details about sampling (7.1), if necessary;

d) a brief description of the chromatographic technique (i.e., direct detection technique or whether a post-column reaction was used);

e) the dry matter of the organic or organo-mineral fertilizer in percent (%) to the nearest 0,1 %;

f) the recovery rate in percent (%);

g) details of any deviations from the procedure;

h) date when the analysis was performed and finished.

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[9] Under preparation

# Annex A
(informative)

# Results of the inter-laboratory study

## A.1 Inter-laboratory tests

## A.2 Statistical results for the determination of Cr(VI)

# Annex B
(informative)

# Chromatographic conditions for direct detection method

## B.1 General

As the instrumental equipment of the laboratories can vary, no generally applicable instructions can be provided for the ion chromatographic analysis. The following parameters have been successfully tested and used.

The method used should be verified using the recovery rate determination (7.5).

## B.2 Example of ion chromatographic conditions

### B.2.1 Mobile phase reagents

All reagents used shall have at least analytical grade purity.

**B.2.1.1 Anhydrous ammonium sulfate,** $(NH_4)_2SO_4$ (CAS 7783-20-2).

**B.2.1.2 Sodium hydroxide,** NaOH (CAS 1310-73-2).

**B.2.1.3 Mobile phase stock solution**

Dissolve 33,00 g of anhydrous ammonium sulphate and 0,40 g of sodium hydroxide in a volumetric flask and make up to 1 000 ml with water (5.7). This solution contains 250 mmol of ammonium sulphate and 10 mmol of sodium hydroxide. Its pH is 8,2. From this solution the eluent for chromatography (A.2.1.4) is prepared weekly. The shelf life is up to four months at 4 °C.

**B.2.1.4 Mobile phase**

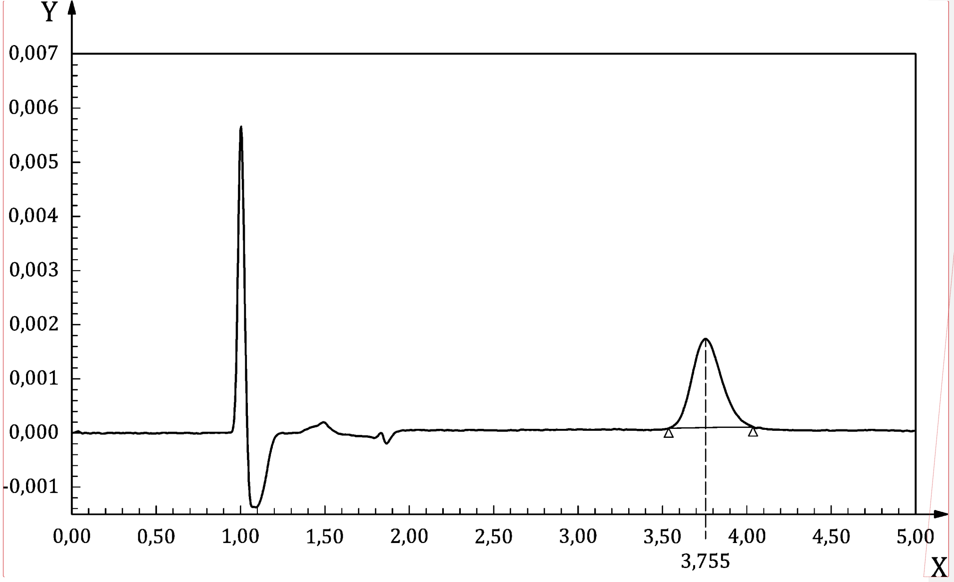
Transfer 100 ml of mobile phase stock solution (A.2.1.3) into a 1 000 ml volumetric flask and make up to the mark with water (5.7). This solution contains 25 mmol of ammonium sulphate and 1 mmol of sodium hydroxide. Check that pH is (8,0 ± 0,2). Filter the solution through a membrane filter. The shelf life is up to one week at room temperature.

### B.2.2 Instrumental conditions

- Column oven: 30 °C
- Mobile phase: 25 mmol ammonium sulphate and 1 mmol sodium hydroxide (A.2.1.4)
- Column: Anion-exchange column (polymethacrylate resin with quaternary ammonium functional groups), 4,6 mm × 75 mm, with 1 mm pre-column
- Range of wavelength (only for DAD): record the UV spectrum in the range 200 nm to 550 nm
- Wavelength of extracted chromatogram: 372 nm
- Flow rate: 0,9 ml/min
- Injection volume: 50 µl

- Run time of chromatograph: 5 min
- Equilibrate between injections: 6 min

A DAD diode array detector allows the reliable confirmation of chromate identity by comparing the UV spectrum of the detected peak with a standard chromate spectrum. Figure B.1 shows the example of chromatogram and UV spectrum obtained from one commercial sample that contains 3,9 mg/kg of Cr(VI).



**Commenté [MC8]:** To substitute with a chromatogram relating to organic/organo-mineral fertilizers (this is referred to ISO 17075-2:2017

**Key**

X min

Y AU

NOTE Peak: Chromate, 3,755 min.

**Figure B.1 — Chromatogram obtained from one sample that contains 3,9 mg/kg of chromium (VI) (EN ISO 17075-2:2017 [4], Figure B.1)**

# Annex C
(informative)

# Chromatographic conditions for method with post-column reaction

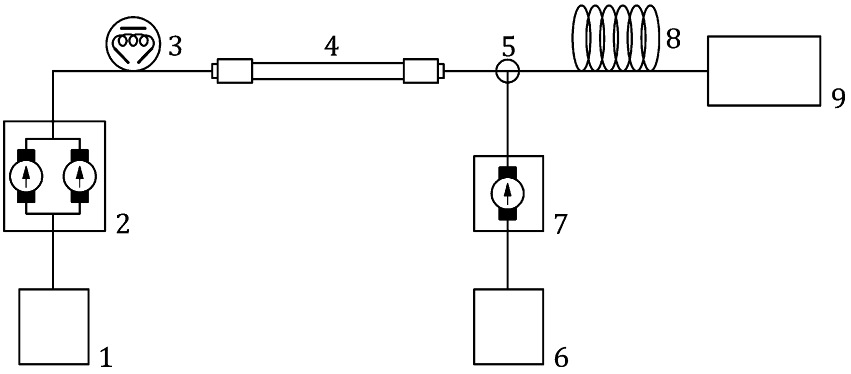
## C.1 General

As the instrumental equipment of the laboratories can vary, no generally applicable instructions can be provided for the ion chromatographic analysis. The following parameters have been successfully tested and used.

The method used should be verified using the recovery rate determination (7.5).

## C.2 Chromatographic system and apparatus required

An example of the ionic chromatography method with post-column reaction is summarised in Figure C.1.



**Key**

1 mobile phase
2 LC pump
3 injection loop
4 analytical column (and guard column)
5 zero dead volume tee
6 post column reagent
7 post column reagent pump (or dosing device)
8 reaction coil
9 detector (MWD or DAD)

**Figure C.1 — Diagram of a system for ionic chromatography with post-column reaction (EN ISO 17075-2:2017 [4], Figure C.1, modified)**

The chromium (VI) content is determined using chromatographic column packed with an anion exchange stationary phase.

Post-column reagent, containing 1,5-diphenylcarbazide, is added between the column and the reactor coil with the help of a zero dead volume tee.

The reaction coil ensures the proper mixing of the eluent from the column and the post column reagent and the chromium (VI) in solution oxidises 1,5-diphenylcarbazide to 1,5-diphenylcarbazone. This forms a red/violet complex with chromium, which can be quantified at 540 nm with the help of multiple wavelength detector (MWD) or a diode array detector (DAD).

**C.2.1 Two suitable liquid chromatoraphy (LC) pumps.** One is used to deliver the mobile phase in the system, the other is used to deliver the post-column reagent before the reaction coil. The last pump can be substituted by a dosing device.

**C.2.2 Autosampler** or **manual injection valve** equipped with a sample loop for the injection of a sample.

**C.2.3 Thermostated column compartment.**

**C.2.4 Analytical column** packed with an anion exchange stationary phase and suitable guard column.

**C.2.5 Zero dead volume tee.**

**C.2.6 Suitable reaction coil.**

**C.2.7 Detector,** either MWD or DAD with the capability to detect at 540 nm.

NOTE In order to maintain the inertness of the configuration, the column and all the capillaries (including the injection loop) are in polyether ether ketone (PEEK).

The usage of a guard column is highly recommended in order to extend the life span of the column. A guard column in PEEK packed with polystyrene-divinylbenzene particles is applicable.

## C.3 Example of analytical conditions

### C.3.1 Mobile phase and post-column reagents

All reagents used shall have at least analytical grade purity.

**C.3.1.1 Ammonium sulfate,** $(NH_4)_2SO_4$ (CAS 7783-20-2).

**C.3.1.2 Ammonium hydroxide,** $NH_4OH$ (CAS 1336-21-6) as 28 % $NH_3$ in water.

**C.3.1.3 1,5-Diphenylcarbazide,** $C_{13}H_{14}N_4O$ (CAS 140-22-7).

**C.3.1.4 Methanol,** $CH_3OH$ (CAS 67-56-1).

**C.3.1.5 Sulphuric acid,** $H_2SO_4$ (CAS 7664-93-9) at 98 % mass fraction.

### C.3.2 Preparation of the mobile phase

Dissolve (33,0 ± 0,1) g of ammonium sulphate (C.3.1.1) and 8,0 ml of ammonium hydroxide (C.3.1.2) in a 1 000 ml volumetric flask, fill to the mark with water (5.7).

### C.3.3 Preparation of post-column reagent

In a 1 000 ml volumetric flask, dissolve 28 ml of sulphuric acid (C.3.1.5) in about 500 ml of water (5.7) and let it stand cooling.

During this time, dissolve (0,50 ± 0,01) g of 1,5-diphenylcarbazide (C.3.1.3) in 100 ml of methanol (B.3.1.4).

When the acidic solution is cooled, stirring with a magnetic stirrer and mix the diphenylcarbazide solution with the acidic solution and fill to the mark with water (5.7).

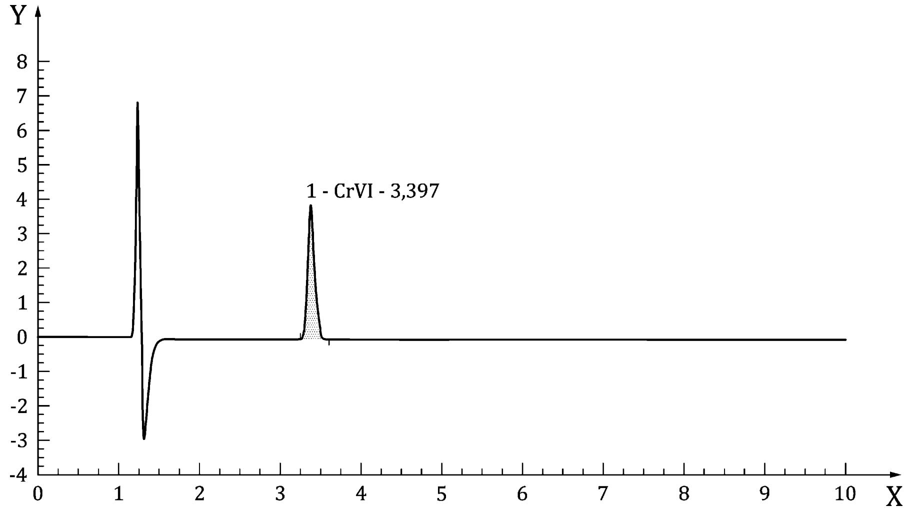
### C.3.4 Instrumental conditions

- Analytical column: Anion exchange column with alkyl quaternary ammonium as functional group. Length: 250 mm. Internal diameter: 4 mm
- Guard column of length 35 mm and internal diameter 4 mm
- Reaction coil volume: 750 µl
- Injection volume: 100 µl
- Mobile phase flow rate: 1 ml/min
- Post-column reagent flow rate: 0,33 ml/min
- Run time: 10 min

## C.4 Examples of chromatograms

Figure C.2 shows the chromatogram of a 20 µg/l standard and Figure C.3 shows a chromatogram of a real sample of organo-mineral fertilizer NPK 6-5-13.

**Commenté [MC9]:** TO evaluate if to substitute with chromatograms deriving from ILS

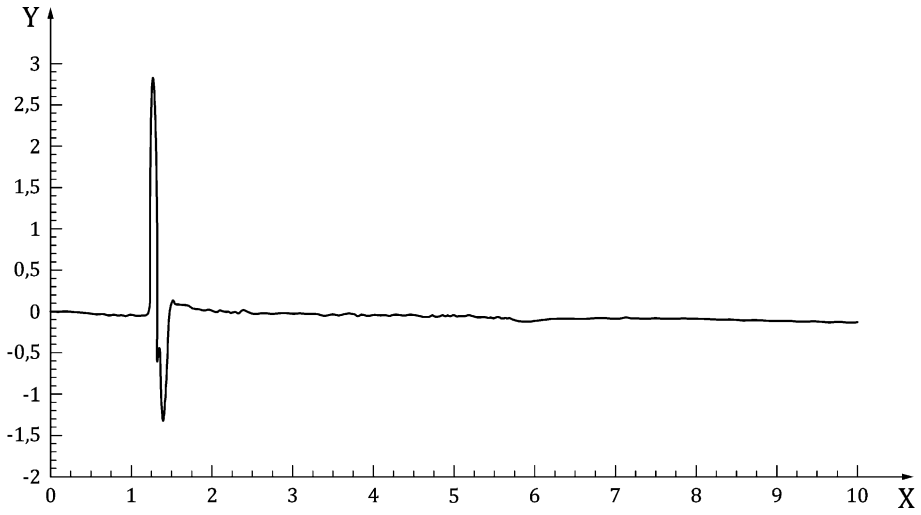


**Key**

X min

Y mAU

**Figure C.2 — Chromatogram of a 20 µg/l standard**



**Key**

X min

Y mAU

**Figure C.3 — Chromatogram of a real sample of organo-mineral fertilizer NPK 6-5-13**

# 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.

**Commenté [MC10]:** To add the new amendment of the M/564

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, PFC 1(A), item 2(b)<br>Annex I, Part II, PFC 1(B), item 3(b) | 8 9 | 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.

# Bibliography

[1] EN ISO 17075-2:2017, *Leather — Chemical determination of chromium(VI) content in leather — Part 2: Chromatographic method (ISO 17075-2:2017)*

[2] 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

[3] EN ISO 15192:2021, *Soil and waste - Determination of Chromium(VI) in solid material by alkaline digestion and ion chromatography with spectrophotometric detection (ISO 15192:2021)*

[4] EN 16318:2015+A1:2016, *Fertilizers and liming materials - Determination of chromium (VI) by photometry (method A) and by ion chromatography with spectrophotometric detection (method B)*

[5] EN 17768:—[10], *Organic and organo-mineral fertilizers - Digestion by aqua regia for subsequent determination of elements*

[6] EN 17770:—[11], *Organic and organo-mineral fertilizers - Determination of the total content of specific elements by ICP-AES after digestion by aqua regia*

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[10] Under preparation

[11] Under preparation