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Water quality — Determination of chromium(VI) and chromium(III) in water — Method using liquid chromatography with inductively coupled plasma mass spectrometry (LC-ICP-MS) after chelating pretreatment

Qualité de l'eau — Dosage du chrome (VI) et du chrome (III) dans l'eau — Méthode par spectrométrie de masse avec plasma à couplage inductif (LC-ICP-MS) après un prétraitement par agents de chélation

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Foreword

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This document was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Chromium (Cr) exists in natural resources and is also widely used in industries as plating agents, paints, dyes, catalysts, and dietary supplements. The Cr(VI) compounds are highly harmful and recognized to be a human carcinogen. The Cr(III) compounds are recently used as a substitute for Cr(VI) compounds in industries, e.g. plating. In wastewater, surface water, or drinking water, chromium mainly exists in two oxidation states: +3 [Cr(III)] and +6 [Cr(VI)]. However, the proportion between Cr(VI) and Cr(III) is quite variable. Therefore, the determination of the individual oxidation states of chromium is crucial to evaluate and control the risk of chromium to human and environmental health. This document will be beneficial to perform a robust, simple, and rapid determination of chromium of the individual oxidation states.

Water quality — Determination of chromium(VI) and chromium(III) in water — Method using liquid chromatography with inductively coupled plasma mass spectrometry (LC-ICP-MS) after chelating pretreatment

WARNING — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices.

IMPORTANT — It is absolutely essential that tests conducted according to this document be carried out by suitably qualified staff.

1 Scope

This document specifies a method for the determination of hexavalent chromium [Cr(VI)] and trivalent chromium [Cr(III)] in water by liquid chromatography with inductively coupled plasma mass spectrometry (LC-ICP-MS) after chelating pretreatment.

This method is applicable to the determination of Cr(VI) and Cr(III) dissolved in wastewater, surface water, groundwater, or drinking water from 0,20 µg/l to 500 µg/l of each compound as chromium (Cr) mass. Samples containing Cr at concentrations higher than the working range can be analysed following appropriate dilution of the sample.

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.

ISO 8466-1, *Water quality — Calibration and evaluation of analytical methods — Part 1: Linear calibration function*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminology 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/

4 Principle

The chemical forms of various Cr(III) species in water samples are unified to a stable Cr(III) complex by a chelating pretreatment with 2,6-pyridinedicarboxylic acid (PDCA) or ethylenediaminetetraacetic acid (EDTA) after adjusting the sample solution pH to 6,9 ± 0,1.[1][2] Liquid chromatography combined with inductively coupled plasma mass spectrometry (LC-ICP-MS) determines the chromatographically separated Cr(VI) and the Cr(III)-PDCA complex or Cr(III)-EDTA complex in the pretreated sample solutions.

5 Interferences

5.1 General

If any of the interferences described in 5.2 to 5.5 are recognized or can be expected due to additional information about the sample, the sum of Cr(VI) and Cr(III) concentrations determined by the proposed method should be compared with the total Cr concentration which can be determined by ICP-MS.

If the Cr(III) and Cr(VI) species are properly determined by the proposed method, the sum of Cr(III) and Cr(VI) should agree with the total Cr with a difference of ≤30 %. An exceedance of this limit (30 %) indicates the occurrence of interferences.

NOTE The water sample can contain Cr^0 or Cr(III) species that cannot be complexed via EDTA or PDCA (e.g. nanoparticles, colloids). These stable species can be so small that they are not retained even by filtration through a 0,2 µm filter membrane. However, as they are uncomplexed, they will not be detected by the LC-ICP-MS analysis and can thus result in a greater difference between the sum of Cr species determined by LC-ICP-MS and the total Cr determination.

5.2 Samples

Reductants or oxidants in the sample may lead to false results for the Cr(VI) and/or Cr(III) concentration through the reduction of Cr(VI) to Cr(III) or oxidation of Cr(III) to Cr(VI), respectively. For example, divalent iron ions or ascorbic acid at 1 mg/l or 10 mg C/l, respectively, cause the reduction within 10 min.[1] Organic matter at high concentrations may slowly reduce Cr(VI) to Cr(III). For example, tartaric acid at 100 mg C/l reduces Cr(VI) to Cr(III) in one night at room temperature, however, the same compound at 10 mg C/l does not cause the reduction. The sample pH also changes the redox equilibrium between Cr(VI) and Cr(III) and the complexation equilibrium between Cr species with coexisting inorganic and organic substances.[3]

5.3 Sample storage and sample preparation

Potential sources of Cr contamination during sampling, sample storage, and sample preparation include: labware, containers, sampling equipment, reagents, water and human contact. Potential unexpected redox reaction between Cr(III) and Cr(VI) may occur during these operations. All apparatus and labware shall be cleaned using the cleaning procedure (see Clause 7).

5.4 Chelating pretreatment

Transition metal cations at high concentrations in the sample solution may lead to a negative biased value for the Cr(III) concentration because these metals decrease the concentration of free chelating agents due to complex formation with chelating agents. For example, neither cobalt(II) nor nickel(II) up to 10 mg/l interfere, however, these metal ions[1] and also calcium ion interfere above 100 mg/l.

Large amounts of organic matter which strongly binds to Cr(III) species in the sample solution may lead to a negatively biased value for the Cr(III) concentration. For example, Cr(III)-PDCA complex formation in the PDCA chelating pretreatment is depressed by the presence of EDTA as an interfering substance at 100 mg/l level, although not depressed at 10 mg/l.[1] The opposite is also true and Cr(III)-EDTA complex formation in the pretreatment of Cr(III) with EDTA is inhibited by the presence of large amounts of PDCA as an interfering substance. The depression at the chelating procedure can be evaluated by recovery experiments (10.3).

5.5 LC-ICP-MS measurements

Polyatomic ions are formed in the argon plasma of the ICP-MS by the reaction among argon, water, reagents and sample matrix, etc. The formation of $^{40}Ar^{12}C^+$ and $^{37}Cl^{16}O^+$ may interfere with the ICP-MS detection of Cr at mass-to-charge ratio (*m/z)* 52 and at *m/z* 53, respectively. Some of the interferences such as $^{40}Ar^{12}C^+$ can be reduced by using a collision-reaction cell of the ICP-MS. On the other hand, some of the interferences such as $^{37}Cl^{16}O^+$ can be reduced by chromatographic separation of chloride (Cl) ions from the Cr species.[1] High-resolution or tandem mass spectrometers, e.g. sector-field or MS/MS-type mass spectrometers, can also reduce the polyatomic interferences very effectively.

LC retention time of Cr species may shift for water samples containing salts or organic matter at high concentrations. If this causes the peak of Cr(III)-PDCA complex [or Cr(III)-EDTA complex] to overlap with Cr(VI), it may cause positive and negative interferences to Cr(VI) and Cr(III), respectively. In such cases, adjust elution conditions such as composition of eluent to ensure adequate separation.

The metallic parts of LC-ICP-MS instruments, e.g. LC columns, tubes and connectors, potentially contaminate the eluent if in contact. Therefore, metallic parts or pathways should be avoided or reduced to a minimum.

6 Reagents and standards

Unless otherwise indicated, reagents of purity grade "for analysis" or "for trace analysis" are used as reagents. If available, use only reagents of pro analysis grade (or purer) free of compounds containing Cr. Weigh the reagents with an accuracy of ± 1 % of the nominal mass, unless stated otherwise.

Prepare alternative concentrations and volumes of solutions as described hereafter, if necessary. Alternatively, use commercially available stock solutions of the required concentration.

6.1 Water, with an electrical resistivity of ≥ 18,2 MΩ cm (25 °C).

The water shall not contain any measurable quantity of Cr(III) and Cr(VI) or interfering compounds at or above one-third the method quantification limit.

6.2 Nitric acid, $w(HNO_3)$ = 650 g/kg, $\rho(HNO_3)$ = 1,4 g/ml.

NOTE Nitric acid is available as $\rho(HNO_3)$ = 1,38 g/ml [$w(HNO_3)$ = 610 g/kg] and $\rho(HNO_3)$ = 1,42 g/ml [$w(HNO_3)$ = 690 g/kg] as well as $\rho(HNO_3)$ = 1,40 g/ml [$w(HNO_3)$ = 650 g/kg].

6.3 Nitric acid stock solution, $c(HNO_3)$ = 6 mol/l.

Transfer 25 ml of water (6.1) to 100 ml volumetric flask (7.14), and add 27 ml of nitric acid (6.2) and then fill up to mark with water (6.1).

6.4 Nitric acid solution for pH adjustment, $c(HNO_3)$ = 2 mol/l.

Transfer 33 ml of nitric acid stock solution (6.3) to a 100 ml volumetric flask (7.14) and fill up to mark with water (6.1).

6.5 Sodium hydroxide, NaOH.

6.6 Sodium hydroxide solution for pH adjustment, $c(NaOH)$ = 2 mol/l.

Weigh 8 g of sodium hydroxide pellets (6.5) and transfer them to a 100 ml beaker. Then add approximately 50 ml water (6.1) and stir with a stirrer (7.12) until the pellets have dissolved. Transfer to a 100 ml volumetric flask (7.14) and fill up to the mark with water (6.1). Commercially available solution of sodium hydroxide can be used to dilute them to the required concentration.

6.7 2,6-Pyridinedicarboxylic acid (PDCA), CAS Registry Number®[1)] 499-83-2, $C_7H_5NO_4$.

6.8 Disodium hydrogenphosphate, Na_2HPO_4.

6.9 Ammonium acetate, CH_3COONH_4.

1) Chemical Abstracts Service (CAS) Registry Number® is a trademark of the American Chemical Society (ACS). This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

6.10 Ethylenediamine-*N*,*N*,*N*',*N*'-tetraacetic acid (EDTA) disodium salt dihydrate (CAS 6381-92-6, $C_{10}H_{14}Na_2N_2O_8{\cdot}2H_2O$) **or EDTA dipotassium salt dihydrate** (CAS 25102-12-9, $C_{10}H_{14}K_2N_2O_8{\cdot}2H_2O$).

6.11 Ammonia solution, mass fraction, $w(NH_4OH)$ = 280 g/kg.

6.12 Ammonia solution for pH adjustment, $c(NH_3)$ = 1 mol/l.

6.13 Potassium dichromate, $K_2Cr_4O_7$.

6.14 Chromium(III) nitrate nonahydrate, $Cr(NO_3)_3{\cdot}9H_2O$.

6.15 Cr(VI) stock solution, ρ[Cr(VI)] = 1 000 mg/l.

WARNING — Potassium chromate can be carcinogenic.

Heat 5 g of potassium dichromate (6.13) at 150 °C for 1 h and then cool at room temperature in a dried desiccator. Dissolve 2,829 g of the dried potassium dichromate (6.13) with water (6.1) in a 1 000 ml volumetric flask (7.14) and fill up to mark with water (6.1). Commercially available Cr(VI) stock solution of the required concentration can be used.

6.16 Cr(VI) standard solution, ρ[Cr(VI)] = 10 mg/l.

Transfer 1,00 ml of the Cr(VI) stock solution (6.15) to a 100 ml volumetric flask (7.14) and fill up to the mark with water (6.1). Prepare this solution on the day of use.

6.17 Cr(III) stock solution, ρ[Cr(III)] = 1 000 mg/l.

Dissolve 7,696 g of chromium(III) nitrate nonahydrate (6.14) in 250 ml water and transfer to a 1 000 ml volumetric flask (7.14). Add 50 ml of nitric acid stock solution (6.3) and fill up to the mark with water (6.1). Commercially available Cr(III) stock solution of the required concentration can be used.

6.18 Cr(III) standard solution, ρ[Cr(III)] = 10 mg/l.

Transfer 1,00 ml of the Cr(III) stock solution (6.17) to a 100 ml volumetric flask (7.14) and fill up to the mark with water (6.1). Prepare this solution on the day of use.

6.19 PDCA solution, c(PDCA) 0,02 mol/l.

Dissolve 3,35 g of 2,6-pyridinedicarboxylic acid (6.7), 2,85 g of disodium hydrogenphosphate (6.8), and 38,5 g of ammonium acetate (6.9) to 900 ml of water (6.1) in a bottle (7.16) of 1 000 ml. Adjust the pH of the solution to pH 6,9 ± 0,1 using a pH meter (7.11) by adding the sodium hydroxide solution (6.6). Transfer the adjusted PDCA solution to a 1 000 ml volumetric flask (7.14) and fill up to the mark with water (6.1).

The amount of NaOH solution is approximately 16,5 ml. The pH adjustment from pH 6,0 should be carefully performed by adding of NaOH solution by small degrees (e.g. 0,1 ml), because the change of pH is drastic.

6.20 EDTA solution, c(EDTA) 0,025 mol/l.

Dissolve 9,31 g of ethylenediamine-*N*,*N*,*N*',*N*'-tetraacetic acid disodium salt or 10,1 g of the dipotassium salt (6.10) to 900 ml of water (6.1) in a bottle (7.16) of 1 000 ml. Adjust the pH of the solution to pH 6,9 ± 0,1 using a pH meter (7.11) by adding the sodium hydroxide solution (6.6). Transfer the adjusted EDTA solution to a 1 000 ml volumetric flask (7.14) and fill up to the mark with water (6.1).

6.21 Mobile phase for LC.

A various mobile phase can be used in accordance with the type of LC column used. The composition of the mobile phase depends on the chosen LC column. Examples are given in Annexes A and B. Weigh each mobile

phase reagent according to its composition, transfer the reagent to a bottle (7.16) and dissolve with water (6.1). If pH adjustment is required, adjust the pH of the solution to the optimum pH by adding alkali or acid. Transfer the adjusted mobile phase solution to volumetric flask (7.14) and fill with water (6.1) to adjust to final concentrations.

The pH adjustment by immersing the pH glass electrode in the mobile phase may result in contamination of the mobile phase. For this reason, a small amount of the mobile phase should be taken in advance into a test tube (7.7) with a clean pipette (7.13) to determine the amount of alkali or acid required for pH adjustment. The use of organic solvents in the mobile phase should be avoided because the carbon deteriorates stability of measurements of LC-ICP-MS due to the deposits and the quantification of limit due to the polyatomic interference, e.g. argon-carbon ion on m/z 52.

7 Apparatus

Apparatus or parts which may come into contact with a water sample or an eluent of LC should be non-metallic and free from Cr and interfering substances.

7.1 Liquid chromatograph-inductively coupled plasma-mass spectrometer (LC-ICP-MS), including LC and ICP-MS instruments.

The outlet of the LC column is connected to the inlet of the nebulizer of the ICP-MS using a connector tube. The internal diameter of the connector tube should be less than 0,25 mm and the length should be as short as possible (e.g. less than 100 mm) in order to avoid the separation efficiency obtained with LC. A fluoropolymer or polyetheretherketone (PEEK) tube should be used. Since the operating conditions differ according to the instruments, the operator shall thus refer to the instructions provided by the manufacturer of each instrument. Examples of the operating conditions of LC-ICP-MS are given in Annexes A and B.

7.1.1 LC instrument, equipped with a pump for LC, a sample injector, and an LC column.

A column oven can be used to stabilize the column temperature. The column shall be capable of baseline separation of Cr(VI) and Cr(III)-PDCA complex or Cr(III)-EDTA complex.

NOTE An autosampler or manual injector can be used as the sample injector. Various column types and mobile phases can be used for separating Cr species. An anion exchange column or a mixed-mode-column with anion and cation exchangers are typically used. Examples are provided in Annexes A and B.

7.1.2 ICP-MS instrument, inductively coupled argon plasma-mass spectrometer.

As a mass spectrometer, quadrupole mass spectrometer with a collision/reaction cell, double focusing mass spectrometer, or tandem mass spectrometers (MS/MS-type) is available. Further information on the ICP-MS instrumentation is given in ISO 17294-1:2004, Clause 5[4].

7.2 Sample collection bottles, polyethylene, polypropylene, borosilicate glass, or fluoropolymer of a capacity of 10 ml to 100 ml, with caps, lined with polymers.

7.3 Filter for sampling and LC-ICP-MS measurements, cellulose acetate, cellulose nitrate, or hydrophilic polyvinylidene fluoride (PVDF) of pore size 0,45 µm.

NOTE Inline or automated filtration techniques can be applied for sample filtration or additional protection of the analytical column.

7.4 Microlitre syringe or pipette, materials which are free from Cr, e.g. polypropylene, polytetrafluoroethylene (PTFE), glass, and titanium, of a capacity of 10 µl to 200 µl, used for dosing Cr standard solutions at preparation of calibration solutions, recovery tests, or injection of the pretreated sample solution into an LC-ICP-MS system.

7.5 Screw-capped test tubes, materials which are free from Cr, e.g. polypropylene, of a capacity of 10 ml, with non-metallic material stoppers, e.g. polypropylene or polyethylene, used for chelating pretreatment.

The volume of the test tube can be changed if the temperature of the solution in the test tube is kept at the set temperature given in 9.1.2. Also, the easily oxidizable material (e.g. high-density polyethylene) should be avoided because Cr(VI) possibly reduces to Cr(III) at the heating of chelating treatments.

7.6 Heating block, capable of heating the test tubes (7.5) at (80 ± 3) °C or (70 ± 3) °C, used for PDCA or EDTA chelating pretreatment, respectively.

7.7 Stoppered test tubes, of a capacity of 10 ml, used for pre-examination to adjust the pH of the water sample (9.1.1).

7.8 Whole pipette, of a capacity 10 ml, used for measuring the volume of water sample.

7.9 LC vials, materials which are free from Cr, e.g. polypropylene, of a capacity of 1 ml or 2 ml, with non-metallic material stoppers, e.g. polypropylene.

7.10 Balances, analytical type capable of weighing with an accuracy of 0,1 mg.

7.11 pH meter, equipped with a glass electrode.

7.12 Magnetic stirrer, with PTFE-coated stirring bar of suitable size.

7.13 Disposable pasteur pipettes, borosilicate glass.

7.14 Volumetric flasks, of different capacities (e.g. 20 ml, 100 ml and 1 000 ml), which should be cleaned properly to be free from Cr, used for preparation of samples, standard solutions and mobile phases.

7.15 Pipettes, of a capacity of 0,1 ml to 10 ml, adjustable or fixed with suitable pipette tips, e.g. polypropylene, used for dosing the chelating reagents or for diluting a sample.

7.16 Bottles, of different capacities (e.g. 100 ml and 1 000 ml), which should be cleaned properly to be free from Cr, used for pH adjustment of the chelating solution and mobile phase of LC-ICP-MS.

New bottles (7.2 and 7.16), microlitre syringe (7.4), screw-capped test tubes (7.5), stoppered test tubes (7.7), whole pipettes (7.8), LC vials (7.9), pipettes (7.13 and 7.15), and volumetric flasks (7.14) are cleaned by filling with diluted HNO_3 or HCl (e.g. 1 mol/l) for at least 24 h. They are rinsed at least three times with water (6.1) and kept in or filled with water for at least 24 h. Rinse the bottles with water (6.1) and dry them prior to use.

8 Sampling, preservation and storage of samples

Samples are collected into rigorously cleaned bottles (7.2). Collected samples are filtered onsite through a 0,45 µm filter (7.3). Use a portion of the sample to rinse the filter and collect the required volume of the filtrate. The filtrated samples are shipped to laboratory at (5 ± 3) °C and are then stored at (3 ± 2) °C before the sample preparation (9.1). If immediate filtration on site is impossible, samples may be shipped to laboratory at (5 ± 3) °C from the time of collection until filtration and then the reason and the time shall be added to the test report. The sample preparation (9.1) shall be completed not later than 24 h after sampling. After the chelating pretreatment (9.1.2), the samples shall be stored cool and dark at (3 ± 2) °C. The samples shall be analysed by LC-ICP-MS within two weeks of the PDCA and EDTA chelating pretreatments (9.1.2).

9 Procedure

9.1 Sample preparation

9.1.1 pH-adjustment of water sample

Transfer 10 ml of the filtrated water sample with a whole pipette (7.8) into a 20 ml volumetric flask (7.14). If the pH of the water sample is within 6,9 ± 0,1, no pH adjustment is necessary. If the pH is outside the range, adjust pH of the water sample without contamination of the sample by immersion of the pH electrode. Take another 10 ml of the filtrated water sample with a whole pipette (7.8) into a test tube (7.7) and measure with a pH meter while adding nitric acid solution (6.4) or hydroxide solution (6.6) in small quantities to estimate necessary amount of acid or alkali for pH adjustment to 6,9 ± 0,1. Then, add the estimated amounts of nitric acid solution (6.4) or hydroxide solution (6.6) to the filtrated water sample in the 20 ml volumetric flask. Add 2 ml of PDCA solution (6.19) or EDTA solution (6.20) to the water sample and fill up to mark with water (6.1) and swirl it gently to mix.

It is recommended to use a pH meter which can measure a sample with a small volume (e.g. 0,1 ml) by placing the solution onto the flat sensor in a measuring scoop.

When using a different final solution volume for the chelating procedure (e.g. 10 ml), it is recommended to keep the volume ratios of the filtrated water sample and PDCA or EDTA solution to the final solution at 5:1:10. The exact volumes of the water sample can also be calculated from its weight and density.

If the concentrations of Cr(III) or Cr(VI) in water samples are higher than the working range or large amounts of coexisting substances (e.g. the transition cations described in 5.4) can be expected that interfere with the chelation of Cr(III), the water samples should be diluted with water (6.1) or a smaller aliquot can be transferred into the 20 ml volumetric flask (7.14). Automatic dilution techniques can be used if available.

For samples containing oxidizing or reducing substances that interfere with the determination of Cr(VI) and Cr(III), the pretreatment described in ISO 11083:1994, 5.2,[5] is applied to remove the interfering substances using hydroxide, aluminium sulfate, phosphoric acid, or sulfite.

9.1.2 Chelating pretreatment

Transfer a small amount (e.g. 5 ml) of the solution treated in 9.1.1 to a screw-capped test tube (7.5) and heat the solution in the test tube for 30 min at (80 ± 3) °C for PDCA-chelation or for 60 min at (70 ± 3) °C for EDTA-chelation, respectively, after the temperature of the solution reaches the set temperature. The treated solution is cooled to room temperature.

If the suspension is produced with the pretreatment, the solution is filtrated through a 0,45 µm filter (7.3).

The temperature of the test solution is lower than the set temperature of heater in the case of insufficient heat-transfer from the heater or in the case of a large volume of a test solution (e.g. more than 20 ml). To avoid the problems, the temperature of the test solution should be confirmed to be the same as the set temperature in advance.

Complex formation of Cr(III) with the chelating agents is not always completed within the set time under some sample conditions in the presence of large amounts of coexisting substances (e.g. organic matter) which bind strongly to Cr(III), especially as the chelation rate of Cr(III) with EDTA is slower than that with PDCA. Dilution or reduction of the volume of the water sample according to 9.1.1 is effective to overcome the interference.

9.2 Optimization of operating condition for LC-ICP-MS

The ICP-MS instrument (7.1.2) shall be optimized according to the manufacturer's instructions.

Start up the LC system (7.1.1) in accordance with the manufacturer's instructions, set the pump flow rate and couple the column outlet to the inlet of nebulizer of the ICP-MS instrument (7.1.2) with the connector tube. Ensure that the baseline is stable and the mobile phase flows sufficiently from the column.

If setting up the method for the first time, check the retention time and identity of each Cr species [Cr(VI) and Cr(III)] carefully. It is recommended that the chelating-treated standard solutions including only Cr(VI) or Cr(III)-PDCA [or Cr(III)-EDTA] complex are injected separately for checking the retention time in the chromatogram with ICP-MS detection at m/z 52 and m/z 53. Examples of chromatograms are given in Annexes A and B.

Determine the appropriate LC and ICP-MS condition experimentally during method development and validation. Examples of operating conditions of LC-ICP-MS are given in Annexes A and B.

9.3 Identification of Cr(VI) and Cr(III) on LC-ICP-MS

Inject the treated samples (9.1.2) into the optimized LC-ICP-MS system (9.2). Identify the Cr(VI) and Cr(III) chelate complex by comparing the retention times of samples and those of calibration solutions (10.2).

Individual Cr(VI) and Cr(III) chelate complex are regarded as identified in the sample if:

— the relative or the absolute retention time of sample component measured in LC-ICP-MS chromatogram matches the relative or absolute retention time of the reference substances in calibration solution within ± 2,5 % or ± 0,2 min, respectively, measured under identical conditions, and

— two peaks at m/z = 52 and 53 should occur at the reference substance-specific retention time, with the same width and shape, and

— The ratio of peak area (or peak height) at m/z = 52 and 53 for the Cr(VI) and Cr(III) chelate complex in the samples should match the ratio of those in the calibration solutions within ± 30 %.

NOTE The retention time of Cr species can shift for water samples with high content of salts or organic matter. For such samples, identification can be possible by spiking Cr(VI) and Cr(III) species into sample and comparing the retention time of sample component with and without spike after chelating pretreatment. The dilution of sample or the injection of a smaller volume into LC column is also effective to overcome this influence.

9.4 Blank value measurements

Check that the instruments and reagents are in a proper condition by carrying out regular blank tests. To conduct the blank tests, prepare and analyse 10 ml of water (6.1) in the same way as the sample. Procedural blank tests should preferably be carried out with each batch of samples.

If unusual blank values occur, find the reason for this by systematic investigations, so that the source of contamination can be eliminated.

10 Calibration

10.1 General requirements

For routine analysis, only a calibration over the total procedures including chelating pretreatment and LC-ICP-MS measurements shall be applied.

Determine the linear working range using at least five measuring points of different concentration in accordance with ISO 8466-1.

The calibration function for a substance is valid only for the measured concentration range. Additionally, the calibration function depends on the condition of the instrument. A calibration shall be run every day when the LC-ICP-MS is used for Cr speciation. A drift check sample (e.g. a calibration solution that is prepared at described in 10.2) can be used to check the response factor for drifting during a longer sequence (more than 20 samples). Table 1 explains the subscripts used in the formulae and in the following text.

Table 1 — Explanation of subscripts

Subscript	Meaning
e	Calibration step
d	Determination step for the sample
i	Identity of the target substance
j	Consecutive figure for the pairs of values

10.2 Calibration covering the total procedure

Prepare a series of mixed calibration solutions for Cr(VI) and Cr(III) by mixing and diluting 10 mg/l of Cr(VI) standard solution (6.16) and 10 mg/l of Cr(III) standard solution (6.18).

Transfer 10 ml of each of these calibration solutions to a 20 ml volumetric flask (7.14) with a whole pipette (7.8), add 2 ml of PDCA solution (6.19) or EDTA solution (6.20), and fill up to the mark with water (6.1).

NOTE 1 The volume of the chelating solution (6.19 or 6.20) and the total volume of the calibration solution can be respectively reduced if the ratios of the volumes are kept the same.

Transfer a small volume (e.g. 5 ml) of each solution to a screw-capped test tube (7.5) and heat the solution in the test tube for 30 min at (80 ± 3) °C for PDCA-chelation or for 60 min at (70 ± 3) °C for EDTA-chelation, respectively, after the temperature of the solution reaches the set temperature.

To prevent changes in the oxidation number of Cr(VI) and Cr(III), chelating pretreatment should be performed as soon as possible (within 1 hour at the most) after the preparation of the mixed calibration solutions.

NOTE 2 The preparation of calibration solutions can be automated by the automatic inline dilution of one chelate-pretreated calibration solution with the chelating solution (6.19 or 6.20) diluted 10-fold with water (6.1) at the LC-ICP-MS measurements if the accuracy and precision of the measurements are not respectively lost.

The temperature of the solution in the test tube is lower than the set temperature of heater in the case of insufficient heat-transfer from the heater or in the case of a large volume of the solution (e.g. more than 20 ml). To avoid the problems, the temperature of the solution should be confirmed to be the same as the set temperature in advance.

Inject the calibration solutions for Cr(VI) and Cr(III) after the chelating pretreatment into the LC-ICP-MS system (7.1).

Plot the values of $y_{i,e,j}$ for each Cr species i on the ordinate and the associated mass concentrations $\rho_{i,e,j}$ on the abscissa.

Determine the linear regression function using the corresponding pairs of values $y_{i,\,e,\,j}$ and $\rho_{i,\,e,\,j}$ of the measured series in accordance with Formula (1):

$$y_{i,e} = m_{i,e}\rho_{i,e} + b_{i,e} \tag{1}$$

where

$y_{i,e}$ is the dependent variable corresponding to the measured response, expressed in units which will depend on the method, e.g. area value, for a given $\rho_{i,e}$ of target substance i in the calibration;

$\rho_{i,e}$ is the independent variable corresponding to the mass concentration, expressed in micrograms per litre, of the substance i in the calibration solution;

$m_{i,e}$ is the slope of the calibration curve from $y_{i,\,e}$ as a function of the mass concentration $\rho_{i,e}$, often called the response factor;

$b_{i,e}$ is the ordinate intercept of the calibration.

10.3 Recovery test of target substances

When analysing an unknown sample or a sample susceptible to matrix effects, e.g. loss or redox conversion of Cr(VI) or Cr(III), a recovery test should be carried out. This recovery test comprises the analysis of an unspiked sample and the spiked sample with a known target concentration, as well as blanks and standards.

For example, add 10 µl of 10 mg/l standard solution of Cr(VI) (6.16) or Cr(III) (6.18) to 10 ml of a water sample, respectively. Analyse the spiked and unspiked water samples, as well as the standard solution of the Cr species at 10 µg/l and a blank solution as given in Clause 9.

Calculate the recovery A_i in accordance with Formula (2).

$$A_i = 100 \times \frac{\left(y_{i,\mathrm{spk}} - y_{i,\mathrm{unspk}}\right)}{\left(y_{i,\mathrm{std}} - y_{i,\mathrm{blk}}\right)} \tag{2}$$

where

$A_{i,}$ is the recovery of the added target substance *i* in per cent, %;

$y_{i,\mathrm{spk}}$ is the measured response (integration units, e.g. for peak area or peak height) of the substance *i* for spiked water sample;

$y_{i,\mathrm{unspk}}$ is the measured response (integration units, e.g. for peak area or peak height) of the substance *i* for unspiked water sample;

$y_{i,\mathrm{std}}$ is the measured response (integration units, e.g. for peak area or peak height) of the substance *i* for the standard solution;

$y_{i,\mathrm{blk}}$ is the measured response (integration units, e.g. for peak area or peak height) of the substance *i* for the blank solution.

Recovery should preferably be in the range from 80 % up to 120 %. A lower or higher value indicates an insufficient efficiency or redox conversion between Cr(VI) and Cr(III) species at the chelating pretreatment or co-elution of interfering substances for ICP-MS detection, respectively. If the interference according to Clause 5 is observed, operate to reduce the interference according to Clause 9 (e.g. dilution of sample). After the improvement, perform the recovery test again and confirm that it is within the range.

11 Calculation

11.1 Use of the calibration curve to determine the result

Determine the result for each sample using the calibration curve prepared as described in 10.2. The same procedure shall always be used for calibration. The concentrations used to prepare the calibration curve shall cover the concentrations in the samples.

11.2 Calculation of results after calibration

Calculate the mass concentration $\rho_{i,\mathrm{d}}$ of target substance *i* in accordance with Formula (3) after solving Formula (1):

$$\rho_{i,\mathrm{d}} = \frac{\left(y_{i,\mathrm{d}} - b_{i,\mathrm{e}}\right)}{m_{i,\mathrm{e}}} \tag{3}$$

where

$y_{i,d}$ is the measured value, expressed in units which will depend on the method of measurement used, e.g. peak area, for target substance *i* in the sample;

$\rho_{i,d}$ is the mass concentration of target substance *i* in the sample, expressed in micrograms per litre;

$b_{i,e}$ see Formula (1);

$m_{i,e}$ see Formula (1).

When a procedural blank value is observed, subtract it to obtain the mass concentration in the sample.

11.3 Treatment of results lying outside the calibration range

If the concentration of the target substances in the sample exceeds the upper limit of the calibration curve, the water sample is diluted with water (6.1) as described in the procedure given in 9.1.1.

12 Expression of results

The analysis results obtained when applying this document are subject to a measurement uncertainty that is considered in the interpretation of the results (see Annex C).

The results obtained are expressed in micrograms of Cr per litre (µg/l as Cr), applying the dilution factors used for each sample. Give the results with two significant digits.

EXAMPLE

Cr(VI)	0,35 µg/l as Cr
Cr(III)	0,82 µg/l as Cr

13 Test report

The test report shall contain at least the following information:

a) the test method used, together with a reference to this document, i.e. ISO 24384:2024;

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

c) the sample storage protocol;

d) the results obtained for the individual Cr species, expressed in accordance with Clause 12;

e) details of any deviations from this procedure and of all circumstances which may have influenced the results;

f) the date of analysis.

Annex A
(informative)

Example of an operating condition of LC-ICP-MS and chromatogram of Cr(VI) and Cr(III) in the case of PDCA-chelating pretreatment

Table A.1 — Example of an operating condition of LC-ICP-MS

LC[a]	
Column[b]	Column of a mixed-bed of anion and cation exchangers, e.g. IonPac CG5A (i.d.: 4 mm, length: 50 mm)
Mobile phase composition	2 mmol/l PDCA, 2 mmol/l Na_2HPO_4, 10 mmol/l NaI, 50 mmol/l CH_3COONH_4 at pH 6,9
Separation temperature	(25 ± 3) °C
Injection volume	20 µl or 100 µl
Connecting tube between LC and ICP-MS instruments	PEEK tube (i.d.: 0,17 mm, length: 50 mm)
Flow rate	0,2 ml/min
ICP-MS[c]	
Nebulizer	Quartz concentric
RF Power	1 500 W
Plasma gas flow rate	15 l/min
Carrier gas flow rate	1,0 l/min
Make-up gas flow rate	0,1 l/min
Monitored isotope	^{52}Cr and ^{53}Cr under a collision cell mode using 5,0 ml/min He

[a] The other conditions of the LC may be used if they can separate Cr(VI) and Cr(III)-PDCA complex and also be shown to lead to the same results.

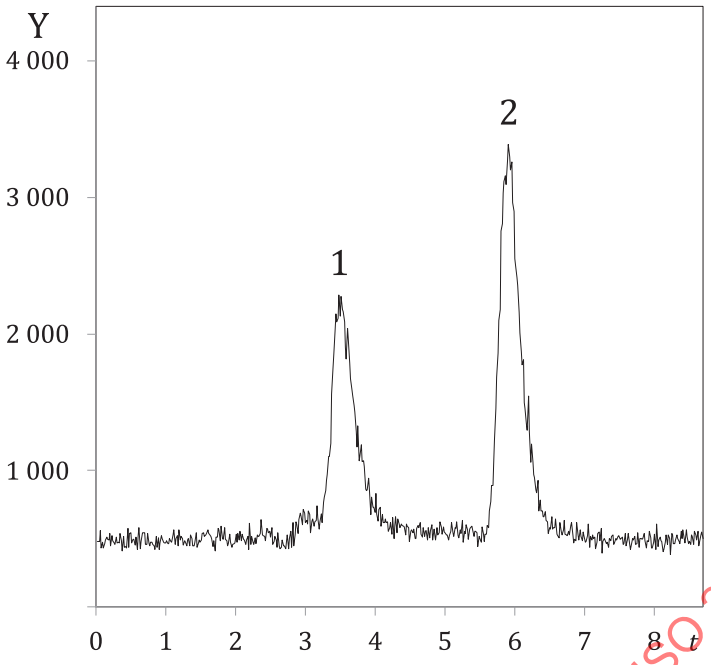
[b] IonPac CG5A is the trade name of a product supplied by Thermo Fisher Scientific, Inc. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

[c] The other conditions of the ICP-MS may be used if they can detect chromium elements of Cr(VI) and Cr(III)-PDCA complex and also be shown to lead to the same results.

Table A.2 — Example of a calibration condition

Number of standards	10
Calibration dynamic range	0,2 µg/l to 100 µg/l at ^{52}Cr with 20 µl injection volume. 0,05 µg/l to 20 µg/l at ^{52}Cr with 100 µl injection volume.
Limit of detection	0,044 µg/l and 0,077 µg/l for Cr(VI) and Cr(III), respectively, at ^{52}Cr and with 20 µl injection volume. 0,012 µg/l and 0,028 µg/l for Cr(VI) and Cr(III), respectively, at ^{52}Cr and with 100 µl injection volume.

NOTE The values of limits of detection for Cr species were calculated using values of 3-times the standard deviation/sensitivity at five repetitive analyses of a standard solution of Cr(III) and Cr(VI) at 0,2 µg/l. The sensitivity is defined as the signal peak area divided by the concentration.



Key

t retention time (min)

Y signal intensity at m/z 52 (cps)

NOTE The chromatogram of a standard solution of (1) Cr(III) and (2) Cr(VI) at 0,2 µg/l; injection volume, 100 µl. ICP-MS measurements were carried out under a collision cell mode with He gas.

Figure A.1 — Example chromatogram of a standard solution containing Cr(VI) and Cr(III)

Annex B
(informative)

Example of operating condition of LC-ICP-MS and chromatograms of Cr(VI) and Cr(III) in the case of EDTA-chelating pretreatment

Table B.1 — Example of operating condition of LC-ICP-MS

LC[a]	
Column[b]	Anion exchange column; e.g. Metrosep Carb 2 (i.d.: 2 mm, length: 100 mm)
Mobile phase composition	100 mmol/l HNO_3, 156 mmol/l NH_4OH at pH 9
Separation temperature	(30 ± 3) °C
Injection volume	200 µl
Flow rate	0,2 ml/min
ICP-MS[c]	
Nebulizer	concentric
RF Power	1 600 W
Plasma gas flow rate	18 l/min
Carrier gas flow rate	1,0 l/min
Make-up gas flow rate	1,2 l/min
Monitored isotope	^{52}Cr and ^{53}Cr under a collision cell mode using 4,2 ml/min He

[a] The other conditions of the LC may be used if they can separate Cr(VI) and Cr(III)-EDTA complex and also be shown to lead to the same results.

[b] Metrosep Carb 2 is the trade name of a product supplied by Metrohm AG. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

[c] The other conditions of the ICP-MS may be used if they can detect chromium elements of Cr(VI) and Cr(III)-EDTA complex and also be shown to lead to the same results.

Table B.2 — Example of calibration condition

Number of standards	10
Calibration dynamic range	0,05 µg/l to 20 µg/l with 200 µl injection volume
Limits of detection	0,022 µg/l and 0,030 µg/l for Cr(VI) and Cr(III), respectively, at ^{52}Cr and with 200 µl injection volume

NOTE The values of limits of detection for Cr species were calculated using values of 3-times the standard deviation/sensitivity at five repetitive analysis of a standard solution of Cr(III) and Cr(VI) at 0,050 µg/l. The sensitivity is defined as the signal peak area divided by the concentration.