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Leather — Chemical determination of chromium(VI) content in leather —

Part 2: Chromatographic method

Cuir — Détermination chimique de la teneur en chrome(VI) du cuir — Partie 2: Méthode chromatographique

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html

ISO 17075-2 was prepared by the Chemical Test Commission of the International Union of Leather Technologists and Chemists Societies (IUC Commission, IULTCS) in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 289, *Leather*, the secretariat of which is held by UNI, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

IULTCS, originally formed in 1897, is a world-wide organization of professional leather societies to further the advancement of leather science and technology. IULTCS has three Commissions, which are responsible for establishing international methods for the sampling and testing of leather. ISO recognizes IULTCS as an international standardizing body for the preparation of test methods for leather.

This first edition of ISO 17075-2, together with ISO 17075-1, cancels and replaces ISO 17075:2007, which has been technically revised.

The main changes compared to ISO 17075:2007 are as follows:

— the chromatographic analytical technique has been added;

— the sample preparation has been revised;

— mechanical shaking in 7.1 and 7.2 has been revised.

A list of all parts in the ISO 17075 series can be found on the ISO website.

Leather — Chemical determination of chromium(VI) content in leather —

Part 2:
Chromatographic method

1 Scope

This document specifies a method for determining chromium(VI) in solutions leached from leather under defined conditions. The method described is suitable to quantify the chromium(VI) content in leathers down to 3 mg/kg.

This document is applicable to all leather types.

The results obtained from this method are strictly dependent on the extraction conditions. Results obtained by using other extraction procedures (extraction solution, pH, extraction time, etc.) are not comparable with the results produced by the procedure described in this document.

If a leather sample is tested with both ISO 17075-1 and this document, the results obtained with this document are considered as the reference. The advantage of the method described in this document is that there are no interferences from the colour of the extract. Nevertheless, interlaboratory trials do not show significant differences (see Annex D) and the results are comparable between both methods.

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 2418, *Leather — Chemical, physical and mechanical and fastness tests — Sampling location*

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 4044:2017, *Leather — Chemical tests — Preparation of chemical test samples*

ISO 4684, *Leather — Chemical tests — Determination of volatile matter*

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:

— ISO Online browsing platform: available at http://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
chromium(VI) content
amount of chromium(VI) in leather determined after extraction with an aqueous salt solution at pH 7,0 to 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.

4 Principle

Extractable chromium(VI) is leached from the sample in phosphate buffer at pH 7,0 to 8,0. An aliquot of the filtered extract is analysed for Cr(VI) using ion-exchange chromatography with UV-VIS detection.

5 Chemicals

All reagents used shall have at least analytical grade purity.

5.1 Extraction solution

Dissolve 22,8 g dipotassium hydrogenphosphate $K_2HPO_4 \cdot 3H_2O$ in 1 000 ml water (5.7), adjusted to pH 8,0 ± 0,1 with phosphoric acid (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 can 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, ρ = 1,71 g/ml, made up to 1 000 ml with water (5.7).

First add approximately 200 ml of deionised 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 deionised water.

5.3 Potassium dichromate ($K_2Cr_2O_7$)

, dried for (16 ± 2) h at (102 ± 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). One millilitre of this solution contains 1 mg of chromium.

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

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). One millilitre 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**, Grade 3 quality as specified in ISO 3696.

6 Apparatus and materials

Usual laboratory equipment and, in particular, the following.

6.1 Suitable mechanical orbital shaker, (100 ± 10) 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 Membrane filter, 0,45 µm pore size [polytetrafluoroethylene (PTFE) or polyamide 66].

6.5 Common laboratory glassware and **pipettes**.

6.6 Ion-exchange chromatograph, with UV detector or **high performance liquid chromatograph (HPLC) with anion-exchange column and UV detector**. It is recommended a photo diode array detector (DAD).

6.7 Analytical balance, capable of weighing to 0,1 mg.

6.8 Suitable vials for HPLC.

6.9 Sharp cutting tool or **blade**, suitable for cutting the leather into 3 mm to 5 mm pieces.

7 Procedure

7.1 Sampling and preparation of samples

If possible, sample in accordance with ISO 2418. If sampling in accordance with ISO 2418 is not possible (e.g. leathers from finished products like shoes, garments), details about sampling shall be given in the test report.

Prepare the leather sample by cutting (6.9) into small pieces according to the method specified in ISO 4044:2017, 6.3.

7.2 Preparation of analytical solution

Weigh (6.7) approximately (2 ± 0,1) g of leather pieces to the nearest 0,001 g. Pipette 100 ml 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 leather and close the flask with a stopper. Record the extract volume as V_0.

Shake the conical flask with the leather pieces for 3 h ± 5 min on a mechanical orbital shaker at (100 ± 10) min^{-1} (6.1) at room temperature to extract the chromium(VI).

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

Immediately after completing the 3 h 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 (6.8).

7.3 Chromatographic conditions

Determination of chromium(VI) is performed using the ion chromatographic technique. As the instrumental equipment of the laboratories may 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 Annexes B and 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) and the results observed should be in the range as listed in Annex A.

Record the injection volume as V_M and record the area of the chromate peak as A.

7.4 Calibration

The content of chromium(VI) in leather is determined with an external standard calibration.

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

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 and transfer a suitable aliquot volume into a vial (6.8).

Prepare calibration levels as specified in Table 1.

Table 1 — Calibration levels preparation

Volume of the standard solution (5.5) (ml)	1,25	2,5	5	12,5	25
Volume of the extraction solution (5.1) (ml)	23,75	22,5	20	12,5	0
Final volume (ml)	25 ml in volumetric flask				
Concentration of hexavalent chromium (µg/l)	50	100	200	500	1 000

Transfer an aliquot to a suitable vial (6.8) corresponding for the chromatography system (6.6).

Inject the standards in the chromatographic system (6.6). 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.

Plot the chromium(VI) concentrations in micrograms of Cr 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.

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. 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 (5.1) (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, this is an indication that the leather contains reducing agents. This leads to the conclusion that this leather has no chromium(VI) content (below detection limit).

8 Calculation and expression of results

8.1 Calculation of chromium(VI) content

$$w_{\mathrm{Cr(VI)}} = \frac{(A - b) \cdot V_0 \cdot V_C}{V_M \cdot m \cdot F} \quad (1)$$

where

$w_{\mathrm{Cr(VI)}}$ is the mass fraction, expressed in milligrams per kilogram (mg/kg), of extractable chromium(VI) in leather;

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

F is the gradient of calibration curve (y/x), expressed in millilitres per microgram (ml/µg);

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

m is the mass of the leather sample 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.

$$w_{\mathrm{Cr(VI)\text{-}dry}} = w_{\mathrm{Cr(VI)}} \cdot D \tag{2}$$

where D is the factor for conversion to dry matter:

$$D = \frac{100}{100 - w} \tag{3}$$

where w is the mass fraction of the volatile matter determined using ISO 4684 with another piece of sample, expressed as a percentage.

8.2 Recovery rate (according to 7.5)

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

where

η is the recovery rate, expressed in percent (%);

V_1 is the volume of sample solution in the spiked solution, expressed in millilitre (ml);

V_2 is the volume of chromate standard in the spiked solution, expressed in millilitre (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 volatile matter, determined according to ISO 4684, is given in percent (%) rounded to the nearest 0,1 %.

The extraction matrix for leather is complex and results below 3 mg/kg show large variations and have limited reliability; therefore the quantification limit shall be 3 mg/kg.

9 Test report

The test report shall include the following information:

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

b) a reference to this document, i.e. ISO 17075-2:2017;

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 volatile matter of the leather in percent (%) to the nearest 0,1%;

f) the recovery rate in percent (%);

g) details of any deviations from the procedure.

Annex A
(informative)

Accuracy

Results obtained from an international interlaboratory trial carried out in June 2015 for two types of leather are summarized in Table A.1, Table A.2 and Table A.3.

Table A.1 — Results for one type of leather (Leather A)

Laboratory	Chromium(VI) content mg/kg			Mean value mg/kg
01	6,41	6,43	6,09	6,31
02	6,20	6,70	6,40	6,43
03	3,57	3,92	3,89	3,79
04	5,90	6,34	5,98	6,07
05	7,10	7,40	6,90	7,13
06	3,86	3,83	4,01	3,90
07	5,80	4,60	5,60	5,33
10	4,20	4,70	4,60	4,50
11	4,10	4,20	3,90	4,07
Mean value: 5,28 Uncertainty: ± 0,97 being k = 2,31 (P 95 %)				

Table A.2 — Results for one type of leather (Leather B)

Laboratory	Chromium(VI) content mg/kg			Mean value mg/kg
01	30,12	29,48	29,89	29,83
02	29,80	28,90	29,20	29,30
03	26,40	25,40	27,30	26,37
04	29,30	28,64	30,03	29,32
05	30,80	31,00	30,50	30,77
06	24,18	22,61	23,03	23,27
07	24,40	23,80	25,50	24,57
09	29,60	29,40	28,50	29,17
10	21,90	23,70	23,80	23,13
11	26,60	25,10	24,60	25,43
Mean value: 27,1 Uncertainty: ± 2,1 being k = 2,26 (P 95 %)				

Table A.3 — Precision data from interlaboratory trial with two types of leathers

Values in milligrams per kilogram (mg/kg)

Chromium(VI) content [a]	Repeatability [b]	Reproducibility [b]
5,28	0,68	2,96
27,1	1,60	6,67

[a] Mean values.

[b] *P* 95 %, factors 2,31 for the first and 2,26 for the second rows respectively.

Annex B
(informative)

Chromatographic conditions for direct detection method

B.1 General

As the instrumental equipment of the laboratories may 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) and the results observed should be in the range as listed in Annex A.

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 is 250 mmol in ammonium sulphate and 10 mmol in sodium hydroxide. Its pH is 8,2. From this solution it is prepared weekly the eluent for chromatography (B.2.1.4). The shelf life is up to four months at 4 °C.

B.2.1.4 Mobile phase

Transfer 100 ml of eluent stock solution (B.2.1.3) into a 1 000 ml volumetric flask and make up to the mark with water (5.7). This solution is 25 mmol in ammonium sulphate and 1 mmol in 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 (B.2.1.4)

— Column: Anion-exchange column (polymethacrylate resin with quaternary ammonium functional groups), 4,6 × 75 mm, with 1 mm pre-column

— Range of wavelength (only for DAD): record the UV spectrum in the range 200–550 nm

— Wavelength of extracted chromatogram: 372 nm

— Flow rate: 0,9 ml/min

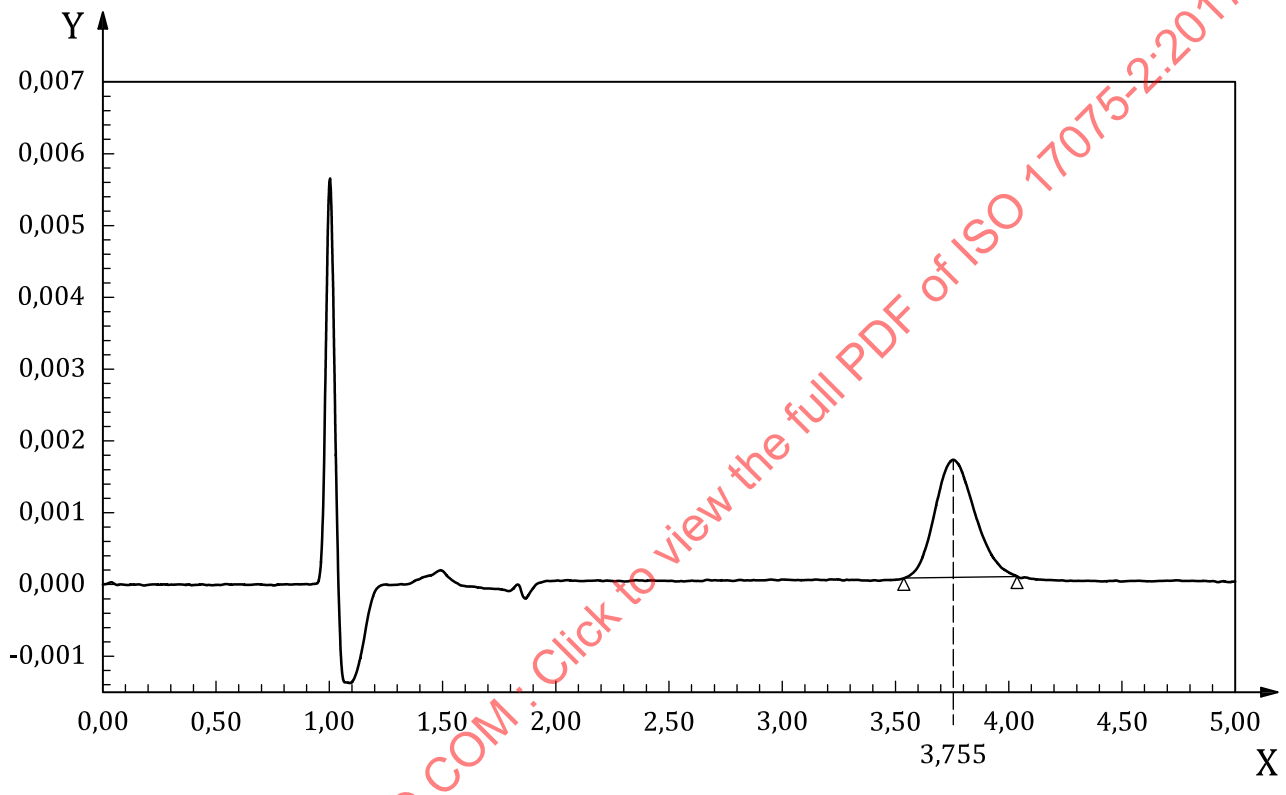
— Injection volume: 50 µl

— Run time of chromatogram: 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 chromatogram obtained from one sample that contains 3,9 mg/kg of Cr(VI). Figure B.2 shows the UV spectrum of the chromate anion found in a sample.

B.3 Example of a chromatogram and UV spectrum obtained in the analysis of a commercial sample



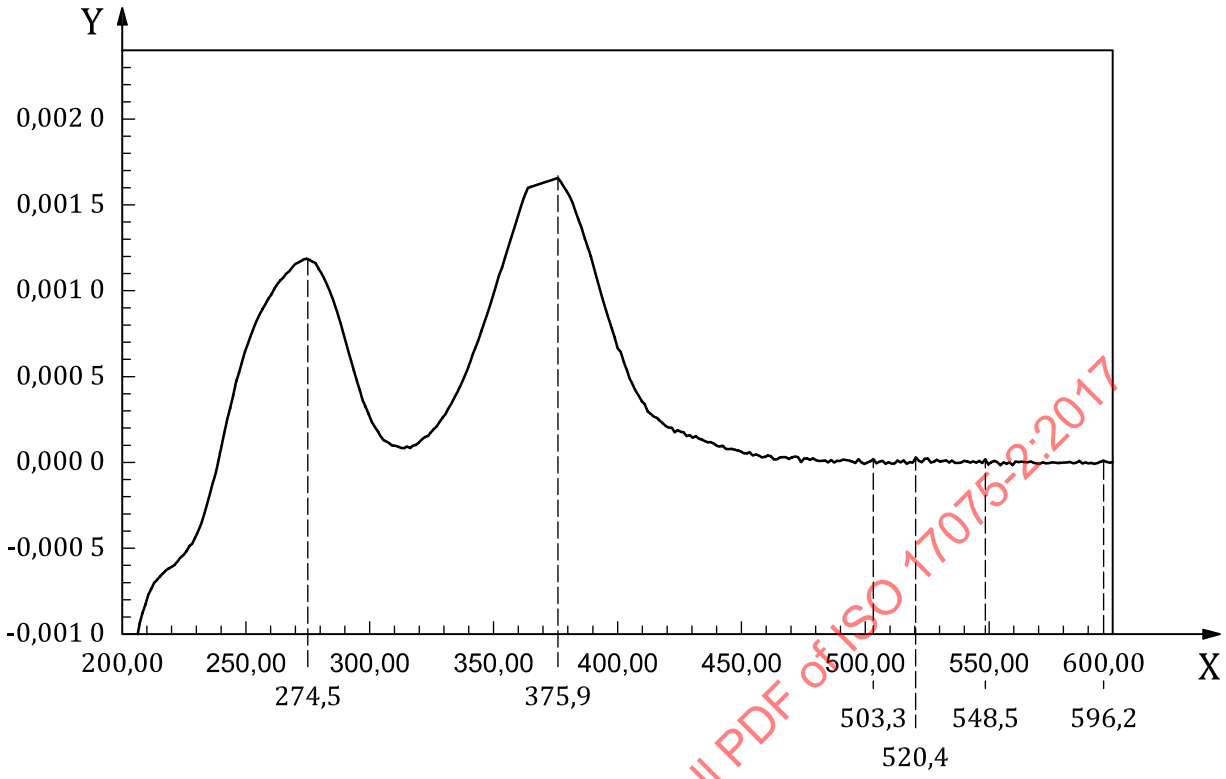
Key

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 Cr(VI)



Key

X nm

Y AU

Figure B.2 — Ultraviolet spectrum of chromate ion captured from the chromatogram of the sample of Figure B.1 at 3,757 min with a DAD detector

Annex C
(informative)

Chromatographic conditions for method with post-column reaction

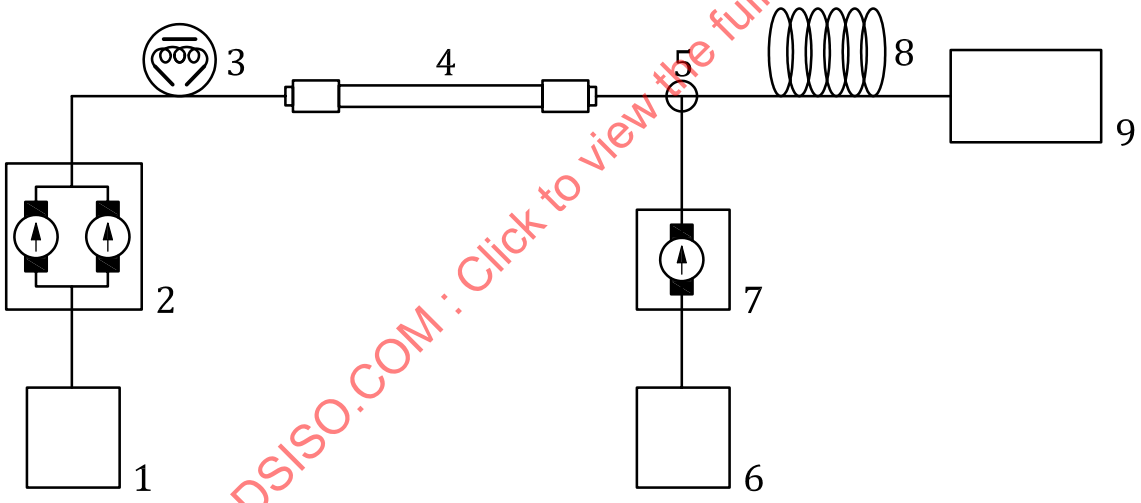
C.1 General

As the instrumental equipment of the laboratories may 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) and the results observed should be in the range as listed in Annex A.

C.2 Chromatographic system and apparatus required

The ionic chromatography method with post column reaction is summarized in Figure C.1.



Key

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

Figure C.1 — Diagram of a system for ionic chromatography with post column reaction

The chromium(VI) is analysed using an analytical 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 ensure the proper mixing of the eluent from the column and the post column reagent and the chromium(VI) in solution oxidizes 1,5-diphenylcarbazide to 1,5-diphenylcarbazone. This gives 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.

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.

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 PEEK.

The use 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 % purity.

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 distilled 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 distilled water (5.7) and let it stand cooling.