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Soil quality — Determination of perchlorate in soil using ion chromatography

Qualité du sol — Détermination du perchlorate des sols en utilisant la chromatographie ionique

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Foreword

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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).

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For an explanation of the voluntary nature of standards, 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 www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 3, *Chemical and physical characterization*.

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

Although perchlorate occurs naturally, it is mainly a manmade anion (ClO_4^-). Usually, it is combined with NH_4^+, Na^+ and K^+ to form ammonium perchlorate, potassium perchlorate, and sodium perchlorate, respectively. It was reported that more than 90 % of perchlorate is used in military activities. Due to the excellent oxidizing capacity of perchlorate, it is added into propellant of rocket, missile, and satellite. We can presume some routes of manmade perchlorate exposure to soil and groundwater. For example, complete or incomplete explosion of the signal bomb (containing about 2 000 µg of perchlorate) in target or impact area, oversupplying of perchlorate for complete combustion in firing point, grand scale of fireworks could be the route of perchlorate exposure to soil and groundwater. In addition to these, other route could come from waste treatment process. Because perchlorate in missile is naturally deteriorated according to time, it should be recharged with a new one. In the past, incineration was preferred for the treatment of deteriorated perchlorate. When the incineration process was carried out in open space and kept as ash on site without any caution, it could be an important route of soil and groundwater contamination. Perchlorate is very stable in water and is not adsorbed easily on soil particle. From that view, surface water or groundwater could be contaminated more often than soil due to surface runoff or leaching process. However, perchlorate can also contaminate soil and vegetation. This kind of contamination could affect high level organisms in food chain. Perchlorate contamination of drinking water and food chain potentially affect human health because it can interfere with iodide uptake by the thyroid gland. Through this kind of interference, thyroid hormone production is decreased and it cause hyperthyroidism. The permitted level of perchlorate concentration in drinking water is below 15 ppb in Korea. Some states in the USA have an advisory level for perchlorate in drinking water. It is very difficult to find a country to regulate perchlorate level in soil because it seems that perchlorate contamination of soil is very rare in normal areas. However, perchlorate could be one of the major contaminants at a target area or firing point in military field and it is needed to manage the perchlorate concentration of soil to protect the vegetation, surface water, and groundwater. For this purpose, a standard method for perchlorate analysis in soil has been developed.

Soil quality — Determination of perchlorate in soil using ion chromatography

1 Scope

This document specifies a method for the determination of perchlorate in soil and soil materials.

Under the conditions specified in this document, a concentration as low as 0,1 mg/kg can be determined.

The working range is restricted by the ion-exchange capacity of the separator column. Dilution of the water extracts to the working range can be necessary.

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 and estimation of performance characteristics — Part 1: Statistical evaluation of the linear calibration function*

ISO 8466-2, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 2: Calibration strategy for non-linear second-order calibration functions*

3 Terms and definitions

No terms and definitions are listed in this document.

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

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

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

4 Principle

A dried and sieved soil sample is used as the test portion. Perchlorate is extracted by distilled or deionised water from the soil sample. Extraction is conducted by mechanical shaking and centrifugation.

After filtering the extract with a 0,45 µm membrane filter (e.g. cellulose acetate, hydrophilic polypropylene or polyethersulphone filter), the filtrate is analysed by ion chromatography to determine perchlorate.

If the adverse effects of anions, cations or organics are not negligible, appropriate pre-treatment for the elimination of these effects should be applied. Selective removal of interfering elements using a cartridge is one of the applicable pre-treatments.

The method requires the application of high-capacity separator columns, which allow the injection of sample volumes up to 1 ml.

Perchlorate is separated by ion chromatography (IC). Detection is conducted by suppressed conductivity (CD).

An anion-exchange resin is used as the stationary phase and an aqueous solution of salts of weak monobasic acids and dibasic acids is used as an eluent for isocratic or gradient elution (e.g. carbonate-, hydrogen carbonate-, hydroxide-eluent, and organic modifiers such as acetone, acetonitrile).

The concentration of perchlorate is determined after calibration of the overall procedure according to ISO 8466-1 or ISO 8466-2.

Control experiments are necessary to check the validity of the calibration function. Replicate determinations can be necessary. Use of a standard addition method can be required if matrix interferences are expected.

NOTE The results of interlaboratory validation study can be found in Annex B.

5 Interferences

Any substance that has a retention time coinciding with perchlorate and producing a detector response can interfere. Co-elution can be solved by changing columns, eluent strength (e.g. gradient elution), modifying the eluent with organic solvents or by selective removal of the interference with sample pre-treatment.

In the case of saline soil, a high concentration of chloride, sulfate, and carbonate in soil extracts can cause interference with the determination of perchlorate. It was reported that an injection of 800 mg/l of chloride, sulfate, and carbonate (about 6 mS/cm as of electrical conductivity) in perchlorate standard solution (0,025 mg/l) resulted in 80 % of recovery for perchlorate (1). Additionally, metals like iron or aluminium in soil extracts can have adverse effects on the performance of ion chromatograph due to binding with the resin material of the separator or suppressor column. These interference can be reduced by sample dilution, with the aid of special cation exchangers (e.g. Na-form, Ag-form, Ba-form, H-form) or resolved by the application of advanced inline cutting or re-injection techniques (see Annexes C, D and E).

Users of this document's method should check their system individually for the significant interfering concentration of anions and cations.

In case of agricultural soil containing phosphate fertilizer, pyrophosphate ($P_2O_7^{4-}$) or tripolyphosphate ($P_3O_{10}^{5-}$) could be coeluted with perchlorate depending on the conditions of ion chromatography (2). This kind of interference could be avoided by using an optimized eluent.

Clay particles (e.g. aluminosilicates) or organic compounds (e.g. humic acids) can plug the column even though the centrifugation and filtering processes are applied. It is recommended to use a pre-column to protect the analytical separator column.

6 Reagents

Use only reagents of pro-analysis grade free of compounds containing perchlorate. Weigh the reagents with an accuracy of ±1 % of the nominal mass, unless stated otherwise. Prepare alternative concentrations or volumes of solutions as described in 6.2 to 6.9, if necessary. Alternatively, use commercially available solutions of the required concentration.

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

6.2 Potassium perchlorate, $KClO_4$.

6.3 Sodium hydrogen carbonate, $NaHCO_3$.

6.4 Sodium chloride, NaCl.

6.5 Sodium sulfate, Na_2SO_4.

6.6 Sodium nitrate, $NaNO_3$.

6.7 Eluents.

6.7.1 General

Degas all eluents used. Take steps to avoid any renewed air pick-up during operation (e.g. by helium sparging and inline degassing).

The choice of eluent (e.g. potassium hydroxide, sodium hydrogen carbonate, sodium carbonate, sodium hydroxide solutions; mixed with organic modifiers if needed) depends on the choice of column and detector. Seek advice from the column supplier. Apply eluents that were prepared manually, automatically or *in situ* electrochemically prepared. The chosen combination of separator column and eluent should conform to the resolution requirements stated in Clause 9. Use eluents as long as the requirements in 8.3.3 and in Clause 9 are met.

One example for an appropriate manually prepared eluent is given in 6.7.2. Additionally, another example for an appropriate eluent prepared using a generating device is given in 6.7.3.

6.7.2 Sodium hydroxide, ρ(NaOH) = 65 mmol/l.

Prepare 65 mmol/l of NaOH by putting 5,2 g of 50 % (mass fraction) aqueous NaOH from the middle portion of the reagent bottle into a 1 000 mL volumetric flask containing about 500 ml of degassed water. Fill it up to the mark with degassed water. Mix this solution gently and degas by sparging with argon or helium or sonicating under a vacuum for 10 min. For the preparation of 50 % (mass fraction) aqueous NaOH, weigh 50 g of sodium hydroxide and transfer into a 100 ml volumetric flask. Dissolve by adding water (6.1) and fill to the mark with water (6.1). Do not shake the 50 % (mass fraction) NaOH bottle to avoid forming carbonate.

NOTE Solutions of sodium hydroxide can be susceptible to carbonate contamination resulting from the adsorption of carbon dioxide from the atmosphere. This contamination can lead to irreproducible perchlorate retention times, elevated instrument background conductivity and increased baseline noise/drift.

6.7.3 Potassium hydroxide, ρ(KOH) = 65 mmol/l.

If the ion chromatographic system has a generating device for KOH eluent, generate 65 mmol/l of KOH eluent according to the manufacturer's recommendations.

Depending on the column's properties the eluent composition can be different. According to the manufacturer's instructions, check which kind of eluent is appropriate for analysing perchlorate.

6.8 Standard solutions.

6.8.1 Perchlorate stock standard solution, $\rho(ClO_4^-)$ = 1 000 mg/l.

Dry potassium perchlorate in the oven at 100 °C for 2 h. Weigh (1,393 ± 0,001) g and transfer quantitatively into a 1 000 ml volumetric flask. Dissolve by adding water (6.1) and fill to the mark with water (6.1). Store this stock standard solution in the refrigerator at 2 °C to 8 °C using polyethylene or glass bottles. This stock standard solution is stable for 12 months.

The use of commercially available certified stock standard solution is also possible.

Other alternative perchlorate compounds (e.g. sodium perchlorate, ammonium perchlorate) may also be used in the preparation of (stock) standard solution.

6.8.2 Perchlorate standard solution I, $\rho(ClO_4^-)$ = 100 mg/l.

Add 10 ml of stock standard solution (6.8.1) into a 100 ml volumetric flask and fill it up to the mark with water (6.1). Store this working standard solution in the refrigerator at 2 °C to 8 °C using polyethylene or glass bottles. This standard solution would be stable for 6 months.

In addition, a working standard solution can be made through the dilution of commercially available certified stock standard solution with water (6.1).

6.8.3 Perchlorate standard solution II, $\rho(ClO_4^-)$ = 1 mg/l.

Add 1,0 ml of perchlorate standard solution I (6.8.2) into a 100 ml volumetric flask and fill it up to the mark with water (6.1). Store this working standard solution in the refrigerator at 2 °C to 8 °C using polyethylene or glass bottles. This standard solution is stable for 3 months.

6.8.4 Perchlorate calibration standard solution.

Prepare the calibration standard solutions through the dilution of perchlorate standard solution I (6.8.2) or perchlorate standard solution II (6.8.3). At least, five levels of concentration should be prepared over the expected working ranges as evenly as possible (e.g. 0,05, 0,1, 0,2, 0,4, 0,8 and 1 mg/l).

6.8.5 Perchlorate system check solution, $\rho(ClO_4^-)$ = 0,5 mg/l.

Add 0,5 ml of perchlorate standard solution I (6.8.2) into a 100 ml volumetric flask and fill it up to the mark with water (6.1). Prepare the solution on the day of use.

6.8.6 Matrix check stock solution, $\rho(HCO_3^-, Cl^-, SO_4^{2-}, NO_3^-)$ each of 1 g/l.

Place 3,44 g of sodium hydrogen carbonate (6.3), 4,13 g of sodium chloride (6.4), 3,72 g of sodium sulfate (6.5) and 3,42 g of sodium nitrate (6.6) into a 100 ml volumetric flask. Dissolve these compounds in approximately 80 ml of water (6.1) and fill the flask up to the mark with water (6.1). This solution is stable for 1 year.

Dilute 4 ml of this solution in 100 ml of water (6.1) to obtain the 1 g/l-stock solution. This solution would be stable for 6 months.

6.8.7 Perchlorate matrix check stock solutions, $\rho(ClO_4^-)$, 2 mg/l.

Depending on the laboratory internal conditions chosen (e.g. separation characteristics), prepare a check solution spiked with an appropriate perchlorate concentration. The composition of this check solution should cover the actual conditions of samples as closely as possible. For example, to make samples with chloride and sulfate concentrations of up to 50 mg/l each and a presumed perchlorate concentration of 2 mg/l, follow the process described below:

Pipette 5 ml of the matrix check stock solution (6.8.6) and 2 ml of the perchlorate standard solution I (6.8.2) into a 100 ml volumetric flask and fill it up to the mark with water (6.1).

The concentrations in this solution are: 50 mg/l of carbonate, chloride, sulfate, and nitrate, respectively, and 2 mg/l of perchlorate. Prepare the solution on the day of use.

6.9 Blank solution.

Fill a volumetric flask (e.g. 100 ml) with water (6.1).

7 Apparatus

7.1 Horizontal mechanical shaker, maintaining a frequency of 100 cycles/min and offering a shaking width of about 10 cm.

7.2 Centrifuge, should be used at a speed setting of 3 000 rpm.

7.3 Membrane filters, with 0,45 µm pore size or smaller (e.g. hydrophilic polypropylene or polyethersulphone filter).

7.4 Cartridges, Ag-form, Ba-form, H-form and Na-form for the selective removal of chloride, sulfate, carbonate and cations (e.g. iron, aluminium), respectively.

7.5 Analytical balance, being capable of making precise measurements of ±0,1 mg.

7.6 Ion chromatographic system.

7.6.1 Eluent reservoir, equipped with a degassing unit.

7.6.2 Pumping system, having an accurate flow rate and pulse-free flow and suitable for the isocratic or gradient technique.

7.6.3 Injection valve, appropriate for reproducible injections into the high-pressure flow path, equipped with sample loop which allow the injection of sample volumes up to 1 ml.

7.6.4 Separator column, with the specified separating performance (9.1).

7.6.5 Pre-column, having the capability to protect the analytical separator column.

NOTE In general, pre-columns contain the same as or similar resin materials to the analytical separator column or non-functionalised resin.

7.6.6 Conductivity detector, thermally controlled and sensitive with a suppressor device.

7.6.7 Recording device.

8 Procedure

8.1 Pre-treatments

General pre-treatments include drying and sieving. The field moist sample is dried in the air or oven. In the case of air drying, spread the soil sample no thicker than 5 cm on the tray. The tray should not absorb any moisture from the soil. Additionally, direct sunlight should be avoided. For oven drying, the temperature in the oven should not exceed 40 °C and a ventilation device should be equipped.

The dried sample is passed through a 2 mm sieve and then it is used as a test portion.

For other details of pre-treatments, refer to ISO 11464.

8.2 Extraction

Weigh (10 ± 0,1) g of dried and sieved soil sample into a 50 ml centrifuge tube. Add 30 ml of deionized water. Shake it using a mechanical shaker (7.1) for 1 h at 100 cycles/min. After the shaking process, centrifuge the mixture at 3 000 rpm for 1 h (Annex A). Filter the supernatant through a 25 mm

diameter and 0,45 µm pore size membrane filter with a syringe (e.g. hydrophilic polypropylene or a polyethersulphone filter). In this step, discard the first portion (e.g. about 0,3 ml) of filtrate and filter the remainder directly into a clean plastic vial. The filtrate is used for ion chromatographic determination. If necessary, cartridges (7.4) are applicable for the removal of anionic and cationic interference in the filtrate according to the manufacturer's instructions.

For the verification of the filtering step, analyse 30 ml of 0,01 mg/l of perchlorate standard solution that has passed through the filter in the same way as that described in 8.2. The recovery of the 0,01 mg/l of perchlorate standard should fall between 80 % and 120 %.

8.3 Ion chromatography

8.3.1 General

Set up the ion chromatographic system (7.6) according to the manufacturer's instructions. Run the eluent, and confirm the baseline stability.

Perform the calibration as described in 8.3.2. Measure the samples and blank solution (6.9) according to 8.3.3. An example of ion chromatography conditions can be found in Annex A.

8.3.2 Calibration

Inject the calibration standard solutions (6.8.4). The measured signal (peak area or peak height) is proportional to the concentration of the perchlorate ion.

When the analytical system is first started up and at intervals afterwards, establish a calibration function (see ISO 8466-1 or ISO 8466-2) for the measurement as follows. This is usually done by a software of the instrument.

Use the data obtained (peak area or peak height) to calculate the regression line as specified in ISO 8466-1 or ISO 8466-2.

Subsequently, verify the continuing validity of the established calibration function (9.2).

NOTE Generally, the calibration method is not restricted to a calibration strategy covering a single concentration decade as specified in ISO 8466-1 or ISO 8466-2 only. When calibrating over a larger range than one concentration decade, a loss of accuracy, compared to that specified in ISO 8466-1 or ISO 8466-2, can occur.

8.3.3 Measurement of perchlorate

Identify the peak of the perchlorate by comparing the retention time with that of the calibration standard solution. Deviation of the retention time should not exceed 10 % within a batch. The use of a pre-column is recommended to protect the analytical separator column.

If the concentration of perchlorate in the sample exceeds the calibration range, dilute the sample and reanalyse it.

In case the concentration of perchlorate in the sample is outside of the calibration range, if necessary, establish a new calibration function for a lower working range.

If matrix interferences influencing the retention time of perchlorate, are expected, check the system suitability using standard addition method to confirm the results (verify the peaks by comparing the retention time of the spiked sample with that of the original sample).

Peak resolution R between the anion of interest and its nearest peak should be checked according to Clause 9.

Measure the blank solution (6.9) in the same way.

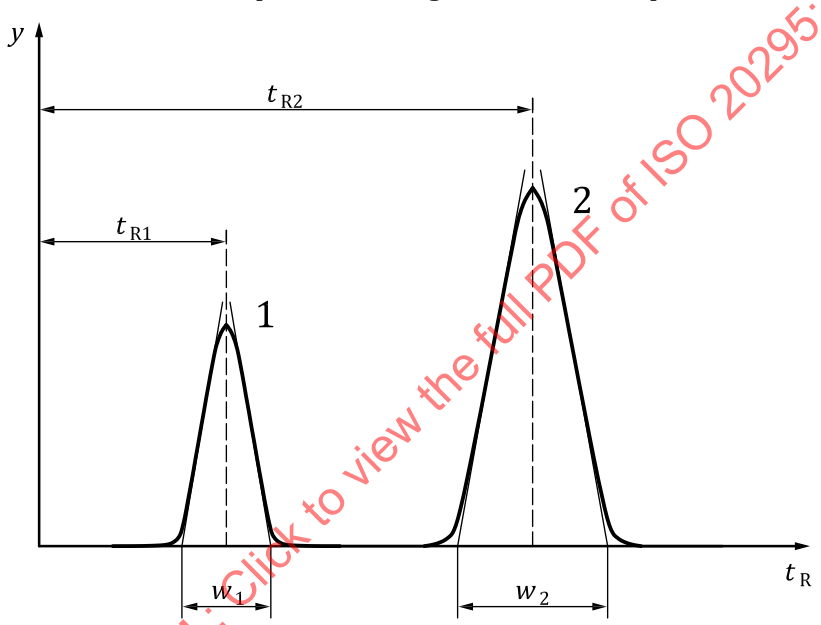
9 Quality control

9.1 Performance of the separator column

In chromatograms of samples and standard solutions, the peak resolution R between the anion of interest and its nearest peak shall not fall below 1,3 [see Formula (1) and Figure 1]. Separation conditions shall be such that possible interfering anions will not interfere with the anion of interest.

If R fails the criteria ≥1,3 or perchlorate elutes as a rider peak (Figure C.1), change the columns and eluent strength (e.g. gradient elution), modify the eluent with organic solvents, remove the interference selectively with sample pre-treatment (Annex C), apply column cut or re-injection techniques (Annex D or Annex E), determine the recovery or apply standard addition.

All the materials used should not add a positive or negative bias to the perchlorate result.



Key

w_1 width of peak 1
w_2 width of peak 2
t_R retention time, in seconds
y signal
1 peak 1
2 peak 2

Figure 1 — Graphical representation of the parameters to calculate the peak resolution *R*

NOTE Within the scope of this document, the calculation of resolution *R* is appropriate for both isocratic and gradient elution.

Calculate the peak resolution for the peak pair 2,1, $R_{2,1}$, using Formula (1):

$$R_{2,1} = \frac{2 \cdot (t_{R2} - t_{R1})}{w_2 + w_1} \quad (1)$$

where

t_{R2} is the retention time, in seconds, of the second peak;

t_{R1} is the retention time, in seconds, of the first peak;

w_2 is the peak width, in seconds, on the time axis of the second peak;

w_1 is the peak width, in seconds, on the time axis of the first peak.

NOTE w_1, w_2 being the base width of the constructed isosceles triangle over each Gaussian peak.

9.2 Validity check of the calibration function

To verify the continuing validity of the calibration function, measure independent standard solutions of different perchlorate concentrations in the lower and upper third of the working range. Proceed accordingly after the set-up procedure and after each sample series at least, but after 20 measurements in all cases. Recovery should be within 85 % to 115 % of the nominal value. If this recovery is not met, a new calibration function should be established.

10 Calculation

Calculate the mass concentration of perchlorate in the soil sample using Formula (2).

$$c_{is} = c_i \times V_T \times \frac{100}{m_f \times w_{dm}} \times f_{dil} \quad (2)$$

where

c_{is} is the mass concentration of perchlorate in the soil sample, in milligrams per kilogram of dry matter (mg/kg);

c_i is the mass concentration of perchlorate in the water extract, in milligrams per litre (mg/l);

V_T is the total volume of the water for extraction, in litres (l);

m_f is the mass of the soil used for extraction, in kilograms (kg);

w_{dm} is the dry matter content of the soil sample, in percent of mass (%);

f_{dil} is the dilution factor of the water extract.

11 Expression of results

Results shall be reported to a maximum of two significant figures [e.g. perchlorate(ClO_4^-) 55 mg/kg, perchlorate(ClO_4^-) 700 mg/kg].

12 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 20295:2018;

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

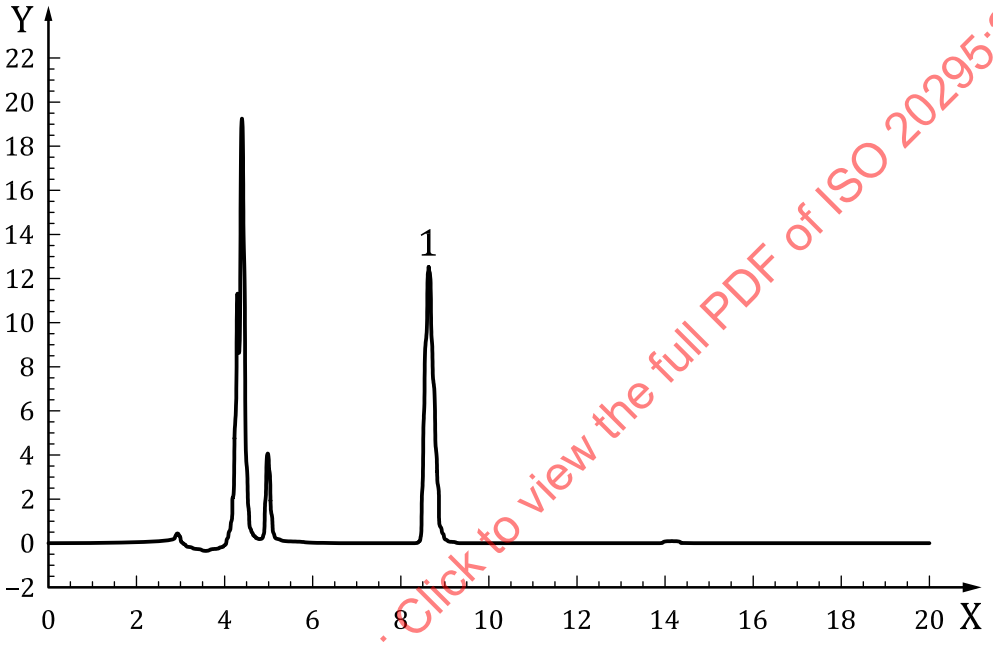
c) the results of the determination according to Clause 10;

d) any details not specified in this document or that are optional, as well as any other factors that may have affected the result.

Annex A
(informative)

Example of ion chromatography conditions and the selection of extraction method

A.1 Example of ion chromatography conditions



Key

X retention time, min

Y conductivity, µS·cm^{-1}

1 ClO_4^- (8,633 min)

Figure A.1 — Example of ion chromatography conditions

Conditions for the chromatogram shown in Figure A.1.

Column:	Anion exchanger
Eluent:	65 mmol/l KOH
Temperature:	30 °C
Flow rate:	1,0 ml/min
Injection volume:	1 000 µl

Detection: Suppressed conductivity

Sample preparation: Filter extract

Peaks: Perchlorate 400 µg/l

A.2 Experimental result for the selection of extraction ratio (soil to water ratio)

This experiment was carried out for the section of extraction ratio (soil to water). Matrix spiked sample (nominal concentration of 2 mg/kg) was used. The conditions of the ion chromatographic system were the same as shown in A.1. When considering the precision and accuracy, soil to water ratio (1:2) showed excellent results. However, with the consideration of practical aspect to take supernatant from soil and water mixture, the soil to water ratio (1:3) was implemented in this document. The results are presented in Table A.1.

Table A.1 — Experimental results for the selection of extraction method (soil to water)

Extraction ratio (soil to water)		1:2	1:3	1:5
Duplicates	D1	2,00	1,82	1,34
	D2	2,02	1,78	1,45
	D3	2,00	1,80	1,45
	D4	2,00	1,81	1,41
Mean (mg/kg)		2,00	1,80	1,41
Standard deviation (mg/kg)		0,00	0,01	0,01
Nominal concentration (mg/kg)		2	2	2
Precision (%)		0,43	0,82	3,18
Accuracy (%)		100,25	90,13	70,63

A.3 Experimental results for the selection of extraction method, devices and time

This experiment was carried out for the selection of extraction methods. Matrix spiked sample (nominal concentration of 2 mg/kg) was used. The conditions of the ion chromatographic system were the same as shown in A.1. Compared with other methods, 1 h of mechanical shaking and 1 h of centrifugation method showed good results from the view of precision and accuracy. The results are presented in Table A.2.

Table A.2 — Experimental results for the selection of extraction method, devices and time

Extraction methods		Centrifugation (1 h at 3 000 rpm)	Mechanical shaker (5 h at 100 cycles/min)	Sonication (1 h)	Mechanical shaker (1 h at 100 cycles/min) **and centrifugation** (1 h at 3 000 rpm)
Duplicates	D1	1,74	1,69	1,39	1,84
	D2	1,76	1,68	1,40	1,98
	D3	1,70	1,72	1,38	2,02
	D4	1,68	1,70	1,35	2,20
Mean (mg/kg)		1,72	1,70	1,38	2,01
Standard deviation (mg/kg)		0,03	0,01	0,02	0,13

Table A.2 *(continued)*

Extraction methods	**Centrifugation** (1 h at 3 000 rpm)	**Mechanical shaker** (5 h at 100 cycles/min)	**Sonication** (1 h)	**Mechanical shaker** (1 h at 100 cycles/min) **and centrifugation** (1 h at 3 000 rpm)
Nominal concentration (mg/kg)	2	2	2	2
Precision (%)	1,84	0,87	1,36	0,15
Accuracy (%)	86,00	85,00	69,00	100,31

Annex B
(informative)

Results of interlaboratory validation study

National Instrumentation Center for Environmental Management (NICEM) in Seoul National University organized an interlaboratory validation study for ISO 20295.

The test materials were collected from the perchlorate free site and were grounded to less than 150 µm of particle size. Each sample was prepared by adding perchlorate (ClO_4^-) standard solution to test materials at a certain concentration. Samples were homogenized with V-mixer for sufficient time.

Ten laboratories from 6 countries participated in the validation study (see Table B.1). All the participants received 3 samples (1 bottle of known sample and 2 bottles of unknown sample). Each sample was filled with about 50 g of soil (particle size < 150 µm) in brown glass bottles (see Table B.2).

For the unknown samples, the participants carried out triplicate analysis per sample. Table B.3, Table B.4, Table B.5, Figure B.1 and Figure B.2 show the summarized results of the interlaboratory validation study. Data evaluation has been performed according to ISO 5725-2.

In summary, the variation coefficient of repeatability ($C_{V,r}$) for all samples was less than 4 % and the variation coefficient of reproducibility ($C_{V,R}$) for all samples was less than 7 % (Table B.5). Thus, when it was considered that this validation study was for soil sample, repeatability and reproducibility of the analytical results were very acceptable.

Table B.1 — List of participants in this interlaboratory validation study

Country	Institute
Czech Republic	Central Institute for Supervising and Testing in Agriculture (UKZUZ)
France	The French Geological Survey (BRGM)
France	Institute National de Recherche Agronomique (INRA)
Germany	Fraunhofer Institute for Molecular Biology and Applied Ecology (IME)
Japan	Railway Technical Research Institute, Japan Railways
USA	Thermo Fisher Scientific
Korea	National Institute of Environmental Research (NIER)
Korea	Seoul National University (NICEM, Chromatography laboratory)
Korea	Seoul National University (NICEM, Chromatography laboratory)
Korea	Gachon University (Dept. of Civil and Environment engineering)

Table B.2 — List of the samples distributed to participants

Type	Sample name	Number of sample	Mass
Known sample[a]	NICE·RM-Standard 01	1 bottle	About 50 g
Unknown sample	NICE·RM-Standard 02	1 bottle	About 50 g
	NICE·RM-Standard 03	1 bottle	About 50 g

[a] Concentration: (2,61 ± 0,24) mg/kg dry mass.

Table B.3 — Summary of test results for NICE·RM-Standard 02

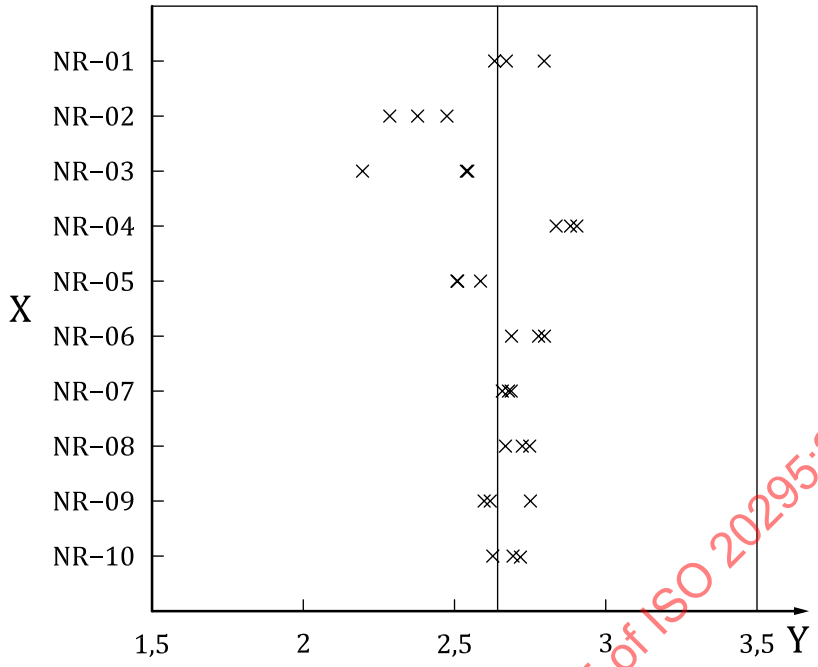
(Unit: mg/kg dry mass)

Laboratory code	Replicate determination			Average	Standard deviation	Relaltive standard deviation (%)
	1	2	3			
NR-01	2,634	2,798	2,673	2,70	0,09	3,17
NR-02	2,380	2,288	2,477	2,38	0,09	3,97
NR-03	2,541	2,546	2,198	2,43	0,20	8,22
NR-04	2,838	2,907	2,886	2,88	0,04	1,23
NR-05	2,589	2,508	2,511	2,54	0,05	1,81
NR-06	2,800	2,780	2,690	2,76	0,06	2,13
NR-07	2,680	2,688	2,659	2,68	0,01	0,56
NR-08	2,669	2,749	2,725	2,71	0,04	1,51
NR-09	2,752	2,619	2,599	2,66	0,08	3,13
NR-10	2,719	2,696	2,629	2,68	0,05	1,74

Table B.4 — Summary of test results (NICE·RM-Standard 03)

(Unit: mg/kg dry mass)

Laboratory code	Replicate determination			Average	Standard deviation	Relaltive standard deviation (%)
	1	2	3			
NR-01	4,491	4,360	4,384	**4,41**	0,07	1,58
NR-02	3,634	3,765	3,729	**3,71**	0,07	1,82
NR-03	4,075	3,914	3,767	**3,92**	0,15	3,93
NR-04	4,503	4,581	4,575	**4,55**	0,04	0,95
NR-05	4,245	4,125	4,275	**4,22**	0,08	1,88
NR-06	4,280	4,340	4,330	**4,32**	0,03	0,74
NR-07	4,176	4,287	4,330	**4,26**	0,08	1,86
NR-08	4,163	4,150	4,223	**4,18**	0,04	0,93
NR-09	4,210	4,247	4,241	**4,23**	0,02	0,47
NR-10	4,374	4,211	4,202	**4,26**	0,10	2,27

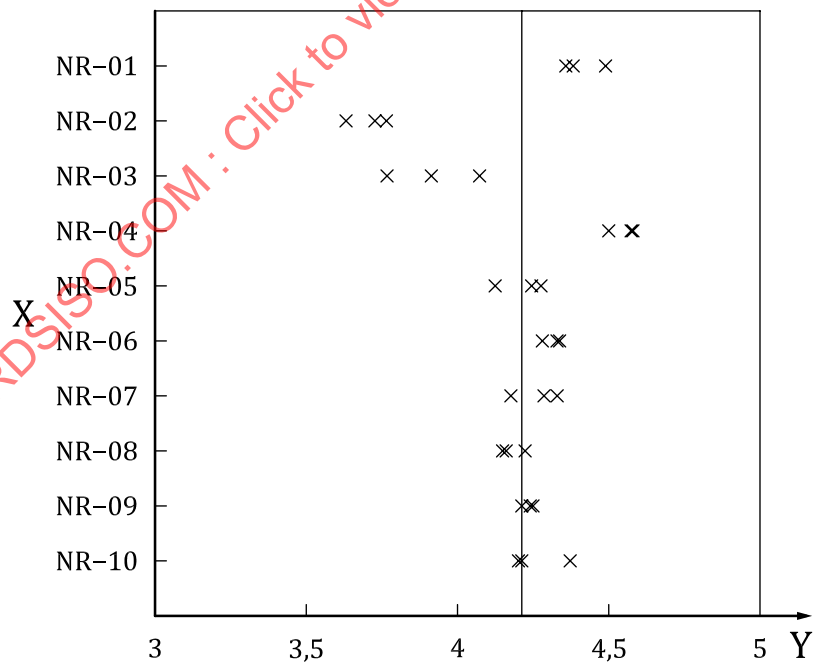


Key

X laboratory code

Y concentration (mg/kg, dry mass)

Figure B.1 — Scatter plots of test results (NICE·RM-Standard 02)



Key

X laboratory code

Y concentration (mg/kg, dry mass)

Figure B.2 — Scatter plots of test results (NICE·RM-Standard 03)

Table B.5 — Data evaluation of test results for both samples

Sample name	l	n	n_A	o %	$\overline{\overline{x}}$	s_R	$C_{V,R}$	s_r	$C_{V,r}$
NICE·RM-Standard 02	9	27	3	10,0	2,64	0,17	6,30	0,09	3,25
NICE·RM-Standard 03	10	30	0	—	4,21	0,25	5,87	0,08	1,84

Explanation of symbols

l	number of laboratories after outlier rejection
n	number of individual test results after outlier rejection
n_A	number of outliers
o	percentage of outliers (%)
$\overline{\overline{x}}$	overall mean of results(without outlier) in mg/kg dry mass
s_R	reproducibility standard deviation in mg/kg dry mass
$C_{V,R}$	coefficient of variation of reproducibility in %
s_r	repeatability standard deviation in mg/kg dry mass
$C_{V,r}$	coefficient of variation of repeatability in %

Annex C
(informative)

Elimination of dissolved sulfate, chloride, hydrogen carbonate, carbonate and metals

This modification is applicable for samples with higher loads of dissolved salts (e.g. chlorides, sulfates, carbonates) causing interference with the perchlorate determination using cation exchange SPE-cartridges. Consider applying the following when using ion exchange cartridges.

Carry out the following elution steps with a constant flow rate of between 1 ml/min to 2,0 ml/min. In addition, purge the sample with an inert gas (e.g. nitrogen or helium) to eliminate carbon dioxide (formed from carbonate and hydrogen carbonate salts), if necessary. The cartridges can be used for different samples as long as the chromatographic resolution R between perchlorate and its nearest peak does not fall below R = 1,3.

The Ba-ions of the respective resin need to be mobilized for the precipitation reaction with sulfate. This can happen either by calcium ions being present in the sample or, in case of low calcium containing sample, by a calcium chloride solution (approximately 10 g/l) added to the sample prior to the SPE-treatment. The chloride salt of the displacing calcium cation is used because the chloride counter ion can be trapped on the Ag-form resin. As long as the chromatographic resolution does not fall below R = 1,3, a calcium chloride solution shall not be added.

Prepare the sample preparation cartridges (one each being Ba-loaded, Ag-loaded and in the H-form or a cartridge combining all three resins in one housing) according to the manufacturers recommendation.

Connect the individual cartridges in order of Ba, Ag, H (Figure C.1) and rinse the cartridges with water according to the manufacturer's recommendation before use.

Spike the sample with an appropriate calcium concentration, if necessary. For example, adjust a concentration to 100 mg/l of Ca^{2+}, dissolving 1 ml of the calcium chloride solution (approximately 10 g/l) in a 100-ml-volumetric flask and dilute to volume with sample.

NOTE Ca^{2+}-concentrations below 100 mg/l can lead to an incomplete elimination of sulfate ions. It is helpful to check the Ca^{2+} content of the sample before spiking additional calcium chloride to the sample as long as the resolution R falls below 1,3.

Apply the spiked sample to the cartridge train at less than 2 ml/min. Follow the manufacturer's recommendation e.g. discard the first 3 ml for a 1,0 ml cartridge and 6 ml for a 2,5 ml cartridge.

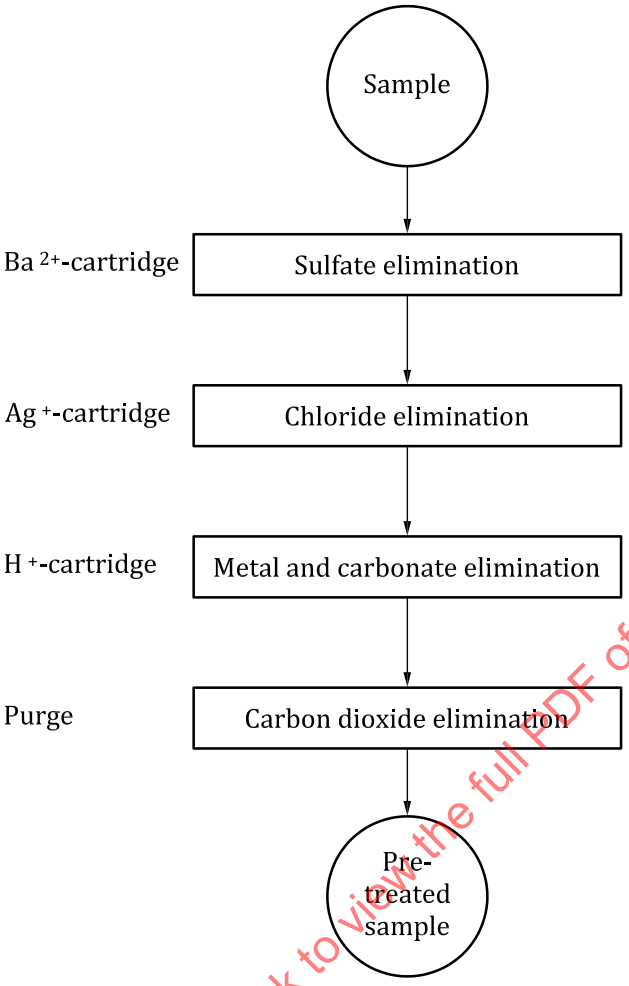
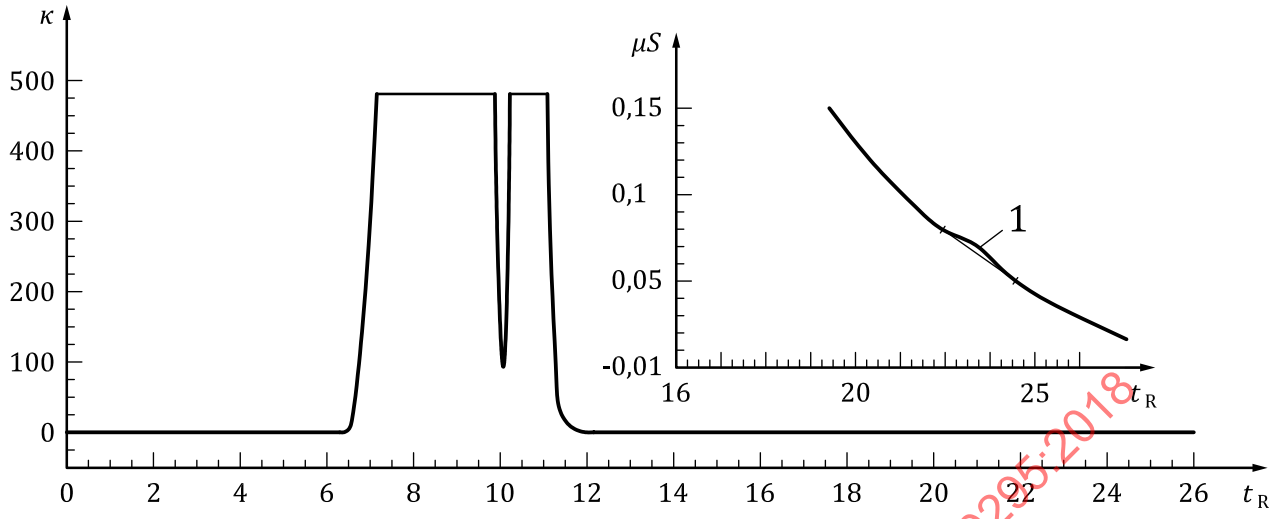


Figure C.1 — Pre-treatment steps for samples

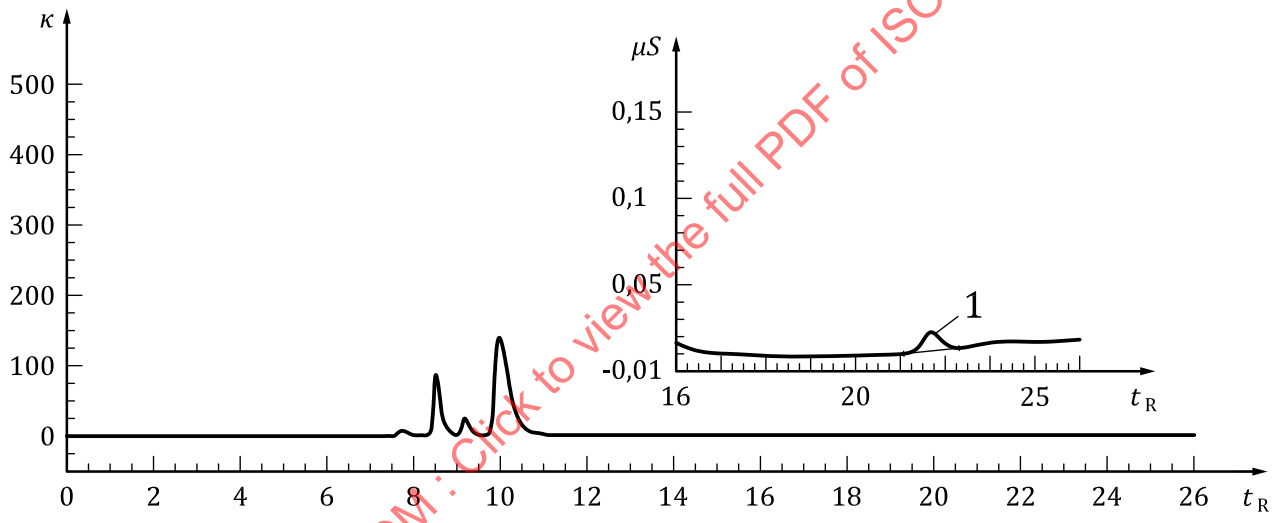
Figure C.2 gives an example for the elimination of interference derived from anions.

Conditions for the chromatogram shown in Figure C.2.

Sample:	River water, spiked with 3 µg/l ClO_4^- (matrix: 660 mg/l chloride, 260 mg/l sulfate, 14 mg/l nitrate)
Column:	Ion exchanger, 2 mm × 250 mm format
Eluent:	35 mmol/l KOH
Sample injection volume:	750 µl
Eluent flow rate:	0,25 ml/min
Detection:	Suppressed CD
Column oven temperature:	30 °C



a) Untreated trace sample



b) Treated trace sample

Key

1 perchlorate

t_R retention time, min

κ conductivity, $\mu S \cdot cm^{-1}$

Figure C.2 — Example of a chromatogram of an untreated and a treated sample

Annex D
(informative)

Determination of perchlorate using inline matrix elimination and applying re-injection analysis

This modification is applicable for samples with higher loads of dissolved salts (e.g. chlorides, sulfates, carbonates) causing interference with the determination of perchlorate. This modification utilizes inline techniques to reduce dissolved salts concentrations in the sample matrix and to collect perchlorate on a concentrator column or in a loop (Figure D.1).

Decreasing of dissolved salt concentrations can be obtained by using an inline matrix elimination step after getting a separated perchlorate from the major ions on the separator column.

Matrix elimination is achieved by diverting the volume of suppressed eluent containing perchlorate to a concentrator or loop. The volume containing the matrix ions is directed to waste.

Elute the diverted eluent fraction from the concentrator or loop to the same separator column, where perchlorate is separated from the remaining anions and detected by suppressed conductivity.

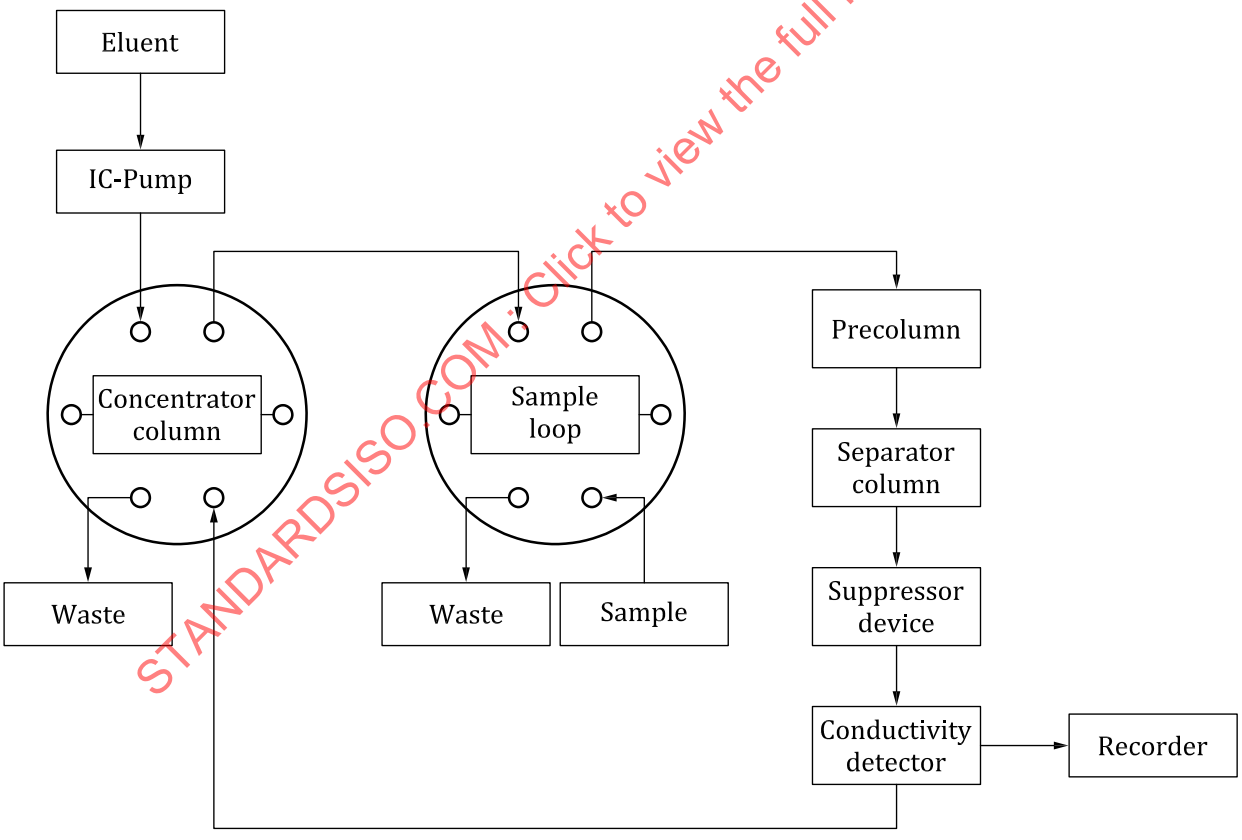


Figure D.1 — Schematic representation of an ion chromatographic system including an inline matrix elimination system and applying re-injection analysis

Figure D.2 gives an example for the separation of perchlorate after matrix elimination.

Conditions for the chromatogram shown in Figure D.2.

Sample:	Artificial ground water (matrix = 100 mg/l chloride, hydrogen carbonate, nitrate, sulfate) spiked with 2 µg/l perchlorate
Column:	Ion exchanger
Eluent:	10 mmol/l Na_2CO_3
Sample injection volume:	1 000 µl
Eluent flow rate:	0,8 ml/min
Detection:	CD
Column oven temperature:	60 °C