

BS EN ISO 12782-5:2012



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Soil quality — Parameters for geochemical modelling of leaching and speciation of constituents in soils and materials

Part 5: Extraction of humic substances from aqueous samples (ISO 12782-5:2012)

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The UK participation in its preparation was entrusted to Technical Committee EH/4, Soil quality.

A list of organizations represented on this committee can be obtained on request to its secretary.

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English Version

Soil quality - Parameters for geochemical modelling of leaching
and speciation of constituents in soils and materials - Part 5:
Extraction of humic substances from aqueous samples (ISO
12782-5:2012)

Qualité du sol - Paramètres pour la modélisation
géochimique de la lixiviation et de la spéciation des
constituants des sols et des matériaux - Partie 5: Extraction
des substances humiques des échantillons aqueux (ISO
12782-5:2012)

Bodenbeschaffenheit - Parameter zur geochemischen
Modellierung der Elution und Speziation von Bestandteilen
in Böden und Materialien - Teil 5: Extraktion von
Huminstoffen aus wässrigen Proben (ISO 12782-5:2012)

This European Standard was approved by CEN on 31 May 2012.

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COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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Foreword

This document (EN ISO 12782-5:2012) has been prepared by Technical Committee ISO/TC 190 "Soil quality" in collaboration with Technical Committee CEN/TC 345 "Characterization of soils" the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by December 2012, and conflicting national standards shall be withdrawn at the latest by December 2012.

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

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Endorsement notice

The text of ISO 12782-5:2012 has been approved by CEN as a EN ISO 12782-5:2012 without any modification.

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

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ISO 12782-5 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 7, *Soil and site assessment*.

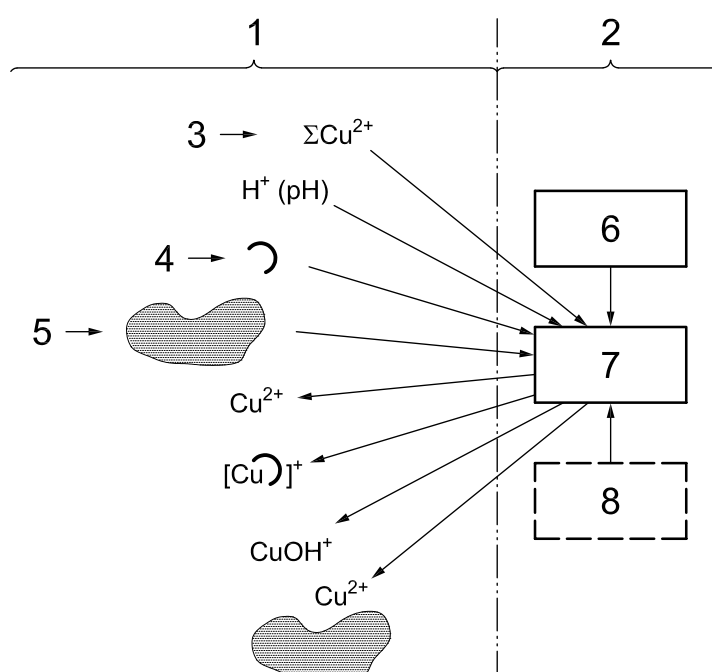
ISO 12782 consists of the following parts, under the general title *Soil quality — Parameters for geochemical modelling of leaching and speciation of constituents in soils and materials*:

- *Part 1: Extraction of amorphous iron oxides and hydroxides with ascorbic acid*
- *Part 2: Extraction of crystalline iron oxides and hydroxides with dithionite*
- *Part 3: Extraction of aluminium oxides and hydroxides with ammonium oxalate/oxalic acid*
- *Part 4: Extraction of humic substances from solid samples*
- *Part 5: Extraction of humic substances from aqueous samples*

Introduction

In addition to leaching procedures for subsequent chemical and ecotoxicological testing of soil and other materials including waste, predictive models are becoming indispensable tools in the environmental risk assessment of these materials. Models are particularly required when the results of laboratory leaching tests are to be translated to specific scenarios in the field, with regard to assessing the risks of both contaminant migration and bioavailability.

In the past few years, geochemical models have been shown to be valuable tools to be combined with the data obtained from characterization leaching standards, such as pH-dependence and percolation tests. These models have the advantage of being based on fundamental thermodynamic parameters that have a general validity. In order to enable extrapolation of laboratory leaching data to the mobility and/or bioavailability of a constituent in a specific field scenario, these models require additional input parameters for specific soil properties (see Figure 1).



Key

- 1 experiment
- 2 geochemical speciation modelling
- 3 available metal concentration
- 4 dissolved humic substances
- 5 reactive (solid) surfaces
- 6 database with stability constants
- 7 computer program
- 8 assumptions

Figure 1 — Relationships between experimental data, as obtained from laboratory leaching/extraction tests, and geochemical modelling of the speciation of a heavy metal in the environment (modified after M. Gfeller & R. Schulín, ETH, Zürich)

Characterization leaching standards provide information on the concentrations of the contaminant of interest as a function of, in particular, pH and liquid/solid (L/S) ratio. In addition, a more complete analysis of the leachates also provides information on the major ion composition and dissolved organic carbon (DOC), parameters that are particularly important for the chemical speciation of constituents through processes such as precipitation, complexation and competition for adsorption on reactive mineral and organic surfaces in the soil. As illustrated

in Figure 1, for the example of copper, geochemical modelling enables calculation of the metal distribution among these different chemical species in the system of interest. This provides necessary information for risk-assessment purposes, as these different chemical forms play distinct roles in the mobility and bioavailability of the metal in the soil. In addition to information obtained from the leaching standards (in their current state of development/definition), two additional types of information are required.

- a) The “available” (sometimes also referred to as “active” or “exchangeable”) concentration of the constituent in the solid phase, as opposed to the total concentration determined by acid destruction of the solid matrix. This “available” concentration can be obtained by leaching at low pH, a condition that can be obtained by extending the pH range in the pH-dependent leaching test (ISO/TS 21268-4) down to $\text{pH} \approx 0,5$ to $\text{pH} \approx 1$.
- b) The concentration of reactive organic and mineral surfaces in the soil, which constitute the major binding (adsorption) sites for most constituents in the soil matrix.

The major reactive surfaces that control the binding of constituents by sorption processes to the soil matrix are particulate organic matter and iron and aluminium (hydr)oxides. It is generally accepted that the reactivity of these mineral and organic surfaces can strongly vary as a function of their specific surface area/crystallinity [iron and aluminium (hydr)oxides] and composition (organic matter). When the results are intended to be used for the above-described purposes of geochemical modelling in conjunction with leaching tests, it is important that the methods be selective for reactive surfaces for which generic thermodynamic adsorption parameters are also available for the most important major and trace elements.

These reactive surfaces have been identified in soils, as well as in a variety of other materials for which the leaching of constituents is of relevance. It has been shown that the binding properties of these surfaces play a generic role in the speciation and leaching of constituents among these different materials. As an example, a similar geochemical modelling approach, using model input from the partial or complete ISO 12782 series, has been successfully applied to different soils^[4], amended soils^{[5][6]}, municipal incinerator bottom ash^[7], steel slag^{[8][9]}, bauxite residues^[10], and recycled concrete aggregate^[11]. Hence, the scope of the ISO 12782 series extends from soils to materials including soil amendments and waste materials.

This part of ISO 12782 aims to determine important reactive organic surfaces in soil and materials, for which generic thermodynamic adsorption parameters exist, i.e., humic and fulvic acids. The procedure is based on Reference [12], while generic thermodynamic adsorption parameters for humic and fulvic acids are available in References [13] and [14].

Thermodynamic parameters for adsorption models other than those used in References [13] and [14] are also available in the literature and may also be used to model the binding of constituents to humic and fulvic acids.

The method^[15] is based on a conventional isolation and purification method^[12] that is also used by the International Humic Substances Society (IHSS).

Soil quality — Parameters for geochemical modelling of leaching and speciation of constituents in soils and materials —

Part 5: Extraction of humic substances from aqueous samples

1 Scope

This part of ISO 12782 specifies a procedure to determine the concentration of humic substances in aqueous samples. These samples may be obtained as such or as eluates from leaching procedures applied to soil or other materials. Other materials also include waste. The content of humic substances can be used as input in geochemical models.

2 Normative references

The following referenced documents are indispensable for the application 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 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 5667-3, *Water quality — Sampling — Part 3: Preservation and handling of water samples*

ISO 8245, *Water quality — Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1

dissolved organic carbon

DOC

sum of organically bound carbon present in water originating from compounds (including cyanate and thiocyanate) which will pass a membrane filter of pore size 0,45 µm

3.2

humic substance

HS

(partial) decomposition product from plant and animal tissue

NOTE 1 Humic substances form a series of relatively high-molecular-weight, brown-to-black-coloured substances formed by secondary synthesis reactions.

NOTE 2 The term is used as a generic name to describe coloured material or its fractions (e.g. humic and fulvic acids) obtained on the basis of solubility characteristics.

3.3

humic acid

HA

fraction of a humic substance that is not soluble in water under acidic conditions (pH <1 to 2) but is soluble at higher pH values

NOTE Humic acids are dark brown to black in colour.

3.4
fulvic acid

FA

fraction of a humic substance that is soluble in water under all pH conditions

NOTE 1 Fulvic acids remain in solution after removal of humic acid by acidification.

NOTE 2 Fulvic acids are light yellow to yellow-brown in colour.

3.5
hydrophilic organic carbon

Hy

organic carbon compound consisting of non-humic and humic-like substances

NOTE In this part of ISO 12782, Hy is essentially regarded as the extractable organic carbon fraction that is not identified as humic acid, fulvic acid or hydrophobic neutral organic carbon in accordance with the procedure specified in Clause 8. Hydrophilic organic carbon generally consists of molecules with a lower molecular weight and higher COOH/C ratios than humic acids and fulvic acids. Examples of compounds are: oxidized carbohydrates with carboxylic acid groups, low-molecular-weight carboxylic acids, and sugar phosphates.

3.6
hydrophobic neutral organic carbon

HON

difference between the amount of adsorbed fulvic acid and hydrophilic organic carbon and the amount of desorbed fulvic acid

NOTE Hydrophobic neutral organic carbon can include non-humic and humic-like compounds.

3.7
laboratory sample

sample intended for laboratory inspection or testing

[ISO 11074:2005]

3.8
test sample

sample, prepared from the laboratory sample, from which the test portions are removed for testing or for analysis; this portion of material, resulting from the laboratory sample by means of an appropriate method of sample pretreatment, and having the size (volume/mass) necessary for the desired testing or analysis

NOTE Adapted from ISO 11074:2005.

3.9
test portion
analytical portion

quantity of material, of proper size, for measurement of the concentration or other property of interest, removed from the test sample

NOTE 1 The test portion may be taken from the primary sample or from the laboratory sample directly if no preparation of sample is required (e.g. with liquids), but usually it is taken from the prepared test sample.

NOTE 2 A unit or increment of proper homogeneity, size, and fineness, needing no further preparation, may be a test portion.

[ISO 11074:2005]

3.10
soil material

excavated soil, dredged material, manufactured soil, treated soil and fill material and other relevant materials, including soil amendments and waste materials

4 Principle

Specific dissolved organic carbon species are isolated based on defined operational conditions. Humic acids are precipitated at pH 1 and fulvic acids (and the hydrophobic organic neutral fraction) are adsorbed onto DAX-8 resin. The organics remaining in solution after resin addition are classified as hydrophilic organic substances. The DOC concentrations are measured after every step, from which the individual concentrations of humic and fulvic acids, hydrophobic organic neutrals, and hydrophilic organic substances, are calculated.

5 Apparatus

The following apparatus shall be used. All materials that come in contact with the sample (material or reagents) should not contaminate the compounds to be determined or adsorb the compounds of interest.

5.1 Balance, with an accuracy of 0,1 g.

5.2 Usual laboratory glass or plastic ware, rinsed in accordance with ISO 5667-3.

5.3 pH-meter, with a measurement accuracy of at least $\pm 0,05$ pH units.

5.4 End-over-end shaking machine (5 min^{-1} to 10 min^{-1}).

NOTE Other shaking methods can be used, provided that they can be shown to provide equivalent results.

5.5 Filtration apparatus, either a vacuum filtration device (between 2,5 kPa and 4,0 kPa) or a high-pressure filtration apparatus ($< 0,5$ MPa). Cleaning is compulsory.

5.6 Filters, pore size 20 μm , for use in the Büchner-funnel filtration device (5.7).

5.7 Büchner-funnel filtration device.

5.8 Membrane filters, for the filtration device, fabricated from inert material with a pore size of 0,45 μm . Filters shall be pre-washed with demineralized water in order to remove DOC.

5.9 Soxhlet extraction device.

5.10 Soxhlet extraction thimbles, glass-fibre extraction thimbles for Soxhlet extraction device (5.9).

5.11 Centrifuge, preferably at 3 000g. For other appropriate conditions, see Annex C.

5.12 Centrifuge bottles, e.g. polycarbonate, of capacity 250 ml, cleaned with distilled and demineralized water and diluted acid (HNO_3) before use.

5.13 Crushing equipment: jaw crusher or cutting device.

5.14 Sieving equipment, with a nominal screen size of 2 mm or 4 mm.

6 Reagents

The reagents used shall be of analytical grade and the water used shall comply with grade 3 in accordance with ISO 3696.

6.1 Demineralized water, deionized water or water of equivalent purity ($5 < \text{pH} < 7,5$) with a conductivity $< 0,5$ mS/m according to grade 3 specified in ISO 3696.

6.2 Potassium hydroxide, $c(\text{KOH}) = 0,1 \text{ mol/l}$ and 1 mol/l .

6.3 Hydrochloric acid, $c(\text{HCl}) = 0,1 \text{ mol/l}$ to 6 mol/l .

6.4 Sodium hydroxide, $c(\text{NaOH}) = 0,1 \text{ mol/l}$ to 5 mol/l .

6.5 Acetonitrile, (CH_3CN), suitable for liquid chromatography.

6.6 Methanol, (CH_3OH), suitable for liquid chromatography.

6.7 DAX-8 resin, e.g. Sigma-Aldrich¹⁾.

NOTE Various documented methods for HS isolation and purification make use of XAD-8 resin to adsorb HA and/or FA. This resin is no longer commercially available; therefore, the comparability of the substitute resin DAX-8 was tested. See Annex B for information.

6.8 Nitric acid, $c(\text{HNO}_3) = 0,1 \text{ mol/l}$.

7 Sampling

7.1 Laboratory sample

The laboratory sample shall consist of a volume to at least 50 ml.

7.2 Test sample

The samples shall be tested on filtered ($0,45 \mu\text{m}$) liquids. The test samples can be of diverse origins and should be stored in a refrigerator. To avoid changes in the samples during storage, NaN_3 (0,1 % in solution) can be added.

Samples that have been spiked with NaN_3 should be processed in a fume hood. NaN_3 decomposes under acidic conditions, releasing toxic gasses.

7.3 Test portion

Based on eluate volume requirements for analysis, the test portion size shall be 50 ml (with a tolerance of $\pm 10 \%$).

8 Procedure

8.1 Preparation of DAX-8 resin

Clean every new batch of DAX-8 resin (6.7) to remove organic impurities with five $0,1 \text{ mol/l}$ hydrochloric acid (6.3) extractions (for 24 h). Renew the solution after each extraction. Repeat this cycle with $0,1 \text{ mol/l}$ sodium hydroxide (6.4). Then, clean the resin thoroughly by Soxhlet extractions (5.9) with acetonitrile (6.5) and methanol (6.6), each for 24 h. The cleaned resin is stored in methanol (6.6) until use.

Prior to use, remove the methanol by placing the DAX-8 resin (6.7) in a Büchner funnel (5.7) with a filter (5.6) and wash the resin under vacuum with water (6.1) that has a volume 20 times that of the resin. Subsequently, rinse the resin similarly with $0,1 \text{ mol/l}$ hydrochloric acid (6.3) having 10 times the resin volume.

NOTE It has been demonstrated^[15] that 250 g of DAX-8 resin (6.7) can be cleaned sufficiently by rinsing with 2 l of water (6.1) and 1 l of $0,1 \text{ mol/l}$ hydrochloric acid (6.3). This cleaning sequence can be used to obtain a DOC-free (DOC generally $< 2 \text{ mg C/l}$) and acidic (pH 1) resin.

1) DAX-8 resin from Sigma-Aldrich is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

8.2 Determination of total humic acid (HA), fulvic acid (FA) and hydrophilic organic carbon (Hy) content in aqueous samples

Weigh the test portion (7.3) in a centrifuge bottle (5.12). Acidify the test portion (M_w) with 1 mol/l hydrochloric acid (6.3) to pH 1 to 2. Adjust the solution volume to 200 ml ($L/S = 10$) with 0,1 mol/l hydrochloric acid (6.3) and record the total volume of added hydrochloric acid (1 mol/l and 0,1 mol/l) (V_6). Close the centrifuge bottle, equilibrate the suspension by continuous shaking (5.4) for 1 h and centrifuge for 30 min at 3 000g or at appropriate centrifugation conditions as given in Annex C. Remove the supernatant (FAHyHON₁) from the residue by decantation into a 250 ml bottle (5.2) and record the water volume (V_7). Store the sample in a refrigerator until DAX-8 treatment (see below).

Neutralize the test portion that remains in the centrifuge bottle with 1 mol/l sodium hydroxide (6.4) to pH = 7,0. Add 0,1 mol/l sodium hydroxide under a N₂ atmosphere to a final volume of 200 ml ($L/S = 10$). Check that the final pH is ≥ 12 to ensure high HA solubility. If necessary, add 1 mol/l sodium hydroxide. Close the centrifuge bottle, equilibrate the suspension overnight by continuous shaking (5.4) and centrifuge the suspension for 30 min at 3 000g or at appropriate centrifugation conditions as given in Annex C. Remove the supernatant from the residue by decanting into a clean 250 ml centrifuge bottle (5.12) and record the volume of the decanted eluate (V_1).

Acidify the supernatant to precipitate HA by adding 6 mol/l hydrochloric acid (6.3) (V_2) while continuously stirring until a pH of 1,0 is reached. Allow the suspension to stand overnight and centrifuge for 30 min at 3 000g or under appropriate centrifugation conditions as given in Annex C. Remove the supernatant by decantation (V_3) into a 250 ml bottle (5.2). Store the solution (FAHyHON₂) in a refrigerator for DAX-8 treatment. Re-dissolve the HA that remains in the centrifuge bottle in 0,1 mol/l potassium hydroxide (6.2) (V_5) and analyse for DOC (DOC_{HA}).

Filter the stored solutions (FAHyHON_{1,2}) over a membrane filter (5.5, 5.8) and analyse DOC (Clause 10) ($DOC_{FAHyHON1,2}$). Transfer 50 ml ($V_{4,i}$) of the filtered solutions to (separate) 100 ml bottles (5.2) and add 10 g of moist DAX-8 (8.1) ($m_{DAX,i}$) to both samples. Equilibrate for 1 h by continuous shaking (5.4) and filter (5.6, 5.7) the suspensions. Analyse DOC (Clause 10) in both solutions ($DOC_{Hy1,2}$). In order to desorb FA, transfer the filtered resins to separate 50 ml bottles (5.2), add 20 ml of 0,1 mol/l potassium hydroxide (6.2) and equilibrate by continuous shaking for 1 h (5.4). Filter (5.6, 5.7) the suspensions and collect the filtrates in 100 ml bottles. Transfer the filtered resins to 50 ml bottles (5.2), add 20 ml of 1,0 mol/l potassium hydroxide (6.2) and repeat the desorption of FA in three additional steps (1 h each). The pH should be > 11 and can be adjusted with 1 mol/l potassium hydroxide (6.2) if necessary. Collect the four fractions of filtered potassium hydroxide in the same 100 ml bottle, record the total volume ($V_{9,10,i}$) and analyse DOC (Clause 10) ($DOC_{FA,i}$).

A schematic overview of the procedure is given in Annex A.

9 Eluate treatment and storage

Preserve the eluate sub-samples depending on the elements to be analysed and store them in accordance with the requirements in ISO 5667-3.

10 Analytical determination

Analyse the samples in accordance with ISO 8245.

11 Blank test

Perform a blank test to determine the DOC contribution from each batch of cleaned DAX-8 resin (8.1). Add 10 g of moist DAX-8 resin ($m_{DAX,BL}$) to 50 ml of 0,1 mol/l hydrochloric acid (6.3) ($V_{4,BL}$) after previous DOC analysis (Clause 10) (DOC_{BL1}). Allow the resin to settle for 5 min after 1 h of equilibration by continuous shaking (5.4), and measure DOC (Clause 10) (DOC_{BL2}).

12 Calculation

12.1 General correction factors for the calculation of humic acid (HA), fulvic acid (FA), hydrophilic organic carbon (Hy) and hydrophobic neutral organic carbon (HON) in aqueous samples

Correction factor for acid addition used in HA precipitation:

$$f_1 = \frac{(V_1 + V_2)}{V_1} \quad (1)$$

Correction factors for moisture content from DAX-8, $f_{2,i}$ can be used for aqueous samples ($i = L$) and the blank experiments ($i = BL$):

$$f_{2,i} = \frac{m_{DAX,i} \times w_{m,DAX} \times 0,01}{V_{4,i}} + 1 \quad (2)$$

DOC contribution of DAX-8 in blank (BL) experiment, in milligrams of carbon per litre (mg C/l):

$$Bl_{DAX} = (DOC_{BL2} \cdot f_{2,BL} - DOC_{BL1}) \quad (3)$$

where

- V_1 is the sample volume used for the determination of HA, FA, Hy and HON in aqueous samples, in millilitres (ml);
- V_2 is the added volume of hydrochloric acid for precipitation of HA, in millilitres (ml);
- $m_{DAX,i}$ is the wet mass of DAX-8 applied for the adsorption of FA or used in the blank experiment, in grams (g) (see the last paragraph of this subclause);
- $w_{m,DAX}$ is the moisture content of the cleaned DAX-8, as a percentage (%);
- $V_{4,i}$ is the sample volume taken into account for the DAX-8 adsorption experiment, in millilitres (ml), after removal of HA, or the amount of 0,1 mol/l hydrochloric acid used in the blank experiment (see the last paragraph of this subclause). $i = L$ for aqueous samples and $i = BL$ in the blank experiments;
- DOC_{BL1} is the DOC concentration in the 0,1 mol/l hydrochloric acid solution used in the blank experiment (mg C/l); when $DOC_{BL1} < DTL$, $DOC_{BL1} = 0$;
- DOC_{BL2} is the DOC concentration in the 0,1 mol/l hydrochloric acid in the blank experiment after 1 h of equilibration with DAX-8 (mg C/l).

For simplicity, it is recommended that both $m_{DAX,i}$ and $V_{4,i}$ be kept constant (10 g and 50 ml, respectively) in both the samples ($i = L$) and the blank experiments ($i = BL$). In this case, the factor $f_{2,i}$ will be constant in all calculations.

12.2 Concentration of total humic acid (HA), fulvic acid (FA), hydrophilic organic carbon (Hy) and hydrophobic neutral organic carbon (HON) concentrations in aqueous samples

Concentration of HA in solution, in milligrams of carbon per litre (mg C/l), corrected for DOC concentration of remaining water in HA pellet after centrifugation (containing small concentration of FA + Hy). Only apply the correction if $V_1 - V_3 > 0$.

$$HA = \frac{DOC_{HA} \times V_5}{V_1} - \frac{(V_1 - V_3) \times DOC_{FAHyHON} \times f_1}{1000} \quad (4)$$

Concentration of Hy in solution, in milligrams of carbon per litre (mg C/l):

$$Hy = DOC_{Hy} \times f_{2,L} - B_{DAX} \quad (5)$$

Concentration of FA + HON in solution, in milligrams of carbon per litre (mg C/l):

$$[FA + HON] = (DOC_{FAHyHON} \times f_1) - Hy \quad (6)$$

Concentration of DOC_{FA} after correction of blank DAX-8 contribution, in milligrams of carbon per litre (mg C/l).

Only apply if $DOC_{FA,measured,i} - DOC_{BL,DAX} > 0$, otherwise 0 is used.

$$DOC_{FA,i} = DOC_{FA,measured,i} - DOC_{BL,DAX} \quad (7)$$

Concentration of FA in solution, in milligrams of carbon per litre (mg C/l):

$$FA = \frac{\sum_{i=1}^4 DOC_{FA,i} \times V_{11,i}}{V_{4,L}} \quad (8)$$

Concentration of HON in solution, in milligrams of carbon per litre (mg C/l):

$$HON = [FA + HON] - FA \quad (9)$$

where

DOC_{HA}	is the measured DOC concentration of HA, in milligrams of carbon per litre (mg C/l);
V_5	is the added volume of potassium hydroxide to dissolve the HA fraction, in millilitres (ml);
V_3	is the volume of supernatant after acid precipitation and centrifugation, in millilitres (ml);
$V_{11,i}$	is the added volume(s) of potassium hydroxide to dissolve FA from DAX-8 in aqueous samples; volumes are registered separately ($i = 1$ to 4);
$DOC_{FAHyHON}$	is the measured DOC concentration of the sample after removal of HA, in milligrams of carbon per litre (mg C/l);
DOC_{Hy}	is the measured DOC concentration of the sample after equilibration with DAX-8, in milligrams of carbon per litre (mg C/l);
$DOC_{FA,measured,i}$	is the DOC concentration(s) in 0,1 mol/l potassium hydroxide after dissolution of FA from DAX-8 in aqueous samples, in milligrams of carbon per litre (mg C/l). DOC concentrations are registered separately ($i = 1$ to 4).

13 Expression of results

Report the concentration of HA, FA, Hy and HON in the sample, in milligrams of carbon per litre (mg C/l).

14 Test report

The test report shall include at least the following details:

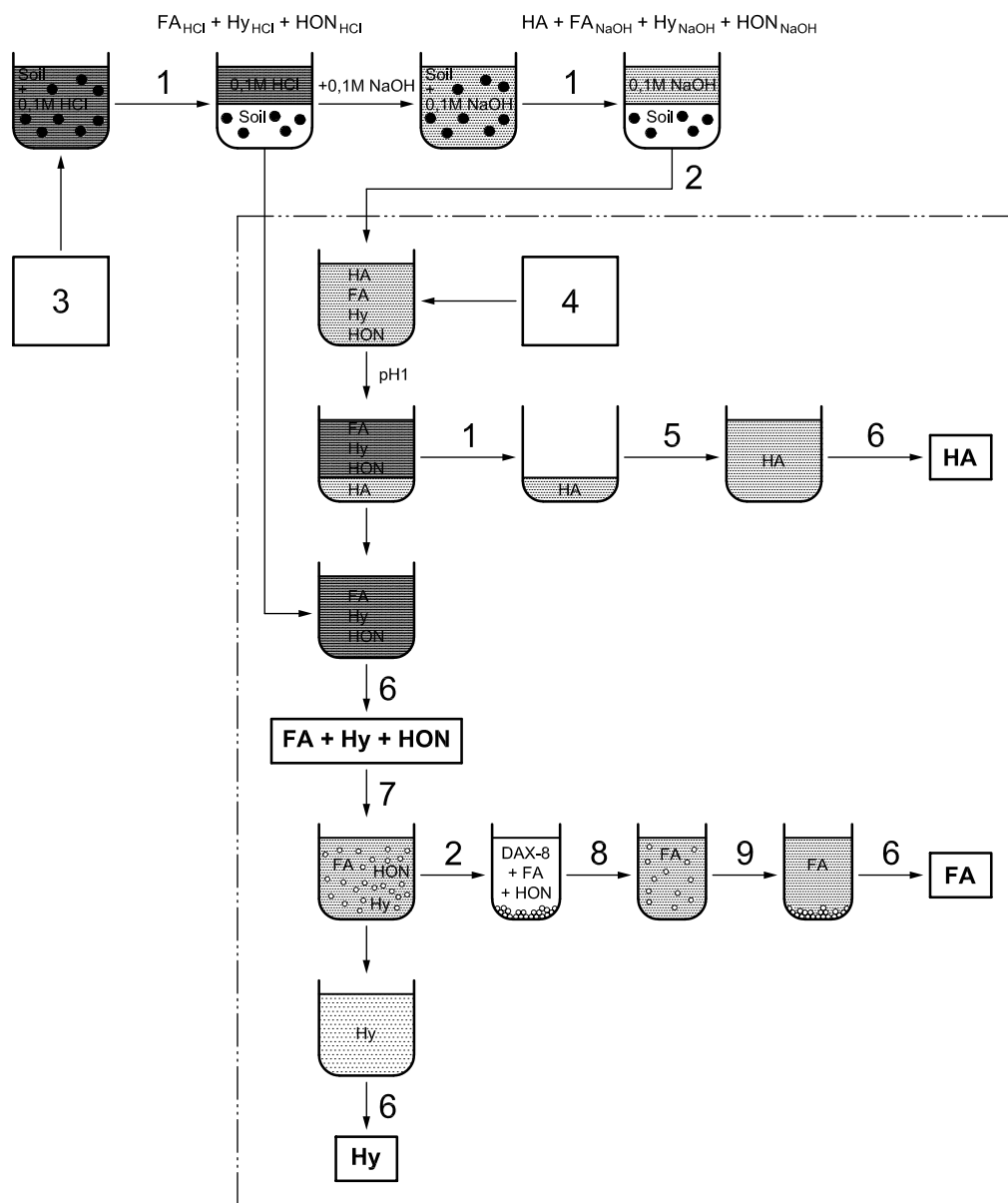
- a) a reference to this part of ISO 12782;
- b) any information necessary for the complete identification of the sample;
- c) a reference to the method used for the analytical determination, i.e. ISO 8245;
- d) the result of the determination;
- e) any details that are optional or deviations from the specifications of this part of ISO 12782, and any effects which may have affected the results.

15 Performance characteristics

The performance characteristics of the method are described in Reference [15].

Annex A
(informative)

Schematic representation of the fractionation procedure



Key

- 1 centrifugation
- 2 filtration
- 3 starting point for solid samples (see ISO 12782-4)
- 4 starting point for liquid samples
- 5 HA dissolution 0,1 M KOH
- 6 TOC analysis
- 7 DAX-8 addition, 1 h equilibration
- 8 FA dissolution 0,1 M KOH, 1 h
- 9 5 min settling

Figure A.1 — Schematic representation of the fractionation procedure

Annex B (informative)

Validation of procedure

B.1 General

The validation of the procedure specified in this part of ISO 12782 is also described in Reference [15]. Several choices made in the development of the procedure are described in this annex.

B.2 Notes on the use of cleaned DAX-8

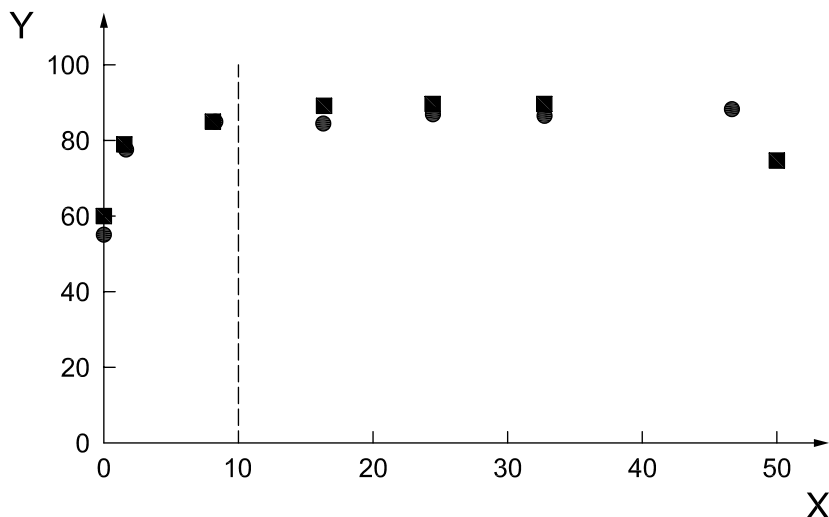
The average moisture content ($w_{m,DAX}$) of the cleaned moist resin was $(60,3 \pm 3,4) \%$ ($n = 5$). Small changes in moisture content have a limited effect on the final results, so this average value was used to correct the measured FA and Hy concentrations for the resin's water content.

After severe cleaning of DAX-8, a blank DOC concentration of about 2 mg C/l was found in the procedure. The molecular size of the residual DOC in cleaned DAX-8 was < 100 u (atomic mass units), based on high-performance size-exclusion chromatography. Therefore, it is assumed that the residual DOC originates from resin bleeding rather than from residual FA.

B.3 Preliminary investigations of DAX-8 and XAD-8 performance

The adsorption of FA on XAD-8 and DAX-8 was studied at different resin additions and the adsorption time was measured. Moreover, the reversibility of the adsorption process was measured because FA are generally identified on the basis of their adsorption and subsequent desorption from XAD-8^{[12][16][17][18]}. The results of these preliminary investigations are given in B.2 and this subclause. It should be noted that the DAX-8 resin tends to adsorb slightly greater amounts (up to 5 %) of FA as compared to XAD-8. These results are consistent with Reference [19]. These studies concluded that the XAD-8 and DAX-8 resins isolate mixtures of components with generally similar structural compositions, although the content of aliphatics within the extracted HS is slightly greater for DAX-8. It should be noted that the results in Reference [19] were based on mixtures of HA and FA, whereas this annex only focuses on the equivalence for FA sorption and desorption. It is concluded that XAD-8 and DAX-8 are equivalent with regard to the estimation of FA concentrations and that the use of this procedure with DAX-8 is compatible with the standard procedures recommended by the IHSS.

See Figures B.1 and B.2.

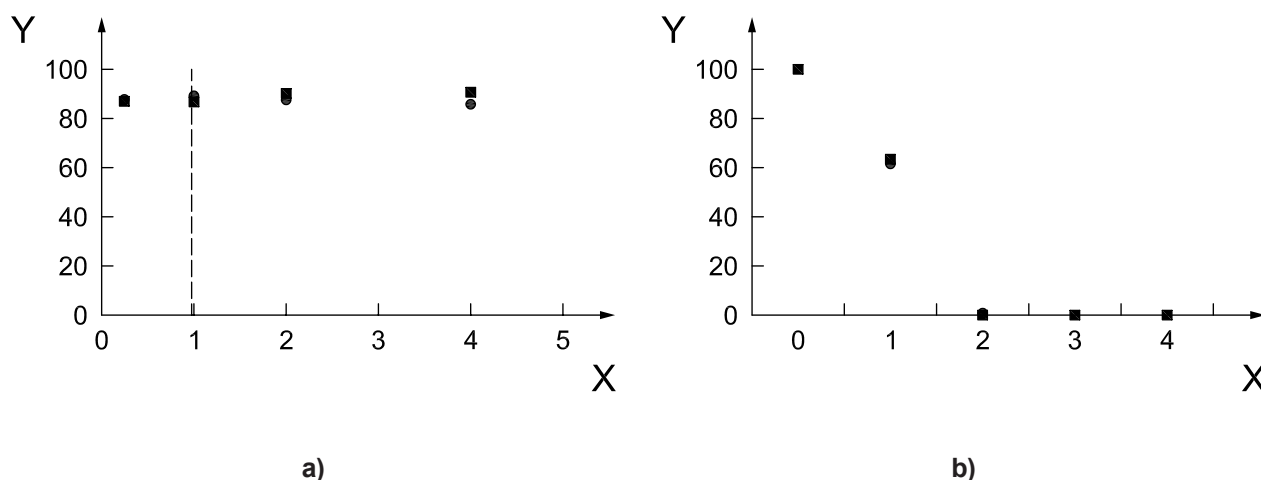


Key

X amount of moist XAD-8/DAX-8, in grams (g)
 Y FA adsorbed, as a percentage (%)

NOTE The circles represent experiments with XAD-8 resin whereas the squares show the results with DAX-8 resin. The vertical line indicates the amount of resin selected for the standard procedure.

Figure B.1 — Adsorption of Elliot soil FA (28 mg C/l) as a function of the amount of XAD-8 or DAX-8 resin (moist)



Key

X adsorption time, in hours (h)
 Y FA adsorbed, as a percentage (%)

Key

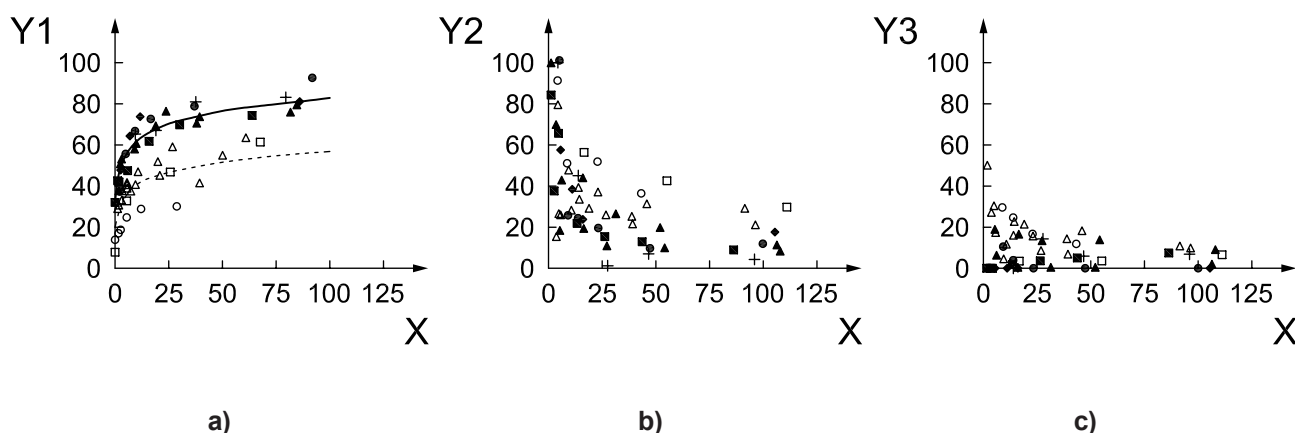
X desorption step
 Y FA adsorbed, as a percentage (%)

NOTE The experiments were performed with 10 g of moist resin for 50 ml of sample solution. The vertical line indicates the selected adsorption time for the standard procedure.

Figure B.2 — Adsorption of FA as a function of time [a]) and desorption characteristics of XAD-8 and DAX-8 [b]) for Elliot soil FA (28 mg C/l)

B.4 Concentration-dependent precipitation behaviour of HA

Figure B.3 reflects the HA recovery from precipitation as a function of the measured HA concentrations. The fitted relationships for solid and aquatic HA can be used to approximately account for the precipitation efficiency. Please note that these general relationships might not be adequate enough in specific samples.

**Key**

- X HA measured (mg C/l)
 Y1 recovery, as a percentage (%) of HA
 Y2 recovery, as a percentage (%) of FA
 Y3 recovery, as a percentage (%) of Hy

NOTE The open symbols represent isolated aquatic HA: Suwannee river (circle), Landfill leachate (square) and Zwanenwater (triangle). The solid line is the fitted curve [$y = 10,46 \ln(x) + 32,93$, $r^2 = 0,86$, $n = 3$; r^2 : coefficient of determination] based on data from peat, soil, compost and the HA derived from the landfill waste mixture; the dashed line is the fitted HA concentration dependency [$y = 8,28 \ln(x) + 12,88$, $r^2 = 0,52$, $n = 27$] based on the data from three aquatic HA samples. Graphs b) and c) show the percentage of DOC (originating from HA) measured as FA and Hy, respectively, as a function of the measured HA.

Figure B.3 — Recovery of purified HA as a function of the measured HA concentration for the peat (black square), Elliot soil (black triangle), Elliot soil after high-speed centrifugation (black diamond), compost (black circle) and the landfill waste mixture (+) solid-source materials (Graph a) (HON was not detected in these samples)

B.5 Chemical characterization of HA precipitates obtained at different concentrations

Additional experiments were performed with HA isolated from compost, to chemically characterize the fractions obtained at different concentrations in the batch method. HA fractions precipitated at five different initial concentrations (10 mg to 100 mg C/l) were again purified by dialysis, freeze-dried and chemically characterized by high-precision size-exclusion chromatography (HPSEC), ultraviolet/visible (UV/VIS) absorbance (254 nm to 665 nm) and elemental analysis (C, H, N, O). The results are shown in Tables B.1 (where ε is the molar extinction coefficient) and B.2. The HPSEC chromatograms are shown in Figure B.4. The E465/E665 ratio seems to be higher in the original sample; this property is not reflected in the other isolates. It is unclear why the absorbance of the original sample at 665 nm is relatively low in comparison with all other results, although it should be noted that very little absorption is measured at this wavelength. The other chemical properties seem to be comparable with each other, irrespective of the sample. The HPSEC chromatograms (Figure B.4) show (other than the peak height due to different concentrations) no significant differences in the apparent molecular size distribution. It is therefore concluded that these techniques reveal no significant differences between the HA fractions that were precipitated from low to high concentrations (0,5 mg to 100 mg C/l).

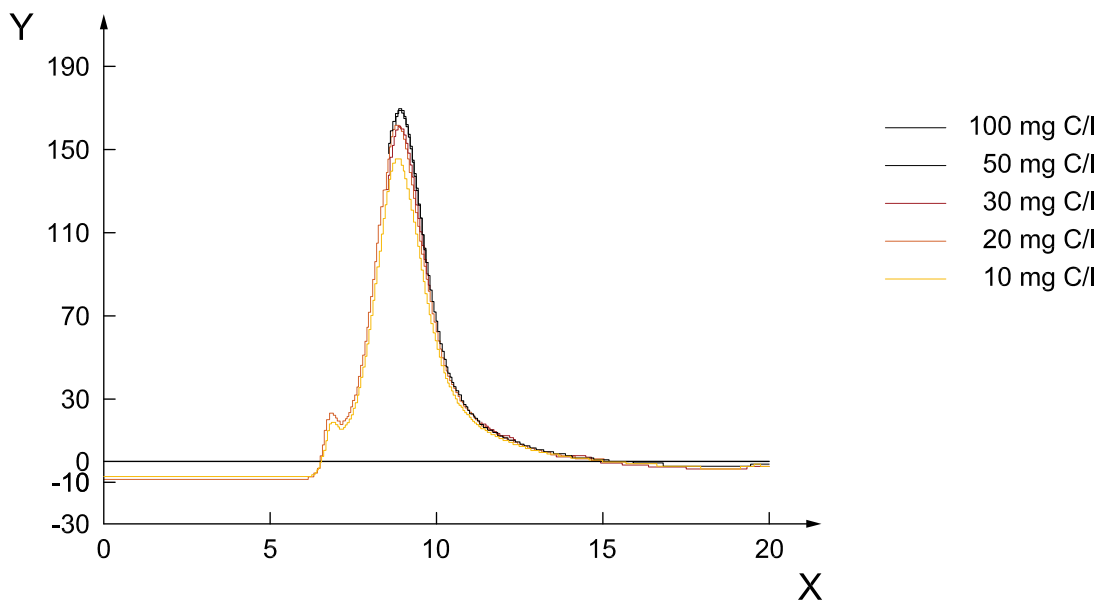
Table B.1 — UV/VIS characteristics of previously purified compost HA (original) and reprecipitated compost HA samples that were subsequently dissolved at different concentrations
(Aromaticity was calculated from the absorbance at 254 nm according to Reference [20].)

Precipitation Concentration	DOC (mg C/l)	254 nm (abs)	280 nm (abs)	300 nm (abs)	400 nm (abs)	465 nm (abs)	665 nm (abs)	E300/ E400 (\square)	E465/ E665 ratio (\square)	ϵ (254) mol/l·cm	Aromaticity %
Original	15,1	0,767	0,668	0,577	0,233	0,124	0,018	2,5	6,9	611	33,3
10 mg C/l	13,5	0,731	0,626	0,541	0,235	0,142	0,058	2,3	2,5	556	34,5
20 mg C/l	15,2	0,769	0,677	0,585	0,261	0,154	0,058	2,2	2,7	534	33,4
30 mg C/l	13,6	0,743	0,645	0,553	0,25	0,145	0,054	2,2	2,7	568	35,1
50 mg C/l	14,4	0,767	0,675	0,584	0,25	0,145	0,055	2,3	2,6	561	34,8
100 mg C/l	14,7	0,803	0,703	0,608	0,255	0,15	0,058	2,4	2,6	572	35,4

Table B.2 — Elemental analysis of previously purified compost HA (original) and reprecipitated compost HA samples that were subsequently dissolved at different concentrations. Elemental composition was determined on a dry-matter basis

(The original material was isolated and purified with the conventional isolation procedure.)

Precipitation concentration	C %	H %	N %	O %	Sum CHNO %	O/C ratio (-)
Original	55,78	4,82	7,77	27,71	96,1	0,61
10 mg C/l	53,31	4,46	6,84	29,17	93,8	0,53
20 mg C/l	54,52	4,83	7,4	29,04	95,8	0,57
30 mg C/l	53,35	4,74	7,26	20,55	85,9	0,56
50 mg C/l	53,86	4,77	7,19	27,1	92,9	0,55
100 mg C/l	54,27	4,79	7,3	27,12	93,5	0,61



Key

X time, in min

Y absorption, in mAU (milli- absorbance unit)

Figure B.4 — HPSEC chromatograms of previously purified compost HA (original) and reprecipitated compost HA samples that were subsequently dissolved at different concentrations

Annex C (informative)

Conditions regarding centrifugation

C.1 General

According to this part of ISO 12782, the first step of the solid/liquid separation shall be done by centrifugation. It is recommended centrifuging at 3 000*g* for 30 min.

However, it is possible to get the same separation efficiency at other centrifugation conditions (shorter centrifugation time at higher centrifugation speed or extended centrifugation time at lower centrifugation speed). In order to ensure reproducibility of the centrifugation, the following principles shall be considered when deviating from the recommended centrifugation procedure.

Generally the relative centrifugal force ($F_{c,r}$, in *g*) depends on the rotor speed, n (revolutions per minute, min^{-1}) and the rotor radius, r (in cm), and is calculated using Equation (C.1):

$$F_{c,r} = 0,000\ 011\ 118 \times (n)^2 \times r \quad (\text{C.1})$$

Drawing on this, each rotor has a specific k -factor that describes its pelleting efficiency. The lower the k -factor, the more efficient the pelleting will be.

This factor can be used to determine the time required for reproducible centrifugation at different rotor speeds. The k -factor can be calculated using Equation (C.2):

$$k = \frac{2,53 \times (\ln r_{\max} - \ln r_{\min})}{(n)^2} \times 10^{11} \quad (\text{C.2})$$

where

- k is the rotor-specific factor;
- r_{\max} is the maximum radius from the axis, in centimetres (cm);
- r_{\min} is the minimum radius from the axis, in centimetres (cm);
- n is the rotor speed, in revolutions per minute (min^{-1}).

To calculate the time needed at different rotor speeds to get the same centrifugation result, the k -factors for both rotor speeds shall be calculated. Using Equation (C.3), the times can be determined:

$$t_a = t_b \cdot \frac{k_a}{k_b} \quad (\text{C.3})$$

where

- k_a is the rotor-specific factor at rotor speed a;
- k_b is the rotor-specific factor at rotor speed b;
- t_a is the centrifugation time for rotor speed a, needed to achieve the same separation efficiency as for rotor speed b in the time t_b , in minutes (min);
- t_b is the centrifugation time for rotor speed b, in minutes (min).

C.2 Example for calculation

Rotor dimension: $r_{\max} = 9$ cm, $r_{\min} = 3$ cm, $\bar{r} = 6$ cm

n needed for $F_{c,r} = 3\,000g$ [see Equation (C.1)]:

$$n = \sqrt{\frac{3\,000}{0,000\,011\,18 \times 6}} = 6\,694 \text{ min}^{-1}$$

Desired n : $3\,000 \text{ min}^{-1}$

Question: $6\,694 \text{ min}^{-1}$ for 30 min correspond to $3\,000 \text{ min}^{-1}$ for x min.

Calculation of k -factors [see Equation (C.2)]:

a) $6\,694 \text{ min}^{-1}$:

$$k_{6\,694} = \frac{2,53 \times (\ln 9 - \ln 3)}{6\,694^2} \times 10^{11} = 6\,203$$

a) $3\,000 \text{ min}^{-1}$:

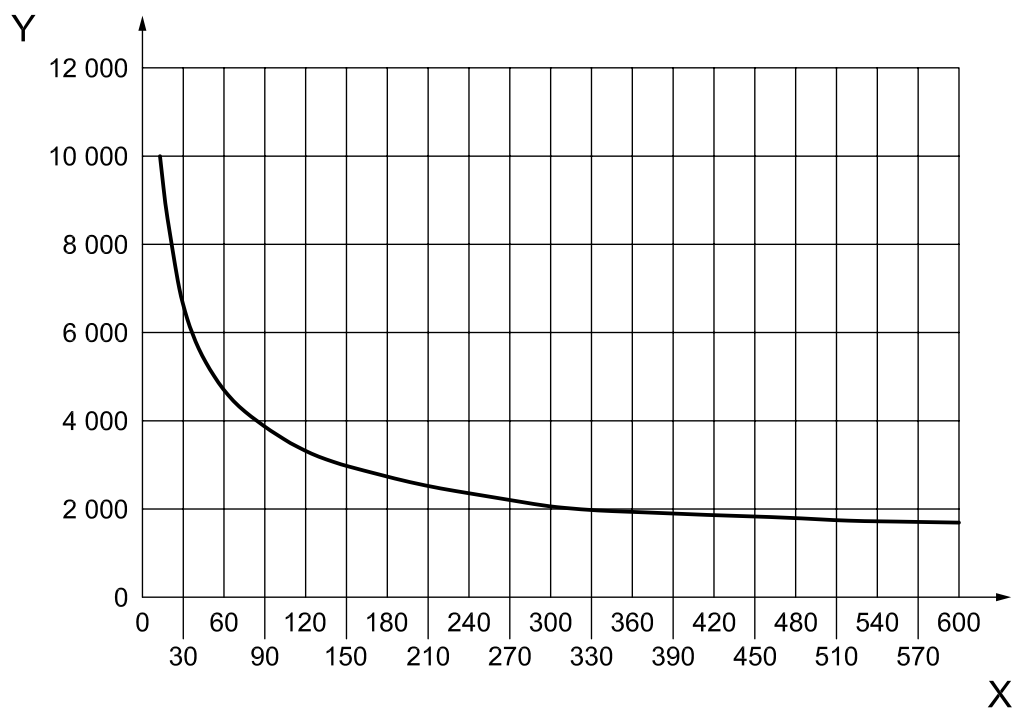
$$k_{3\,000} = \frac{2,53 \times (\ln 9 - \ln 3)}{3\,000^2} \times 10^{11} = 30\,883$$

Time needed at $3\,000 \text{ min}^{-1}$ [see Equation (C.3)]:

$$t_{3\,000} = 30 \times \frac{30\,883}{6\,203} = 149,4 \text{ min}$$

Answer: at $3\,000 \text{ min}^{-1}$ the centrifugation time shall be 149 min.

Figure C.1 shows a plot of the centrifugation time, t , versus the rotation speed, n , for the rotor dimension described above (based on $3\,000g$ for 30 min).



Key

X centrifugation time, in min

Y rotor speed, in r/min

Figure C.1 — Plot of the centrifugation time, t , versus the rotation speed, n , for the rotor dimension described above (based on 3 000g for 30 min)

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