INTERNATIONAL STANDARD

ISO 19822

First edition
2018-08

Fertilizers and soil conditioners — Determination of humic and hydrophobic fulvic acids concentrations in fertilizer materials

Engrais et amendements minéraux basique - Détermination des acides humique et fulvique

Reference number
ISO 19822:2018(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

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 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 134, *Fertilizers and soil conditioners*.

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

Humic substances are present in all ecosystems: oceans, rivers, lakes, and top soils. Quantifying the amount of humic material present in these systems is essential for academic research and commercial applications, specifically agricultural soil and plant management.

The increased use of humic substances in agriculture has generated intense interest among producers, consumers, and regulators for a reliable method for quantification of the active ingredients in raw humic ores and commercial fertilizer products; specifically humic and fulvic acids. As both commercial trade and regulation of humic products are based on percentage (%) of the humic and fulvic acids in commercial humic products, use of % units instead of SI units is warranted, therefore incorporated into this standard.

This document establishes a method for the determination of humic acids (HA) and acidic hydrophobic fulvic acids (HFA). The method is based on an existing preparative procedure use by the International Humic Substances Society (IHSS) for extracting high purity HA and HFA from soil samples[1], which is a modified form of the "classical" technique described in detail by Stevenson[2]. The "classical" methods and the IHSS method were developed as preparative methods for the fractionation of soil organic matter; they were not intended to be used as quantitative analytical methods. The classical method of extracting humic acids and *fulvic acids* from soil humus utilize a "strong base" to extract the alkaline soluble materials, and then the alkaline extract solution is acidified to flocculate the humic acids, which appear to precipitate out of solution. The remaining substances in solution after alkaline and acid treatment were called *fulvic acids*.

This method modifies the "classical" technique in a number of ways:

- it determines the quantity of humic substances on an "ash free" basis (mineral salts excluded);
- the alkali extraction is done under anoxic conditions to reduce oxidation of the analytical sample during extraction;
- it defines the materials that are soluble in both alkali and acid as the Fulvic Fraction;
- it can differentiate products containing certain non-humic materials that some manufactures claim to contain humic substances;
- it further defines HFA as materials of low sulfur content[3] that bind to a hydrophobic resin at pH 1[4][5][6], instead the classical, and perhaps more common definition, for *fulvic acids* as materials that are defined as soluble in both acid and alkali solution. This stricter definition is necessary to distinguish HFA from mineral salts, polysaccharides, amino sugars, amino acids, proteins, acids, and carbohydrates that are extracted along with humic substances when using the "classical" method[1][4].

See Annex B for information on ISO/CD 19822 interlaboratory study.

Fertilizers and soil conditioners — Determination of humic and hydrophobic fulvic acids concentrations in fertilizer materials

1 Scope

This document specifies the procedure for the analysis of humic acids and hydrophobic fulvic acids which is applicable to dry and liquid materials used as ingredients in commercial fertilizers, soil amendments, and geological deposits.

2 Normative References

There are no normative references in this document.

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 https://www.iso.org/obp

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

3.1
hydrophobic fulvic acids
HFA
materials composed of less than 0,75 % elemental sulfur (S) that are soluble in aqueous alkaline and acid solution and are adsorbed at pH 1 onto a polymeric adsorbent resin of moderate polarity. The resin is of a type designed for adsorption of amphiphilic compounds having molecular weights typical of fulvic acids

3.2
fulvic fraction
alkali extracted portions of humic substances that are soluble in both alkali and acid aqueous solutions

3.3
humic acids
HA
alkali extracted humic substances that are insoluble in strongly acidic solution and will precipitate from the alkali extract in acid solutions of pH 1

3.4
humic substance
major organic constituent of natural organic matter consisting of complex heterogeneous mixtures of carbon-based substances formed by biochemical reactions during the decay and transformation of plant and microbial remains

3.5
lignosulfonates
amorphous light to dark brown powder or liquid derived from the sulfite pulping of softwoods. The lignin framework is a sulfonated random polymer of three aromatic alcohols: coniferyl alcohol, p-coumaryl alcohol, and sinapyl alcohol, of which coniferyl alcohol is the principle unit

4 Principles

4.1 This method determines ash-free quantities of HA and HFA gravimetrically after separation from their matrix.

4.2 The method of extracting HA and HFA utilizes a strong base to extract the alkaline-soluble materials, and then, after removal of non-soluble components, the alkaline solution is acidified to flocculate the HA.

4.3 The liquid supernatant remaining after the removal of the HA is called the Fulvic Fraction. The Fulvic Fraction, which can contain Hydrophobic Fulvic Acids (HFA), is treated to determine the quantity of HFA in the Fulvic Fraction by selective adsorption onto a methacrylic-ester resin designed to separate the HFA from non-humic compounds. The material retained by the hydrophobic resin is referred to in the literature as the hydrophobic acid fraction of soluble organic matter[3].

5 Warnings

5.1 Requirements

5.1.1 Good laboratory practices

Related standards (e.g. ISO/IEC 17025) should be followed at all times in regards to personal protective equipment (safety glasses, handling strong acids, hydrochloric acid) and alkali (sodium hydroxide).

5.1.2 Moisture control

Humic and fulvic acids are hygroscopic materials; it is critical to prevent absorption of moisture during the handling of dried materials.

5.3 Lignosulfonates

Lignosulfonates will damage the hydrophobic resin. This analytical method cannot differentiate between hydrophobic fulvic acids and lignosulfonates, therefore pre-screening for the presence of lignosulfonates is recommended for liquid products of unknown origin.

See Annex A.

5.4 Temperature control

Do not exceed 65 °C when drying the humic and fulvic analytes. The analytes are subject to decomposition at higher temperatures.

6 Reagents

6.1 Sodium hydroxide solution, 0,1 M, dilute 3,99 g of 99,99 % purity NaOH in 1 l of deionized water.

6.2 Sodium hydroxide solution, 0,5 M, dilute 19,99 g of 99,99 % purity NaOH in 1 l of deionized water.

6.3 Hydrochloric acid solution, 6 M, dilute 12 M HCl with an equal part of deionized water.

6.4 Hydrochloric acid solution, 1 M, dilute 83,3 ml of 12 M HCl with 1 l of deionized water.

6.5 Hydrochloric acid solution, 0,1 M, perform a 1:10 dilution using 1 M HCl prepared in 6.4 with final volume of 1 l using deionized water.

6.6 Nitrogen gas (UN1066) 99,9 % purity.

6.7 Methacrylic-ester resin, 40–60 mesh, approximately 0,79 ml·g^{-1} pore volume, 225 Å mean pore size, 160 m^2·g^{-1} surface area, for adsorption of materials up to 150 000 MW, e.g. Supelite DAX-8 Resin, or any other available resin meeting equivalent properties.

6.8 Amberlite IR-120 strong cation exchange resin, hydrogen form.

6.9 Deionized water[8].

6.10 Acetone.

7 Apparatus

7.1 Analytical balance with draft guard: capacity 210 g, with readability to ±0,000 1 g.

7.2 Drying oven, capable of 120 °C, precision ±3 °C.

7.3 Centrifuge, minimum relative centrifugal force 1 500 × g, capable of 3 900 × g.

7.4 4 ml to 50 ml or 250 ml polyethylene or HDPE centrifuge tubes, or heavy duty high temperature resistant centrifuge tubes capable of 600 °C (for example; Kimble-Chase, catalog number 45212-50 KIMAX).

7.5 4 ml to 100 ml wide-form crucibles (for example: Fisher Scientific catalog number FB-965-M).

7.6 Rotary evaporator 400 ml capacity.

7.7 Magnetic stir plates and 5 cm to 7 cm long magnetic stir bars.

7.8 pH meter and electrode.

7.9 Electrical conductivity meter with probe having a calibrated cell constant of approximately one, as determined using standard protocols.

7.10 Spectrophotometer, capable of measuring ±0,005 absorbance units at 350 nm.

7.11 Peristaltic pump with a minimum flow rate of 1,2 ml·min^{-1} and tubing.

7.12 Muffle furnace.

7.13 Rotating shaking mixer.

7.14 Desiccator with silica gel (or its equivalent) as desiccant.

7.15 Erlenmeyer flask, 1 000 ml.

7.16 Beaker, 4 l.

7.17 Graduated cylinder, 1 000 ml.

7.18 Glass chromatography column, 4 cm × 25 cm for DAX-8 resin.

7.19 Glass chromatography column, 5 cm × 60 cm for IR120 H+ exchange resin.

7.20 Ceramic mortar and pestle.

7.21 Sieve, 74 µm (#200 US mesh).

7.22 Parafilm®[1].

8 Preparing crucibles, drying, and weighing samples

8.1 Preparing crucibles

8.1.1 If using new crucibles, first wash them with acetone and then dry them in an oven at 105 °C for 2 hours.

8.1.2 Prepare previously used crucibles by washing in acetone, then firing them in a muffle furnace at 500 °C for 2 hours. Cool the crucibles in a desiccator to room temperature. Remove from desiccator when cool, record weight of the crucibles to four decimal places.

8.2 Drying and weighing solid analytical samples

8.2.1 If the analytical sample is a solid material, crush and screen approximately 5 g of the analytical sample to ≤75 µm making sure that the powder becomes well homogenized.

8.2.2 Transfer approximately 5 g analytical sample to a 100 ml crucible prepared according to 8.1.

8.2.3 Place the analytical sample in a drying oven for 24 h at a temperature of 62 ± 3 °C. (do not exceed 65 °C). If any clumping of the sample occurs during drying, break up the clumps with a glass rod. Continue drying until the sample is dried to a constant weight. This can take up to 24 h;

8.2.4 After achieving constant weight, remove the analytical sample from the drying oven and immediately place in a desiccator to cool.

NOTE Both humic and fulvic acids are hygroscopic materials, it is critical to prevent absorption of moisture during the handling of these materials.

8.2.5 Weigh out approximately 2,5 g test portion from the dried analytical sample in a pre-weighed crucible prepared according to 8.1, taking precautions to prevent moisture adsorption while handling. Record the test portion + crucible weight to four decimal places. Proceed to 9.1 immediately or return the crucible with test portion to the desiccator.

8.2.6 Determine the weight of the test portion by subtracting the weight of the crucible from the test portion + crucible; record the result as the Test Portion Dried Weight.

---

[1] Parafilm® is a trademark of Bemis NA, Neenah, Wisconsin, USA. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results. It is commonly used for sealing or protecting vessels. It is a ductile, malleable, waterproof, odorless, translucent and cohesive thermoplastic.

8.3 Drying and weighing liquid samples

8.3.1 For liquid analytical samples, thoroughly homogenize the analytical sample by shaking the sample for one minute in the container in which the liquid was delivered. Weigh out approximately 5 g test portion from the liquid analytical sample to four decimal places. Record this as the Liquid Test Portion Weight.

For liquid analytical samples with an expected HFA content <1 %, use a test portion weight of 10 g.

8.3.2 Follow steps 8.2.2 to 8.2.6 for drying the sample and weighing the test portion of the sample.

9 Extraction procedure

9.1 From this point on, the method is the same for both solid and liquid samples.

Transfer the prepared test portion of the analytical sample to a 1 l Erlenmeyer flask containing a 5 cm to 7 cm long magnetic stir bar. Add 0,1 M NaOH, constantly stirring on a stir plate, to a final volume of 1 l. Evacuate the head space with N_2 and cover with parafilm (or seal the flask with a similar material). Then mix vigorously on a stir plate (e.g. 300 rpm to 400 rpm). Stir liquid samples for 1 hour; stir the solid samples for 16 hours to 18 hours.

NOTE For solid samples, it can be convenient to perform this step late in the day so the solid samples can be stirred overnight.

9.1.1 After stirring, remove the flask from the stir plate, transfer to suitable centrifuge tubes and centrifuge the entire volume at 3 900 × g for 30 minutes to separate any insoluble material from the dissolved materials in the alkaline extract. Carefully transfer the alkaline extract to a clean 1 l Erlenmeyer flask containing a magnetic stir bar. Discard the insoluble materials.

9.1.2 While gently stirring the solution, adjust the pH of the alkaline extract solution to flocculate the HA from the acid soluble materials by adding 6 M HCl (1:1) drop-wise to the alkaline extract, until 1,0 ± 0,1 pH is reached.

9.1.3 Cover the flask with parafilm and mix for 1 h. After 1 hour, check the pH and readjust to pH 1,0 ± 0,05 with additional 6 M HCl, if necessary. If the pH falls below 0,95, adjust the pH back to 1,0 ± 0,05 with 0,5 M NaOH solution. Continue mixing the acidified extract until it stabilizes at pH 1,0 ± 0,05 for exactly 5 minutes. Do not let the acidified extract sit for longer than 5 minutes after the pH stabilizes. Remove the pH electrode.

9.2 Separation of HA

9.2.1 Once the pH is stable, remove the flask from the mixer, and cover the flask with parafilm. Allow the pH-adjusted extract to sit undisturbed for 4 hours ±5 minutes (do not exceed 4 hours). This stage is critical to prevent further partitioning of the HA and HFA constituents. The flocculated HA will drop out of solution.

9.2.2 Immediately centrifuge the extract solution at 3 900 × g for 30 minutes using pre-weighed 50 ml centrifuge tubes to recover the flocculated humic acids portion. Decant the supernatant (Fulvic Fraction), being careful not to include any of the flocculated HA. Typically, about 500 ml of the clarified extract can be decanted without disturbing the flocculated HA. If hydrophobic fulvic acids (HFA) analysis is to be performed, decant the fulvic fraction into a clean 1 l Erlenmeyer flask. [Alternatively: using heavy duty high temperature centrifuge tubes instead of plastic centrifuge tubes eliminates the need to transfer the flocculated HA to a crucible, decreasing labor and increasing precision.]

9.2.3 Centrifuge the tubes containing the flocculated HA again at 1,500 × g for 20 minutes to 30 minutes to further separate the flocculated HA that appears to precipitate from the liquid fulvic fraction. If HFA analysis is intended, add this supernatant to the decanted fulvic fraction supernatant in 9.2.2.

9.2.4 If using high temperature centrifuge tubes, place the tubes containing the flocculated HA in a drying oven at 62 ± 3 °C. If using plastic or low temperature centrifuge tubes, carefully scrape all of the flocculated HA from the centrifuge tubes into 100 ml wide-form crucible(s) prepared according to 8.1. After scraping out the flocculated HA, add a minimal amount of deionized water[8] to the centrifuge tube, secure the caps on the tubes, shake the capped tubes vigorously to get all the flocculated HA out of the tube, adding this deionized water/flocculated HA mixture to the crucible(s). Regardless of using either heavy duty tubes or crucibles, dry the flocculate HA to constant weight (typically overnight) in an oven at 62 ± 3 °C. Break up any clumps that form during drying using a glass rod, taking care to avoid removal of any material from the tubes. Depending on the source of the HA, the drying process can take up to 24 hours.

NOTE The high temperature centrifuge tubes can be supported in the furnace using 50 ml beakers.

9.3.2 Once the flocculated HA is dried to constant weight, remove the tubes/crucibles from the drying oven and immediately place them in a desiccator to cool. Once cooled to room temperature in the desiccator, reweigh the tubes/crucibles containing the flocculated HA. Take precautions to reduce adsorption of moisture during this step. Record the combined weight of the dried flocculated HA + tubes/crucibles, subtract the weight of the tubes/crucibles; record this weight as the Dried Flocculated HA Weight.

10 Determination of ash content

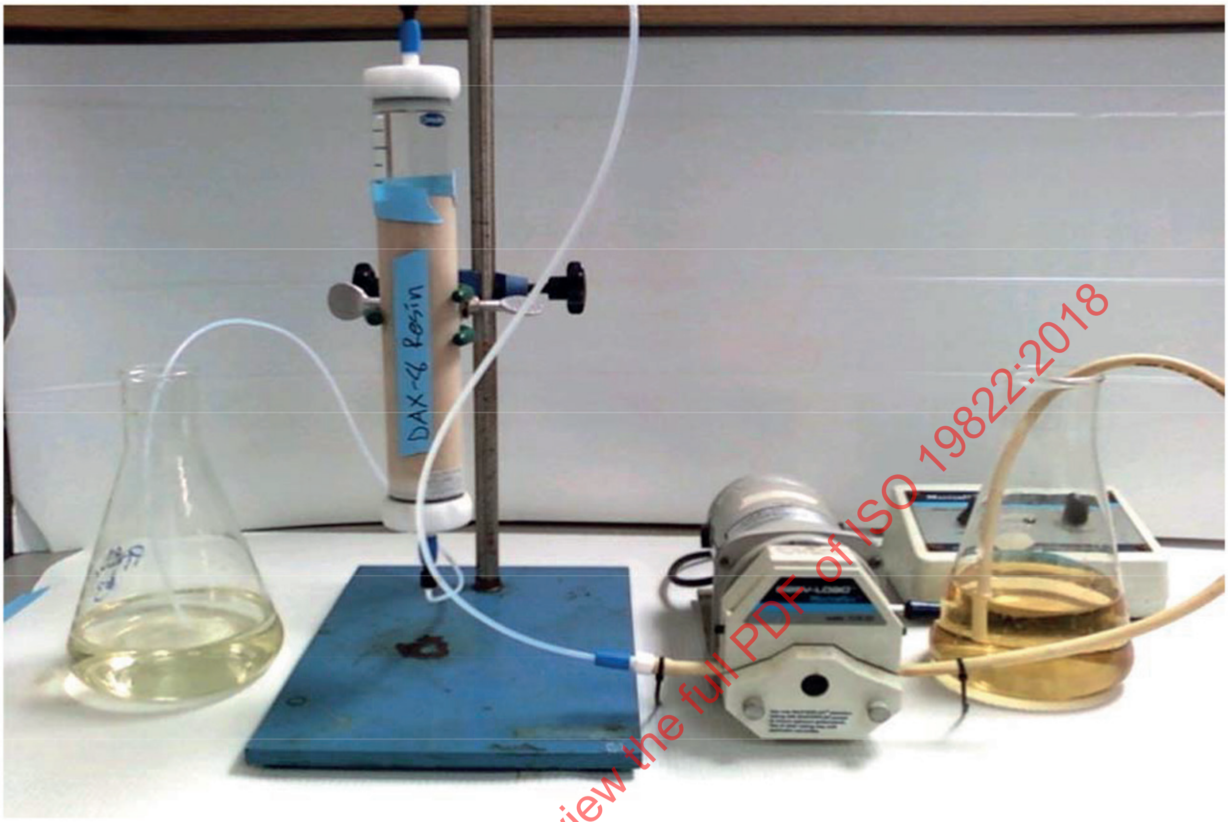
10.1 At this stage, the dried flocculated HA contains some residual ash. The ash content is determined by combusting the dried flocculated HA in either heavy duty centrifuge tubes or crucibles (prepared according to 8.1) in a muffle furnace for 4 h at 500 °C. Wait until weight is constant. If any solid clumps form during ashing, carefully break up the clumps using a glass rod. Do not use a metal rod.

10.2 After achieving constant weight, remove the tubes/crucibles containing the flocculated HA from the muffle furnace and place them in a desiccator to cool to room temperature.

10.3 Once cool, determine the weight of the dried ash by subtracting the weight of the tube/crucible from the combined weight of the tube/crucible + ash. Record the result as Flocculated HA Ash Weight.

11 Separation of Hydrophobic Fulvic Acids (HFA)

11.1 Hydrophobic fulvic acids (HFA) are separated from other acid soluble substances in the Fulvic Fraction by selective adsorption to an acidified hydrophobic resin (i.e. Supelite DAX-8), to which hydrophilic acid soluble components do not bind and can thus be removed. This is accomplished using a 4 cm × 25 cm glass chromatography column partially filled with 280 ml of resin soaked in deionized water[8] to prevent air pockets. Maintain an optimum space of approximately 2,5 cm from the top of the wet resin to the top of the column (see Figure 1). The intent is to leave sufficient space above the resin to visually monitor the flow rate through the column. If using new resin, clean the resin prior to use according to 14.1. After using the chromatography column resin, regeneration of the column resin is performed according to 14.1.2. When finished using the resin at the end of the day, store in methanol. Before reusing the resin, rinse the methanol from the resin with deionized water[8] by using a slurry technique in a large beaker until the resin is free of methanol (less than 2 $\mathrm{mg \cdot l^{-1}}$ dissolved organic carbon). If the resin becomes discolored, or has been used about 15 times, perform a quality control test using a quality control material. If necessary, clean the resin according to 14.1 or discard and replace with new resin.



NOTE The fulvic fraction is in the Erlenmeyer flask on the right side of the photo.

Figure 1 — Example of apparatus used to separate hydrophobic fulvic acids from the fulvic fraction

11.2 The Fulvic Fraction solution is passed through the column via the top of the column using a peristaltic pump set at just enough pressure so that the flow rate is sufficient to completely cover the resin in the column without overflowing the column or allowing the resin to be exposed to air. The suggested flow rate is about 4 ml·min^{-1} to 5 ml·min^{-1}·. Do not allow the solution level to drop below the top of the resin. Discard this effluent.

11.3 The column is then washed with deionized water[8] using a peristaltic pump with enough pressure so that the flow rate (about 4 ml·min^{-1} to 5 ml·min^{-1}) is sufficient to maintain complete coverage of resin. Continue washing until the UV absorbance A = 0,015 (350 nm) or after eluting 2 column volumes, whichever occurs first. Do not exceed 2 column volumes. Discard the effluent.

11.4 The HFA is then desorbed from the resin by back elution (i.e. influent introduced into the bottom of column) with 0,1 M NaOH, which is pumped using the peristaltic pump (flow rate about 4 ml·min^{-1} to 5 ml·min^{-1}). Capture the HFA-containing effluent. The HFA has been desorbed when the absorbance of the effluent is A = 0,030 (350 nm) using a spectrophotometer or after eluting 3 column volumes, whichever occurs first. Do not exceed 3 column volumes.

12 Hydrogen ion exchange

12.1 Using a *hydrogen form* H^+ exchange resin prepared according to 14.2. Pour 500 ml of the prepared H^+ exchange resin into a 5 cm × 50 cm chromatography column according to 14.2.1. Pump the HFA-containing solution into the top of the column allowing it to flow through the column by gravity only. Repeat this step for a total of 2 passes through the column. This step protonates the HFA by exchanging Na^+ on the HFA with H^+.

12.2 Wash the column with 500 ml of deionized water[8]. This wash water effluent is added to the protonated HFA solution from 12.1.

12.3 Concentrate the HFA to a volume of approximately 50 ml to 60 ml by using a rotary evaporator set at 65 °C. Do not exceed 65 °C.

12.4 Dry the HFA concentrate using a crucible that has been prepared according to 8.1. Record the weight of the crucible. Transfer the remaining HFA concentrate to the prepared crucible. Dry the HFA concentrate at 62 ± 3 °C to constant weight in a drying oven, breaking up any clumps that have formed. Do not exceed 65 °C.

12.5 After drying to constant weight, place the crucible with HFA concentrate in a desiccator to cool to room temperature. Record the total weight of the crucible + dried HFA concentrate. Subtract the weight of the crucible from the total weight; this is the Weight of Dried HFA.

12.6 Determine the residual ash content of HFA. The procedure for determining ash content is the same as that described in Clause 10 for HA, using either a prepared crucible or heavy duty centrifuge tubes.

12.7 Calculate % of HFA analyte using the calculations in 13.2 to 13.4.

13 Calculations

13.1 Determination of ash-free HA weight

% Ash = (Flocculated HA ash weight ÷ Dried flocculated HA weight) × 100

Weight of ash-free HA = Dried flocculated HA weight × (1 − % Ash)

13.2 Determination of ash-free HFA weight

% Ash = (HFA ash weight ÷ Dried HFA weight) × 100

Weight of ash-free HFA = Dried HFA weight × (1 − % Ash)

13.3 Determination of % analyte in solid analytical samples

% Analyte in a solid sample = (Weight of ash-free analyte ÷ Test portion dried weight) × 100

13.4 Determination of % analyte in liquid samples

% Analyte = (Weight of ash-free analyte ÷ Liquid test portion weight) × 100

14 Resin regeneration and column preparation

14.1 General

14.1.1 Preparation of chromatography resin

It is paramount that this preparation precedes the use of a new resin or discoloured used resin. Soxhlet-extract sequentially for 8 hours with methanol, diethyl ether, acetonitrile, and methanol at 2 hour intervals. If the resin will not be used immediately after preparation, store it in methanol.

14.1.2 Chromatography column preparation

Before column packing, rinse the methanol from the resin with deionized water[8] using a slurry technique in a large beaker until free of methanol (less than 2 mg·l^{-1} dissolved organic carbon). After packing 280 ml of the resin into a column, rinse 3 times, alternating from 0,1 N NaOH to 0,1 N HC1; this removes impurities which could otherwise be incorporated into the resin.

14.1.3 Chromatography Resin regeneration

Regeneration is accomplished by using a 4 cm × 25 cm chromatography column containing 280 ml of resin, rinse the resin in the column by pumping 2 column volumes of deionized water[8] with a peristaltic pump into the top of the column, allowing the water to flow down by gravity only. Then pass 1 column volume of 0,1 M HCl through the bottom of the column.

14.2 Regeneration of hydrogen form H$^+$ exchange resin

14.2.1 The *hydrogen form* H$^+$ exchange resin is regenerated using a batch process by pouring a minimum of 500 ml of the resin into a 4 l beaker, pour off any liquid if present, cover the resin with 1 M HCl. Let it stand for a minimum of 30 minutes, stirring once every 5 min. Remove the excess HCl from the resin by pouring off the acid and covering the resin with deionized water[8]. Stir vigorously with a stirring rod for 15 seconds, then let the resin and rinse water stand for 5 minutes. Pour off the water, cover the resin again with deionized water[8] and stir vigorously.

14.2.2 Load 500 ml of the regenerated H$^+$ exchange resin into a 5 cm × 50 cm column. Once loaded, wash with deionized water[8] until the rinse water tests negative for the presence of Cl$^-$ using AgNO$_3$.

Annex A
(informative)

Procedure to confirm the presence lignosulfonates

A.1 General

The materials extracted from humified substances that are both alkali and acid soluble meet the classical definition for *fulvic acids*. However, this document defines the alkali and acid soluble materials as the *fulvic fraction* because there are numerous substances that fit that generalized operational definition. The *fulvic fraction* possibly will or possibly will not contain the analyte of interest; *hydrophobic fulvic acids*. As some materials in the market place are represented as *fulvic acids*, especially lignosulfonates, pre-screening for the presence of lignosulfonates might be necessary.

CAUTION — The hydrophobic absorbent resin used in this document is not compatible with lignosulfonates. Lignosulfonates do not quantitatively desorb from the resin and can permanently damage the resin.

A.1.1 Humic substances are either odorless or have a slight petroleum-like smell. Lignosulfonates have a distinctive sulfurous odor because they are derived from wood lignins treated with sulfites. As organoleptic inspection is not conclusive, pre-screening for lignosulfonates is accomplished initially by analyzing for elemental sulfur(S).

A.2 Sulfur concentration

Total sulfur concentration in lignosulfonates is typically ≈5 %. As the concentration of sulfur in humic substances is typically <1 %, with an average of ≈0,6 %. Any product that exceeds 0,75 % total elemental sulfur (S) can be tested by Fourier Transform Infrared Spectroscopy (FTIR) analysis to confirm the absence or presence of lignosulfonates.

A.3 FTIR analysis

Fourier Transform Infrared Spectroscopy (FTIR) is based on the principle that absorption of infrared light causes chemical bonds to bend and stretch. Mid-FTIR records the energy released as wavenumbers between 4 000 cm^{-1} and 400 cm^{-1}. The observed absorption peaks of the infrared spectrum of a substance correspond to the frequencies of vibrations between the bonds of the atoms making up a material. As no two compounds produce the same exact infrared spectra, FTIR analysis is commonly used to positively identify substances. Numerous commercial laboratories offer FTIR spectral analysis and will provide interpretation reports.

A.3.1 Spectra of sulfonate bonds

The sulfur-oxygen bonds in lignosulfonates demonstrate characteristic symmetric stretching of sulfoxide single S=O bonds at 1 030 cm^{-1} to 1 041 cm^{-1}, symmetric stretching of 0=S=O at 1 150 cm^{-1} to 1 200 cm^{-1}, and asymmetric stretching of O=S=O sulfonate bonds at approximately 1 330 cm^{-1} to 1 430 cm^{-1}. The presence of lignosulfonates is difficult to detect by Mid-FTIR at concentrations <500 mg·kg^{-1} in complex mixtures. However, lignosulfonates lack the distinct sharp peak of C=O stretch of aliphatic carboxylic acids at 1 700 cm^{-1} to 1 730 cm^{-1}, and the slightly weaker peak of C=O stretching at 1 680 cm^{-1} to 1 710 cm^{-1} of aromatic carboxylic acids typically associated with carboxylic acids. Therefore, the absence of these peaks can be used to rule out the presence of hydrophobic fulvic acids rather than confirm the presence of lignosulfonates. See A.3.4 for FTIR spectra of lignosulfonates.

A.3.2 Spectra of fulvic acids

Both humic acids and hydrophobic fulvic acids display similar spectral bands, with strong double peaks at approximately 1 620 cm^{-1} and 1 720 cm^{-1}. Wavenumbers in the range of 1 600 cm^{-1} to 1 710 cm^{-1} correspond to aromatic C=O stretching, and 1 700 cm^{-1} to 1 730 cm^{-1} are from aliphatic C=O stretching. The wave number at around 1 720 cm^{-1} is much stronger in fulvic acids than humic acids because of the occurrence of more –COOH groups. These bands are either very weak or absent in spectra for lignosulfonates. Typically there are two weaker but distinct peaks at about 1 220 cm^{-1} and 1 400 cm^{-1} generated by C-O stretching (1 210 cm^{-1} to 1 320 cm^{-1}) and O-H plane bending (1 395 cm^{-1} to 1 440 cm^{-1}) for carboxylic acids. See A.3.5 for spectra of hydrophobic fulvic acids.

A.3.3 Specifications for FTIR analysis

Sample media: Water[8]

Spectral wavenumber Range: Mid-IR (4 500 cm^{-1} to 600 cm^{-1})

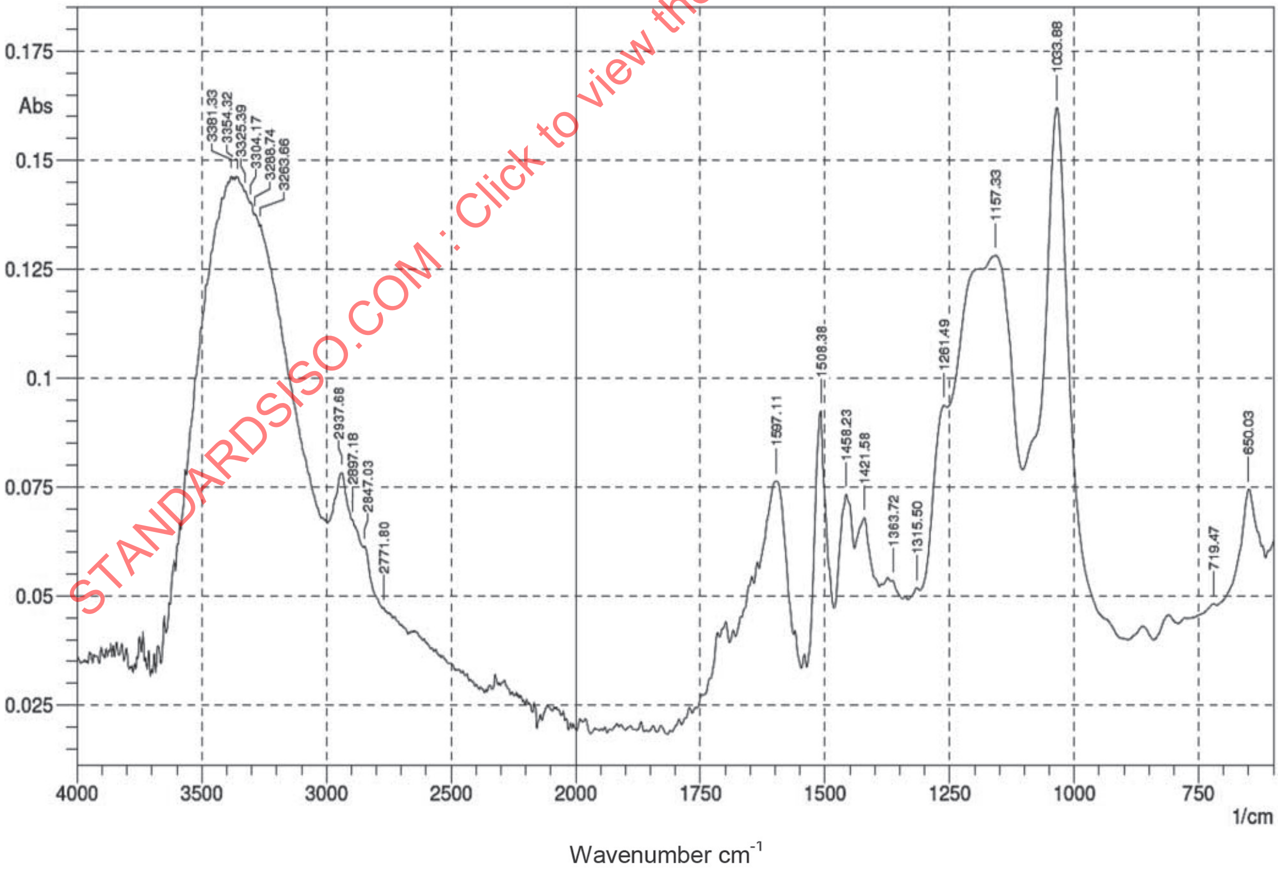
Apodization: Happ-Genzel

Resolution: 4 cm^{-1}

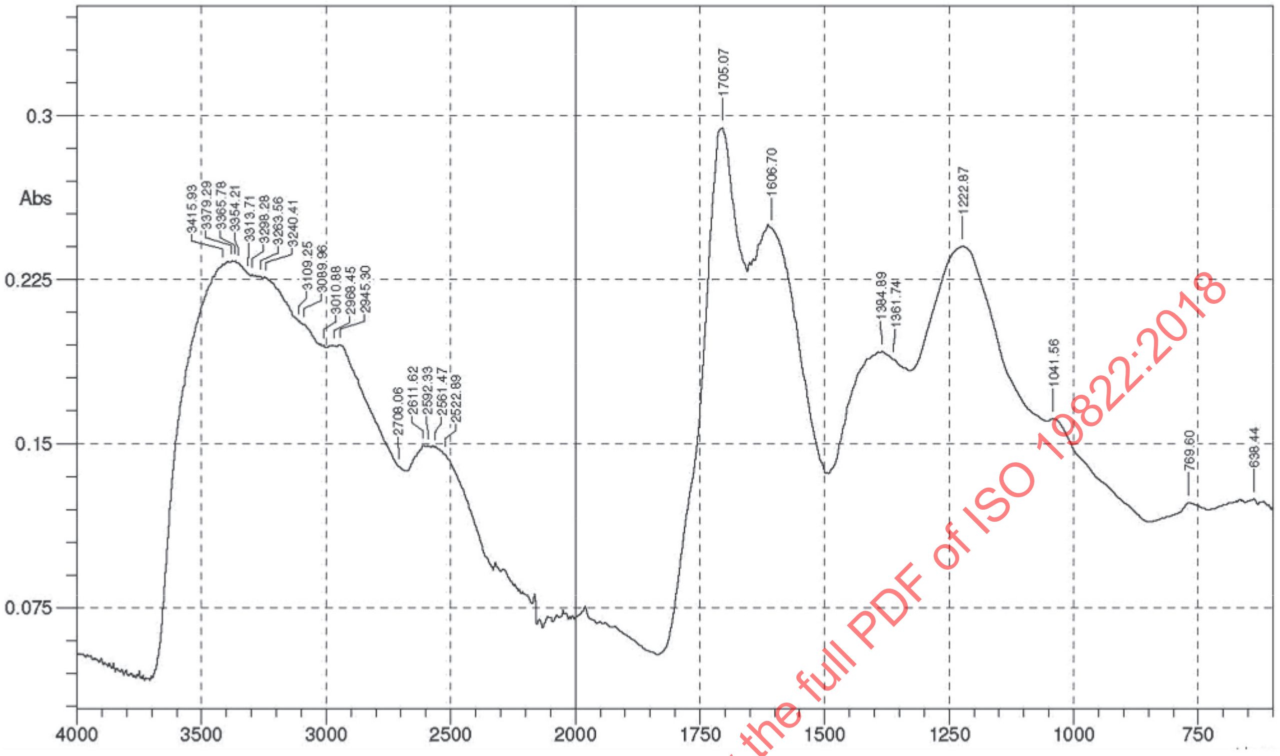
Number of Scans: 16 to 64

Temperature: 296 K

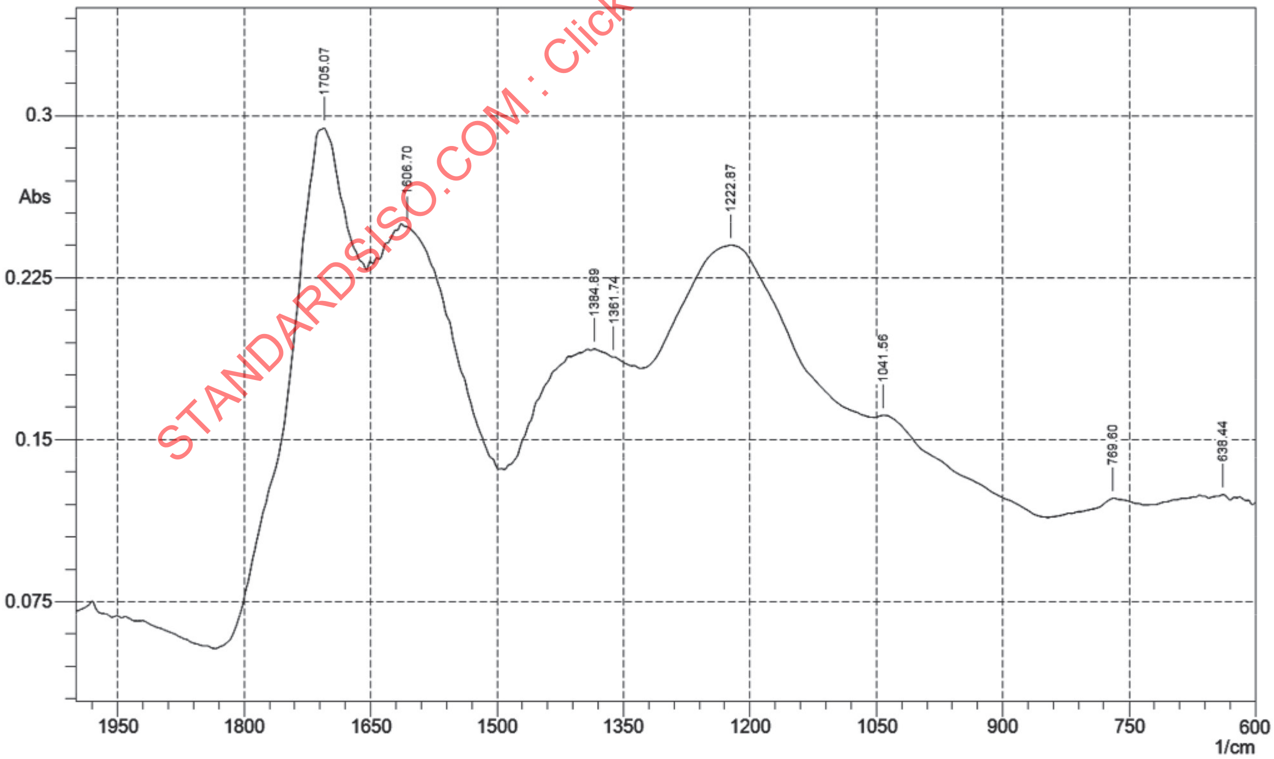
A.3.4 Representative spectra of a commercial lignosulfonate



A.3.5 Spectra of International Humic Substances Society 2S103F Suwannee River Fulvic Acid Standard



A.3.6 Detail of Mid-FTIR Spectra 600 cm⁻¹ to 1 900 cm⁻¹ of International Humic Substances Society 2S103F Suwannee River Fulvic Acid Standard



Annex B
(informative)

ISO/CD 19822 interlaboratory study

The purpose of the ISO/CD 19822 interlaboratory test was to statistically analyze the data generated from the 11 participating laboratories for precision of the method as per ISO 5725-2:1994. “Precision” refers to the closeness of agreement between test results from the participating laboratories using the same method on the same materials to determine what difference in results can occur under actual testing conditions.

In February of 2016, letters of invitation were sent out to numerous laboratories asking them to participate in an interlaboratory study of an ISO analytical method for analyzing humic and hydrophobic fulvic acids in commercial products. 12 laboratories from 6 countries agreed to participate in the study and adhere to the following criteria:

a) all essential apparatus, chemicals and other requirements specified in the method shall be available in their laboratory when the study begins;

b) specified “timing” requirements, such as starting date and finishing date of the program, shall be rigidly met;

c) the analytical protocols shall be strictly adhered to without any modifications

d) a qualified operator shall perform the analysis;

e) each analysis shall be carried out under repeatability conditions, i.e. on different days by the same operator using the same equipment;

f) participants were instructed to report the results from the 4 replicated analyses without discarding or reworking the analyses regardless of how inconsistent the data might appear.

The interlaboratory study commenced in June of 2016 starting with a pre-study Phase 1.

Phase 1 consisted of analyzing pre-*study practice samples* using the ISO/CD 19822 procedure. This phase allowed laboratories to familiarize themselves with the method and to communicate any technical issues to the Study Director. The samples were supplied to the laboratories at no cost by the Humic Products Trade Association of the United States.

Phase 1 involved analyzing two sample materials; a solid mined material and a commercial liquid product. As the solid was previously homogenized and as the liquid product could be homogenized simply by shaking the sample in its container prior to removing an aliquot, sampling variability was reduced substantially. Sufficient materials were supplies to allow for numerous analyses.

Each laboratory was assigned a confidential laboratory number. Each laboratory reported the results of 4 replicate analyses for the two samples, along with comments and observations, to the Study Director. After subjecting the pre-study data to statistical analyses, the data was shared with all of the participating laboratories.

Preliminary statistical analyses of the Phase 1 pre-study results revealed high variability of the analyte reported. After reviewing the reported moisture in the solid sample and the ash reported for humic acids (HA), hydrophobic fulvic acids (HFA) in both samples, column volumes for elution, and quantity of DAX-8 resin used by each lab, some procedures were revised. The revisions were standardization of:

- Moisture determination of solid samples;
- Ash determination of humic acids (HA) and hydrophobic fulvic acids (HFA);