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Quantification of Lignosulfonates and Humic Components in Mixtures by ATR FTIR Spectroscopy

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Abstract: The existing techniques for lignosulfonate (LS) in humate fertilizers lack selectivity to humic substances (HS) as the main component; they involve labor- and time-consuming sample preparation to separate the components at the level of detectable LS concentrations. The procedure based on attenuated total reflectance (ATR) FTIR spectroscopy with simple sample preparation for directly quantifying lignosulfonates in aqueous solutions and lignosulfonates and HS in aqueous solutions of preparations based on HS of coal origin (Sigma Aldrich, Powhumus, and Life Force) was developed. Lignosulfonate quantification is possible by exploiting the bands at 1266, 1192, 1093, and 1042 cm^{-1} with limits of detection of 0.4–2 g/L. Quantifying LS in a mixture with humates includes centrifugation of prepared solutions to separate interfering silicate impurities. LS quantification in the range of 10–100 g/L against HS (up to a 2-fold excess) with an error of up to 5% is possible based on the spectral absorptions at 1093 and 1042 cm^{-1}. Simultaneous quantification of humate in the mixture with an error of up to 10% is possible by exploiting the bands at 1570 and 1383 cm^{-1} (carboxylates). The study shows the possibility of determining lignosulfonate against an HS background several times higher than lignosulfonate. The developed technique is applicable for analyzing fertilizers of simple composition and quality control of pure humates used for plant growth. Obtaining the most accurate results needs calibration solutions from the same brands that make up the test mixture.

Keywords: lignosulfonates; humic substances; aqueous solutions; humate fertilizers; quantification; IR spectroscopy; ATR-FTIR spectroscopy

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1. Introduction

Lignin and its derivatives have found application in various industries [1]. They are used in the drilling of wells [2], in the production of various types of fuel (briquettes, gas, and boiler fuel) [3,4], and as reducing agents for metals [5] and activated carbon [6,7]. Lignin is a raw material for producing phenol, acetic, and oxalic acids. It is a substitute for sawdust and wood flour in the production of bricks and is used as an additive in asphalt concrete and as a filler for plastics and composites. Lignins are used as sorbents for wastewater, oil products, and heavy metals [7]. They are widespread in agriculture as herbicides and in some fertilizers [8,9]. There are medicinal preparations based on lignin [10–13]. Lignosulfonates (LS) are lignin derivatives and waste products from the sulfite processing of wood in the pulp and paper industry. About 1.2 million tons of lignosulfonates are produced annually, approximately 10% of the total lignin mass obtained worldwide [14]. LS are widely used; however, their properties differ from the original lignin due to a different structure. Lignosulfonates are surfactants, so they are mainly used as stabilizers, emulsifiers, and dispersants in industries such as oil [15], coal chemicals [16,17], and in the production of building materials [18]. In agriculture, LS are used to treat soil against erosion and plant protection products and fertilizers [19]. In addition, lignosulfonates create solutions resistant to temperature changes and presence of electrolytes [20].

Lignosulfonates have different molecular weights; their formula is not defined, and they have various functional groups. In addition, lignin (and, thus, LS) obtained from various plants differ from each other in chemical composition and structure [21,22]. Currently, no standards regulate the production of lignin and lignosulfonates and how the quality control of the manufactured LS is conducted. Thus, developing relatively prompt and readily reproducible quantification methods for LS is an imperative task.

The assessment of lignin and LS is commonly based on gravimetric analysis after hydrolysis with sulfuric acid, known as the Klason lignin analysis [23,24] or its variants [25,26]. The results of such gravimetric analysis are strongly affected by the presence of components insoluble in lignin, proteins, and fungal chitins. Moreover, this approach does not distinguish between different structures of lignin. Neither method can be considered independent as an analytical method and requires some extra information [23,24]. Dence [27] proposed a non-aqueous potentiometric titration of lignin in tetrabutylammonium hydrochloride in the presence of 4-hydroxybenzoic acid as an internal standard. The advantage is the assessment of hydroxyl groups in lignin and weakly acidic phenolic hydroxyls. In combination with ion exchange, this titrimetric technique is used to quantify strongly acidic (sulfonic) groups in lignosulfonates [28].

UV spectroscopy has been the most widely used technique for lignin and lignosulfonates for several decades. These methods are based on the LS UV absorption at 200–300 nm and are used for LS quantification in sulfite solutions and LS production wastewaters [29]. Spectrophotometric quantification of LS at 280 nm is used for gravel and drilling fluids [30,31]. Since all substances, especially decomposition products of carbohydrates, interfere with the characteristic bands of lignosulfonate, the most critical step of these methods is sample preparation to avoid superimposing the bands of low-molecular substances on LS bands. An approach is lignosulfonate precipitation; e.g., Haars, Lohner and Hüttermann [29] used precipitation with polyethyleneimine. The advantages of this approach are easy repeatability, rapidity, and affordable equipment. However, an additional component is introduced in precipitation techniques, affecting the spectrum. Precipitation and precipitate dissolution also depend on various parameters (temperature, pH, the presence of cations, the sulfonation degree, and LS molecular weight distribution). UV spectrophotometry is also a common method for quantifying acid-soluble lignin [23]. However, if the biomass is not extracted in suitable quantities, foreign extractive materials can complicate the analysis.

Some methods for LS quantification are based on quantitative analysis of the functional groups of its molecules. For the quantification of molecular forms, in most cases, it is necessary to apply more complex mathematical models, e.g., a PCA method for quantification by IR spectroscopy [32–34]. There are examples of the quantification of lignin monomers by chromatography [35] and densitometry for lignin and its derivatives, including mixtures [36]; the error in the quantification of lignosulfonate is up to 20%. Combining the latter method with thin-layer chromatography allows the estimation of other components simultaneously with lignin, e.g., monosaccharides [36]. The combination of methods provides the quantification of LS in a mixture without separation. Thus, one of the main tasks of biomass analysis for lignin and LS is to develop an analytical tool for the specific quantification of lignin in absolute amounts, which can simultaneously characterize its structural features in a reliable and high-performance way.

Another relevant task in LS assessment is its quantification in various products and mixtures. This is complicated because LS are additives to complex compositions with many components. One of the most relevant tasks in this aspect is fertilizers and other mixtures based on humic substances (HS). The properties of the final product depend on the HS source, admixtures, and the parameters of the production technology. However, the functions of LS and HS in fertilizers differ, and it is necessary to assess and control the concentrations of each component individually. The concentration of additional components, including lignosulfonates, is usually not controlled. The development of techniques is further complicated because both HS and LS are complex continua of macromolecules

of variable composition and irregular structure with different molecular weights. Since humus is formed in nature during lignin decomposition, the structure and properties of HS and LS are similar, and, therefore, quantification methods are the same. As a result, the simultaneous quantification of LS and humates by UV spectroscopy is impossible since humates also absorb in this range [37,38]. The same problem of similar chemical composition arises for densitometric quantification [36]. The potentiometric titration technique also gives false-positive results in lignosulfonate–humate mixtures due to similar functional groups [27]. Lamar, et al. [39] developed a method for the gravimetric quantification of humic and fulvic acids. One of its stages is the adsorption of humates. However, lignosulfonate is also adsorbed, and it becomes impossible to separate humates from LS. Therefore, lignosulfonate additives overestimate the concentration of humic substances in artificial liquid samples [39].

Thus, LS quantification in LS–HS mixtures usually consists of a two-step procedure, first separating lignosulfonates from other components and then assessing the analyte. Extraction with organic solvents is used to separate components, particularly HS [29]. Gel chromatography is often used to isolate lignosulfonates [40]. Most of the efforts are devoted to solving the problem of LS isolation by molecular weight fractions. Sumerskii, et al. [41] described an approach for isolating and purifying LS from a spent sulfite solution. This approach includes sorption on macroreticular nonionic poly(methyl methacrylate) granules (XAD-7 resin) followed by desorption with organic solvents to obtain highly pure lignosulfonates. Similar methods for isolating LS using liquid membranes were used; however, problems arose with the purity of extraction of lignosulfonates, low stability of membranes, and long sample preparation [42].

To sum up, the existing techniques for LS in humate fertilizers lack selectivity to HS as the main component; they involve labor- and time-consuming sample preparation stages to separate the components, and the level of detectable LS concentrations does not entirely fit the task.

IR spectroscopy is already used to characterize the functional group composition of both HS and LS [43–45]. We have previously developed a method for directly quantifying HS in fertilizers by IR spectroscopy [46]. However, there are no studies on the quantification of lignosulfonates in mixtures by IR spectroscopy. Furthermore, as HS are present in fertilizers in relatively high concentrations, their solutions have a high IR absorption, preventing IR spectroscopy in the transmission mode. The disadvantages of transmission IR spectroscopy, such as spectral sensitivity to water and the need for specific sample preparation, e.g., pressing into tablets with KBr, are shown as serious for LS quantification [23]. However, ATR FTIR spectroscopy proves suitable for powdered samples and aqueous solutions of complex mixtures, including soil organic matter [47–49] and its components [50–53].

Thus, this work aims to develop a procedure based on ATR FTIR spectroscopy with simple sample preparation for directly quantifying (1) lignosulfonates in aqueous solutions and (2) lignosulfonates and humic substances in aqueous solutions of fertilizer preparations based on humic substances.

2. Materials and Methods

2.1. Samples and Reagents

Commercial samples of humic substances of coal origin were used: humic acid sodium salt (technical grade, Sigma-Aldrich, St. Louis, MO, USA, CAS Number: 68131-04-4), Powhumus (Humintech GmbH, Grevenbroich, Germany, CAS Number: 68514-28-3), and Life Force (Life Force LLC, Saratov, Russia). These samples are further referred to as Aldrich, Powhumus, and Life Force, respectively. Sodium lignosulfonate (Life Force LLC, Saratov, Russia; referred to as LSNa) was used. Water (specific resistance, 18.2 MΩ·cm) from a Milli-Q purification system (Millipore, France) was used throughout.

2.2. IR Equipment and Measurements

IR spectra of dry samples and aqueous solutions were recorded by a Vertex 70 spectrometer (Bruker Optik GmbH, Germany); beamsplitter, KBr; aperture, 8 mm; detector, room temperature DLaTGS; scanner velocity, 10 kHz; sample and background scan numbers, 64, acquisition mode, double-sided, forward–backward. The spectra were recorded in the range 4000–400 cm^{-1} (2000–800 cm^{-1} for quantification) with a resolution of 2 cm^{-1}. A GladiATR™ single reflection attenuated total internal reflection accessory with a diamond crystal (Pike Technologies, Madison, WI, USA) was used. A background signal was recorded prior to each sample. The spectrometer and accessory were continuously purged with −70 °C dew point air (a PG28L Purge Gas Generator, PEAK Scientific) at 500 L/h. For aqueous solutions, deionized water was used as a background. A drop of the solution (ca. 30 µL) was placed on the ATR crystal. The sample was in the ambient atmosphere during the measurement, and the environment temperature was kept at 23 ± 1 °C by an air conditioner.

The data was processed using OPUS software (Bruker Optik GmbH 2012, version 7.2.139.1294). All spectra were smoothed over 9 points; ATR correction was performed for the spectra of dry samples (refractive index of sample 1.5 was used). After ATR correction, peak intensity correction was carried out in OPUS software by three types of processing. The correction mode (OPUS notation, Type M "Peak intensity relative to the horizontal baseline"), from now on referred to as Method 1, consisted in drawing a baseline through one set point parallel to the *X*-axis. The interval of each peak was set, at which the maximum intensity was found, and the peak height relative to the baseline was obtained. In this mode, we used two approaches, (1a) without accounting for the humate concentration and (1b) taking into account the humate concentration for baseline correction. The second type of correction (OPUS notation, Type P "Intensity at the specified frequency", from now on, Method 2) was to determine the intensity (full peak height) at a given frequency without a baseline. A straight line was constructed for each band by a least-square fit. In the case of Method 1a, the calibration line passes through zero.

We based upon the procedure developed to quantify humic substances in aqueous solutions by ATR FTIR [46] and extended it to aqueous LS–HS solutions. In binary mixtures, the concentration of one component was considered known and used in baseline correction and concentration calculations. Then, the second component was calculated from two equations of the calibration lines (for humate and lignosulfonate). For LS, the intensities of the bands at 1093 and 1042 cm^{-1}; for humate, the intensities of the bands at 1570 and 1383 cm^{-1} were used. The concentrations of LS and HS for each test band were calculated as, respectively,

$$c_{LS} = (I - b_{LS} - b_{HS} - k_{HS}c_{HS})/k_{LS} \quad (1)$$

and

$$c_{HS} = (I - b_{LS} - b_{HS} - k_{LS}c_{LS})/k_{HS}, \quad (2)$$

where I is the band intensity, c_{HS} and c_{LS} are HS and LS concentrations in solution, and coefficients k and b are from individual calibration equations for LS and HS. The values for both wavenumbers corresponding to the same analyte were averaged.

Further data processing was carried out using OriginPro 8.1 software (OriginLab Corp., version 8.1.34.90). The measurement results are presented in accordance with the requirements of ISO/IEC 17025:2005. Coefficients of correlation, confidence limits, standard deviations, relative standard deviations, and limits of detection (LOD) were calculated according to presentation guidelines of IUPAC recommendations 1998 and ISO 5725:1994.

2.3. Other Equipment

Samples were weighed by an Ohaus Discovery DV114C analytical balance (Nänikon, Switzerland; accuracy, 0.0001 g). For quantification, all prepared solutions were centrifuged

in 2-mL polypropylene Eppendorf vials in a CM-50 microcentrifuge (ELMI Ltd., Riga, Latvia) at 4000 rpm for 30 min.

Water concentration in samples was measured using an HG63 infrared thermogravimetric moisture analyzer (Mettler–Toledo AG Laboratory & Weighing Technologies, Greifensee, Switzerland). The automatic cut-off criterion "weight loss per unit time" was used; drying ends automatically when the average weight loss (Δm in mg) per unit time (Δt in seconds) falls below the preset value. All amounts and concentrations of humates and sodium lignosulfonate are recalculated for the water concentration.

2.4. Procedures

2.4.1. General Procedure for Solutions

To prepare individual solutions, a weighed portion of the sample was placed in a polypropylene test tube, and 10 mL of water was added and shaken until complete dissolution. To prepare mixture solutions, weighed portions of humate and lignosulfonate were mixed, and then 10 mL of water was added and shaken by hand until complete dissolution.

2.4.2. Model Mixtures of Lignosulfonate with Humate for Qualitative Analysis

Solutions of mixtures of lignosulfonate with Aldrich HS were prepared according to Section 2.4.1 with a total concentration of components of 100 g/L and an LS: HS ratio of 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, and 4:1. Further centrifugation of solutions was not performed.

2.4.3. Selection of Conditions for Centrifugation of Humate Solutions for Quantification of Lignosulfonates in the Presence of Humate

An aqueous solution of Powhumus HS with a concentration of 75 g/L was prepared according to Section 2.4.1 and divided into several vials. One vial was left without centrifugation, while the remaining vials were centrifugated at 4000 rpm. After 15, 30, and 60 min, one vial at a time was taken out, and the supernatant liquid was collected into a clean vial and the spectrum of this solution was recorded.

2.4.4. Calibration Solutions of Humate and Lignosulfonate

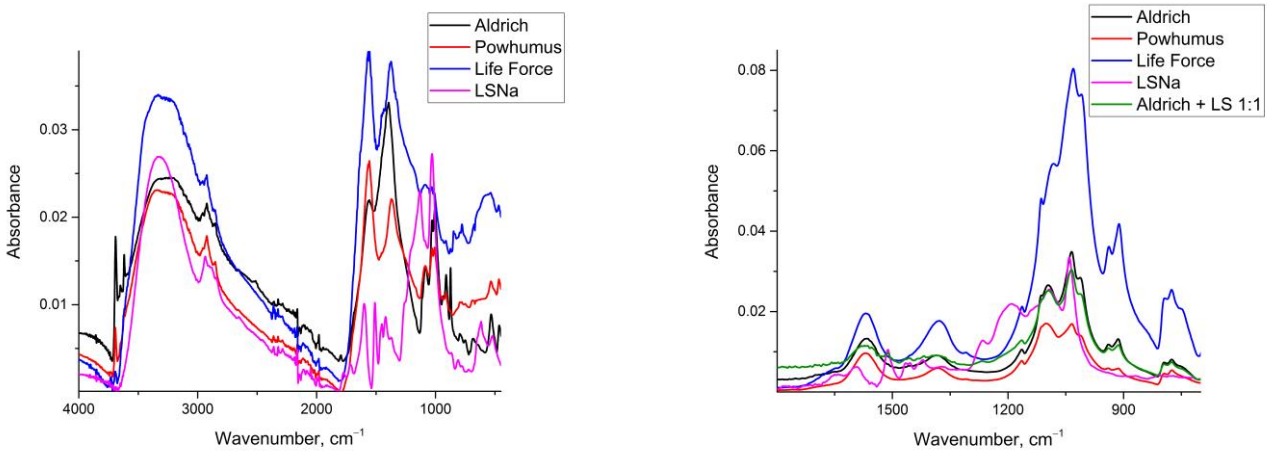
Individual Powhumus HS and lignosulfonate solutions with concentrations 10, 20, 35, 50, 75, and 100 g/L were prepared according to Section 2.4.1. For mixtures, Powhumus HS was used for calibration and Life Force HS was used to test the technique. For both samples, two series of solutions of lignosulfonate/humate mixtures were prepared according to Section 2.4.1: (1) humate of 20 g/L and LS:HS ratios of 1:2, 1:1, 2:1, and 4:1 and (2) humate of 50 g/L and LS:HS ratios of 1:2, 1:1, 3:2 and 2:1. In all the cases, solutions were centrifugated at 4000 rpm for 30 min. The supernatant liquid was collected into a clean vial, and the spectrum of this solution was recorded.

3. Results and Discussion

3.1. Band Identification of Humates and Lignosulfonates

ATR-IR spectra of dry individual samples of all brands and their 100 g/L aqueous solutions (Figure 1) were obtained. Based on many sources, the most significant absorption bands of the spectra of lignosulfonate and humates were identified [54–66]. Table 1 summarizes characteristic bands of lignosulfonate and humates for dry samples and their solutions before and after centrifugation.

The main vibrations characteristic of both humates and LS (Table 1) fall in the ranges 3400–3300, 2935–2850, 1725–1710, 1640–1600, 1570–1560, 1460–1450, 1420–1410, and 1380 cm^{-1}, which correspond to CH_x groups, aromatic C=C bonds, quinone and ketone C=O bonds, and carboxyl groups and carboxylate ions [60,67–70].



(**a**) (**b**)

Figure 1. ATR absorption spectra of (**a**) lignosulfonate and humate powders from Aldrich, Powhumus, and Life Force in the mid-IR region (4000–500 cm^{-1}) and (**b**) aqueous solutions of lignosulfonate and the same humates, and a mixture of Aldrich with lignosulfonate in the mid-IR region (1800–700 cm^{-1}).

Bands at 3690, 1130–1110, 1070, 1035, 1015, 938, 910, and 875 cm^{-1} are characteristic of HS samples only and correspond to the inorganic, silicate part of humates [46,61,71,72]. The mineral composition of HS is confirmed by elemental analysis [72]. The band at 1070–1050 cm^{-1} is present in the IR spectra of both compounds. Although in HS, this band most probably corresponds to Si–OH bending vibrations in silicate impurities [46], as it is revealed only in dry HS samples and HS solutions without centrifugation. After centrifugation, the spectra of the humate do not show any of these bands.

Table 1. Absorption bands of dry sodium lignosulfonate (LSNa) and humates (HS) and their aqueous solutions [43,60,61,66–71,73–82].

Wavenumber, cm^{-1}	Substance	Assignment
3691	HS *	OH stretching of structural hydroxyl groups of SiO_2
3400–3300	HS, LSNa	O–H stretching, N–H stretching (minor), hydrogen-bonded OH; O−H stretching
2935–2925, 2850	HS, LSNa	C–H stretching of CH_2, C−H stretching of $-OCH_3$
1725–1710	HS, LSNa	asymmetric C=O stretching of –COOH
1640–1600	HS, LSNa	aromatic C=C skeletal vibrations, C=O stretching of amide groups (Amide I), C=O of quinone or H-bonded conjugated ketones, –COOH group stretch, C–C stretch, aromatic and nonaromatic
1591	LSNa	aromatic C=C ring breathing
1570–1560	HS	aromatic C=C skeletal stretching; C=O of quinone or H-bonded conjugated ketones; $-COO^-$ antisymmetric stretching
1512	LSNa	aromatic C=C ring breathing
1460–1450	HS, LSNa	C–H scissoring of CH_3 groups
1455	LSNa	aromatic ring stretching, C–H deformation in $–O–CH_3$ group
1420–1410	HS, LSNa	aromatic C=C ring breathing, aromatic skeleton vibrations combined with C–H in-plane deformations; O–H deformation and C–O stretching of phenolic OH
1380	HS, LSNa	Wagging C–H of CH_2 and CH_3 groups, $–COO^-$ symmetric stretching

Table 1. *Cont.*

Wavenumber, cm^{-1}	Substance	Assignment
1370	LSNa, HS (only dry)	methylene bridge, phenolic OH, C–H wagging in methyl groups
1308	HS	CO of phenols, CO and OH of carboxylic acids, aliphatic C–C
1266	LSNa	Ar−O stretching breathing, C–O in guaiacyl ring
1192	LSNa	S=O of SO_3^{2-}
1130	LSNa	Ar–O stretching breathing
1130–1110	HS *	C–O stretching of secondary alcohols or ethers
1093	LSNa	C–O–C and OH of alcohols
1080	HS *	Si–O stretching
1070–1050	HS *, LSNa	alcoholic and polysaccharide CO stretch and OH deformation; CO and OH of polysaccharides and alcohols; Si–OH bend in silicates
1042	LSNa	R–SO_3H, OH groups, or S=O stretching
1015	HS *	Si–O of silicates
938	HS *	OH deformation of the inner-surface hydroxyl group
910	HS *	OH deformation of inner hydroxyl groups
875	HS *	$Si–O^-$ or Si–O–Si bridge; carbonate; polyaromatic bend vibrations

* in dry samples and solutions without centrifugation.

Although the band at 1308 cm^{-1} (CO of phenols and CO and OH of carboxylic acids) is characteristic of HS only, it is very weak, and it cannot be used to quantify humate. The bands at 1560 and 1380 cm^{-1} (carboxylate antisymmetric and symmetric vibrations, respectively) have medium intensities in all the samples. The spectra of all humate brands used in the study have the same set of bands and differ only in the ratio of intensities.

As expected, bands corresponding to sulfonic groups (1192 and 1042 cm^{-1}) are not found in HS samples [43]. Thus, except for carboxylate, no bands in HS spectra correspond to humate organic matter and are present in LS as well.

Spectral features that correspond to lignosulfonates only (Table 1) include bands assigned to aromatic groups at 1591, 1512 (C=C ring breathing), and 1455 cm^{-1} (most probably aromatic ring stretching vibrations), 1266 and 1130 cm^{-1} (Ar−O stretching breathing), 1093 cm^{-1} (C–O–C and OH of alcohols), and sulfonic groups at 1192 and 1042 cm^{-1} [43,66,67,73,74]. However, the aromatic bands at 1591, 1512, and 1455 cm^{-1} are weak and located on the intense and broad humate bands, making them unsuitable for quantifying lignosulfonate in mixtures with humate. On the other hand, the band at 1130 cm^{-1} is weak and appears only in dry lignosulfonate samples. Thus, only the bands at 1266, 1192, 1093, and 1042 cm^{-1} (interactions between C–O and C–O–H vibrations, S=O, R–SO_3–H, OH groups) qualify to be tested for LS quantification [43,60,61,66–71,73–82]. However, these LS characteristic bands cannot be considered pure as they may also correspond to C–O and C–O–H vibrations and because all lignosulfonates have intramolecular interactions that produce intense bands [83] that could entail a possible unavoidable spectral interference from HS. However, intermolecular interactions in humates give rise to vibrations in a broader frequency range and lower intensities [84]; thus, their interference can be considered minor (Figures 1 and 2). Apart from this, the bands at 1266, 1093, and 1042 cm^{-1} overlap with relatively intense silicate bands, which requires separation of the latter.

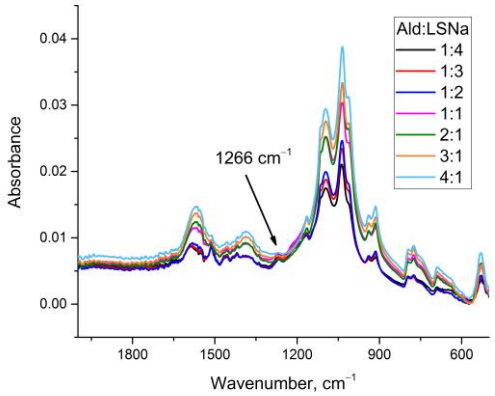


Figure 2. ATR absorption spectra of aqueous solutions of Aldrich HS and LSNa mixtures of different compositions (total concentration of components, 100 g/L) in the range of 2000–500 cm^{-1}.

3.2. Selection of Quantification Conditions

For calibration solutions, we have selected Powhumus humate. Since Aldrich humate has significant silicate amounts [46], it was used to study the effect of silicates on the qualitative tests and lignosulfonate quantification. The third brand, Life Force, was used to verify the correctness of the procedures. Mixtures of Aldrich humate with lignosulfonate were prepared according to Section 2.4.2 (Figure 2). All the characteristic bands of LS at 1266, 1192, and 1042 cm^{-1} are clear. The bands of lignosulfonate appear against the background of humate at a concentration level of 10 g/L at a twofold excess of humate. However, significant silicate impurities in humates make it challenging to quantify lignosulfonate as HS silicate peaks at 3691, 1080, 1030, 1015, 938, 910, and 875 cm^{-1} have high intensities and overlap with most lignosulfonate bands. Thus, without silicate separation, it is possible to detect lignosulfonate only qualitatively by a relatively low-intensity peak in the range of 1265–1270 cm^{-1} (Figure 2). In addition, the intensity of silicate bands in solution depends on the recording time due to the silicate precipitation [46] and, thus, affects the measurement accuracy.

Therefore, silicates were separated by centrifugation (Section 2.4.3). After centrifugation, the spectrum of HS solutions (Figure 3) reveals no characteristic silicate bands at 3691, 1080, 1030, 1015, 938, 910, and 875 cm^{-1}. Figure 4 shows the spectra of centrifuged solutions of humate, lignosulfonate, and their mixture. In the region of 1300–900 cm^{-1}, free of silicate bands, the bands at 1266, 1093, and 1042 cm^{-1} are not overlapped by humate bands. Under these conditions, the 1192 cm^{-1} band (vibrations of C–O–C bonds and OH groups of alcohols) can also be used for LS quantification. The spectra of solutions after 30 and 60 min of centrifugation are identical (Figure 4). Thus, all mixtures for quantification were prepared using centrifugation for 30 min at 4000 rpm.

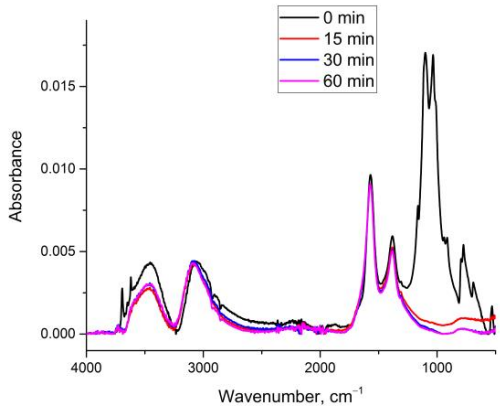


Figure 3. ATR spectra of an aqueous solution of Powhumus humate with a concentration of 75 g/L before and after 15, 30, and 60 min of centrifugation.

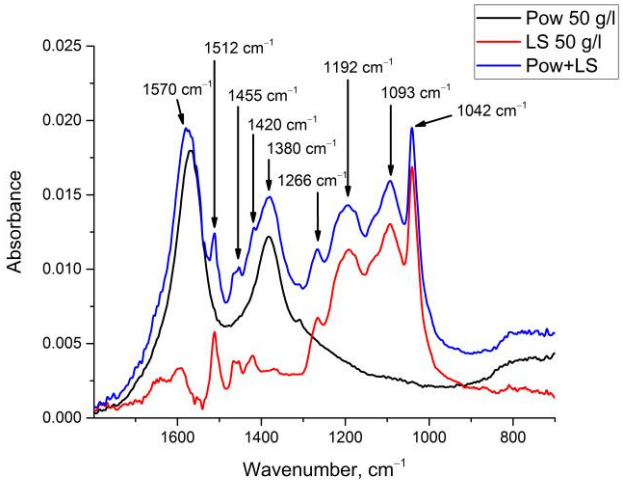


Figure 4. ATR absorption spectra of centrifuged aqueous solutions of sodium lignosulfonate, potassium humate Powhumus (Pow), and their 1:1 mixture, with concentrations of 50 g/L.

As mentioned above, the characteristic bands of lignosulfonate at 1266, 1192, and 1042 cm^{-1} are complex [83] and may overlap with bands of intermolecular interactions in HS. Nevertheless, as lignosulfonates have a more definite functional-group composition and thus give rise to narrower and more intense bands (Figure 1), we can determine LS against the background of humates.

3.3. Band Processing

The bands of the ATR spectra of lignosulfonate solutions are resolved (Figure 1), and band integration does not affect the quantification result. However, peak integration is essential for mixtures of lignosulfonate with humate since the LS and HS bands overlap (Table 1 and Figure 4). Therefore, even though a mixture purified from silicate has no distinct bands in the region of 1300–1000 cm^{-1}, humate still contributes to the total spectrum with broadbands in the entire range and can lead to a significant error in quantifying lignosulfonate. Therefore, when quantifying lignosulfonate, it is strictly necessary to correct it for the background content of HS. Upon preliminary studies, we selected three approaches towards this correction. They are Method 1 (peak intensity relative to horizontal baseline, in two variants) and Method 2, taking into account the humate concentration without a baseline correction (full peak height), Section 2.2.

These calculation methods were compared using the spectra of centrifuged solutions of mixtures of lignosulfonate and Powhumus humate with different component ratios (Table 2). The lignosulfonate concentration was calculated using the bands at 1266, 1192, 1093, and 1042 cm^{-1} that refer to the spectra of LS only.

In Method 1a, we manually set the boundaries of each peak to calculate the maximum absorption in each range without taking into account the background absorption of the humate. In this case, the straight calibration line passed through zero, and we did not consider the background humate concentration in the further calculation of the LS concentration. This processing resulted in the most significant errors among the tested methods, which are acceptable only for HS concentrations of 20 g/L. The quantification error is positive (overestimation). In Method 1b, in contrast to Method 1a, the second component concentration was taken into account. This approach led to relatively low negative errors for three bands, except for positive errors of 40% for the band of 1042 cm^{-1}. Thus, exploiting the 1093 cm^{-1} band is possible, which can be used for quantifying lignosulfonate with an error of up to 10% in the studied concentration range.

Table 2. Concentrations and the error of lignosulfonate quantification in mixtures with Powhumus humate by the bands at 1266, 1192, 1093, and 1042 cm^{-1} by different peak processing methods ($n = 6$, $p = 0.95$).

Added, g/L		Method 1a				Method 1b				Method 2			
LSNa	**Powhumus**	**1266**	**1192**	**1093**	**1042**	**1266**	**1192**	**1093**	**1042**	**1266**	**1192**	**1093**	**1042**
						Found LS, g/L							
10	20	24	15	15	16	5.0	6.5	9.2	14	7.7	8.3	10	10
20	20	32	24	25	30	13	15	19	28	17	18	21	21
40	20	54	45	47	56	36	37	40	56	38	38	41	41
80	20	91	84	89	109	74	76	80	111	76	78	80	81
25	50	61	39	37	42	17	19	25	35	23	23	26	25
50	50	85	63	64	75	41	43	50	70	46	47	51	51
75	50	109	87	90	108	66	68	75	104	71	71	76	76
100	50	132	111	116	140	89	92	100	138	94	96	100	101
						Error, %							
10	20	140	53	46	63	−50	−35	−8	39	−23	−17	1.3	0.2
20	20	62	20	26	49	−33	−23	−3	41	−13	−10	4	5
40	20	35	13	18	41	−11	−8	0.4	40	−5	−4	2	1.6
80	20	14	5	11	36	−8	−5	−0.2	39	−5	−3	0.5	0.9
25	50	146	55	48	66	−31	−26	−0.4	40	−9	−9	3	1.3
50	50	70	26	27	49	−18	−14	0.6	39	−8	−6	1.5	1.3
75	50	46	16	20	43	−12	−10	0.7	39	−6	−5	1	1
100	50	32	11	16	40	−11	−8	0.1	38	−6	−4	0.5	0.7

When using Method 2, we do not draw a baseline and set the peak boundaries but take the absolute intensity value at a given wavelength. This approach gives a minor error in determining the lignosulfonate compared to Method 1b (Table 2) and is suitable for all four LS bands within the specified error range (up to 10%). Thus, the results for all four bands can be compared; the bands at 1266 and 1190 cm^{-1} give a negative error, and 1090 and 1040 cm^{-1} give a positive error.

Thus, the selected peak processing method consists of taking the absolute intensity at a given wavelength, while the available background counterpart concentration is considered to minimize the quantification error when calculating the concentration of the test compound. The processing was used in all the subsequent experiments.

3.4. Lignosulfonate Quantification in Neat Solutions

In setting up a procedure for LS in aqueous solutions, we based upon the procedure developed to quantify humic substances in aqueous solutions by ATR FTIR [46]. For all characteristic bands of lignosulfonate, the calibration relationships are linear over the entire LS range of 10–100 g/L (Table 3). As expected, the highest sensitivity (slopes and LODs) among all the characteristic bands of lignosulfonate is achieved for the most intense bands at 1266, 1192, 1093, and 1042 cm^{-1} that do not overlap with humate bands. The minimum attainable LOD is 0.4 g/L at 1042 cm^{-1}. Therefore, these four bands were selected to quantify lignosulfonate against HS. The quantification error of lignosulfonate in aqueous solutions by these bands does not exceed 3% and, at concentrations below 50 g/L, is lower than 1% (Table 4).

Table 3. Parameters for the quantification of sodium lignosulfonate ($n = 6$, $p = 0.95$).

Wavenumber, cm^{-1}	Slope, L/g × 10^5	Correlation Coefficient	LOD, g/L
1642	3.7 ± 0.2	0.9898	5
1591	5.8 ± 0.3	0.9974	3
1512	9.5 ± 0.3	0.9991	4
1466	6.7 ± 0.2	0.9992	4
1455	6.7 ± 0.2	0.9986	5

Table 3. *Cont.*

Wavenumber, cm^{-1}	Slope, L/g × 10^5	Correlation Coefficient	LOD, g/L
1420	7.3 ± 0.1	0.9994	4
1266	12.3 ± 0.5	0.9998	2
1192	21.4 ± 0.6	0.9999	1
1093	25.1 ± 0.6	0.9999	0.5
1042	32.5 ± 0.7	0.9999	0.4

Table 4. Error in determining lignosulfonate in neat solutions (n = 6, p = 0.95).

Added LS, g/L	Error of Calculation of LS Concentration, g/L			
	1266 cm^{-1}	1192 cm^{-1}	1093 cm^{-1}	1042 cm^{-1}
20	−3.0	−3.0	−1.0	1.0
50	0.1	−0.1	0.6	0.7
100	−0.7	−0.5	0.02	0.8

3.5. Lignosulfonate Quantification in Humate Mixtures

For LS quantification against an HS background, mixtures of LS with Powhumus humate centrifuged to separate silicate impurities served as calibration solutions. For bands at 1192 and 1266 cm^{-1}, a quantification error of up to 10% is achievable using the bands at 1192 and 1266 cm^{-1} for lignosulfonate concentrations above 20 g/L (Table 2; the corresponding error for neat solutions up to 3%, Table 4).

For bands of 1093 and 1042 cm^{-1}, the quantification error does not depend on the LS:HS ratio and HS level (Table 2). These bands provide the most accurate quantification of lignosulfonate with an error of up to 5% in the entire investigated concentration range and at any investigated background humate concentration. For LS concentrations over 75 g/L, an error of even less than 1% is possible, which is comparable to LS quantification from neat solutions (Section 3.4).

We tested this procedure for HS samples different from the humate used to prepare the calibration solutions. The lignosulfonate concentration in the mixture was determined using the calibration for Powhumus humate, while Life Force HS was used to prepare model HS/LS mixtures (Figure 5). The results are presented in Table 5.

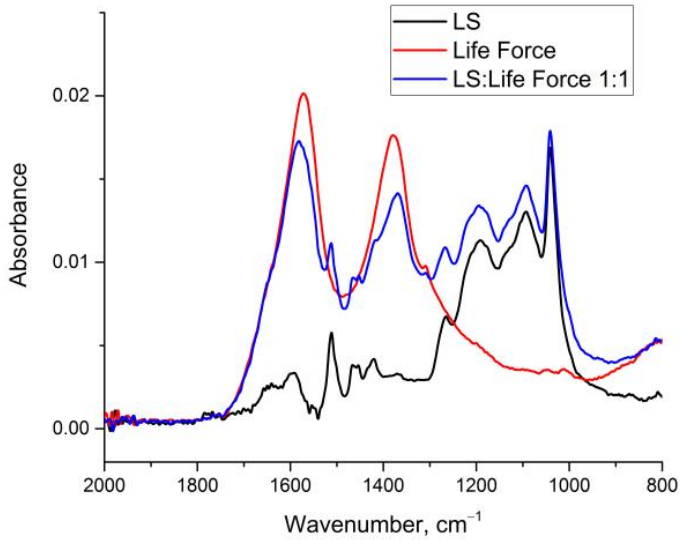


Figure 5. ATR absorption spectra of centrifuged aqueous solutions of sodium lignosulfonate, sodium humate Life Force, and their 1:1 mixture.

Table 5. Concentrations and errors of lignosulfonate quantification in mixtures with Life Force humate using a calibration by Powhumus at different bands (n = 6, p = 0.95).

Added, g/L		Found, g/L			
LSNa	**Life Force**	**1266 cm^{-1}**	**1192 cm^{-1}**	**1093 cm^{-1}**	**1042 cm^{-1}**
		Found, g/L			
10	20	5.1	6.4	8.0	8.2
20	20	15	16	18	18
40	20	32	34	37	37
80	20	68	71	75	75
25	50	19	20	22	22
50	50	42	42	45	46
75	50	59	64	69	69
100	50	74	83	90	91
		Error, %			
10	20	−49	−36	−20	−18
20	20	−24	−21	−12	−11
40	20	−20	−15	−8	−8
80	20	−15	−11	−6	−6
25	50	−24	−21	−14	−14
50	50	−15	−15	−9	−9
75	50	−21	−15	−9	−8
100	50	−26	−17	−10	−9

All calculated LS concentrations, in this case, were underestimated. The bands at 1266 and 1192 cm^{-1} give significant quantification errors, more than 15% in the entire range of concentrations and LS:HS ratios. On the contrary, the error by the bands at 1093 and 1042 cm^{-1} does not exceed 10% for LS concentrations above 40 g/L. At low lignosulfonate concentrations and a 1–2-fold humate excess, the quantification error increased to 20%. At high concentrations of lignosulfonate and its 2-fold excess relative to humate, the error in the most sensitive bands at 1093 and 1042 cm^{-1} was ca. 10%. Thus, using different humates as the calibration standard, it is possible to determine lignosulfonate in a humate fertilizer with a satisfactory bias.

For a more accurate quantification of lignosulfonate using a humate preparation that is different from the calibration one, it is necessary to calculate the error in determining humate, which can be used to calculate the lignosulfonate content. Indeed, this conclusion works for samples of humates of the same origin. All humates used in this work are of coal origin and produced from leonardite. It is expected that when using humates of a different origin (e.g., peat), the quantification error becomes higher, and the quantification itself may not be possible.

Thus, the procedure for LS quantification in humate fertilizers by ATR FTIR by the bands at 1093 and 1042 cm^{-1} can be employed in the range of 10–100 g/L and for a 2-fold excess humate concentration with an error of up to 5%. The presence of silicates requires centrifugation of the solutions before measurements.

3.6. Humate Quantification in Lignosulfonate Mixtures

The data obtained for LS makes it possible to estimate the accuracy with which to determine HS against LS. For assessing humate in a mixture with lignosulfonate, the characteristic humate bands at 1570 and 1383 cm^{-1} were selected, which were previously shown to provide the smallest error in determining humate in solution [46]. For consistency's sake, the spectra were processed similarly to LS without drawing the baseline and considering the lignosulfonate concentration. The quantification parameters for Powhumus humate are presented in Table 6; the results for model mixtures are summed up in Table 7.

Table 6. Quantification of humate (Powhumus) by the bands 1560 and 1383 cm^{-1} without baseline correction (n = 10, p = 0.95).

Wavenumber, cm^{-1}	Slope, L/g × 10^5	Correlation Coefficient	LODs, g/L
1570	2.9 ± 0.1	0.9823	0.7
1383	5.8 ± 0.4	0.9984	1

Table 7. The error of humate quantification in the mixtures with lignosulfonate by the characteristic bands of both humate and lignosulfonate.

Added, g/L		1570 cm^{-1}	1383 cm^{-1}
Powhumus	LSNa		
		Found, g/L	
20	10	20	19
20	20	20	19
20	40	19	19
20	80	19	18
50	25	50	49
50	50	49	48
50	75	48	47
50	100	47	47
		Error, %	
20	10	−1	−3
20	20	−1	−3
20	40	−3	−5
20	80	−7	−10
50	25	0	−3
50	50	−3	−4
50	75	−4	−5
50	100	−6	−7

At concentration levels of 20 and 50 g/L, the quantification error is up to 10%. For concentrations of lignosulfonate less than 75 g/L, the quantification error does not exceed 5%. Quantification of HS is possible in the range of 20–180 g/L with an error of 7%; for 50 g/L, with an error of 5%. Thus, even though HS quantification in a mixture with LS is possible with less accuracy than in humate-only solutions [46], an error of 10% is acceptable for most tasks involving LS assessment in humate fertilizers. This conclusion holds true when the humate of the same brand as in the mixture itself is used for the calibration solutions. Simultaneous quantification of LS and HS of different brands requires a more detailed study, which was beyond the scope of this work.

The previously proposed methods did not allow the simultaneous quantification of lignosulfonate and humate in the same mixture without separation; humic substances isolated from the mixture were still contaminated with lignosulfonate, which introduces an error in the quantification of their content, the additionally introduced components influenced the type of the spectrum, and the level of the determined concentrations was limited by the transmission of solutions [28–30,39]. The developed technique loosens these limitations and allows simultaneous quantification of humate and lignosulfonate without preliminary separation in highly absorbing solutions (at the level of 10–100 g/L).

4. Conclusions

The study shows the possibility of quantifying lignosulfonate in aqueous solutions against an HS background, which is several times higher than lignosulfonate. It may help regulate LS standard products and quantify LS in humate-based fertilizers. The developed technique is applicable for analyzing fertilizers of simple composition and quality control of pure humates used for plant growth, although with the selected brands of LS and HS at this stage. Obtaining the most accurate results needs calibration solutions from the

same brands that make up the test mixture. As LS and HS are chemically complex objects with non-stoichiometric and variable chemical compositions, it is essential not to become constrained to specific brands of LS. Furthermore, humates of coal origin of Life Force, Powhumus, and Aldrich brands were used. As humate fertilizers can be based on HS of various origins, further work needs to check the applicability of the approach for peat and soil humates. It is also feasible to build a chemometric model to quantify LS and HS simultaneously to possibly decrease the error furthermore compared to the values achieved in this study. In addition, fertilizers based on HS often contain other organic and inorganic components, which may significantly complicate their analysis. Therefore, it is necessary to develop methods that make it possible to separate the accompanying components before analysis and quantify HS and LS in multicomponent systems.

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