analytical chemistry

Article

pubs.acs.org/ac

Enumeration of Labile Hydrogens in Natural Organic Matter by Use of Hydrogen/Deuterium Exchange Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

Yury Kostyukevich,[†,§] Alexey Kononikhin,[†,§] Igor Popov,[‡,§] Oleg Kharybin,[∥] Irina Perminova,[⊥] Andrey Konstantinov,[⊥] and Eugene Nikolaev*[,†,‡,∥]

[†]Institute for Energy Problems of Chemical Physics, Russian Academy of Sciences, Leninskij pr. 38 k.2, 119334 Moscow, Russia

[‡]Emanuel Institute for Biochemical Physics, Russian Academy of Sciences Kosygina st. 4, 119334 Moscow, Russia

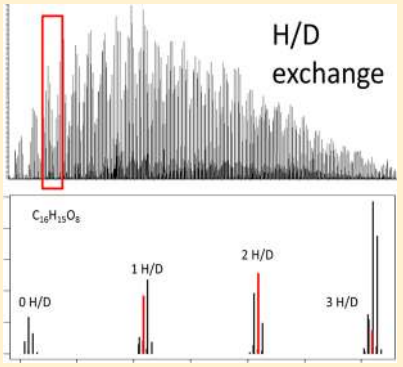
[§]Moscow Institute of Physics and Technology, 141700 Dolgoprudnyi, Moscow Region, Russia

[∥]Orekhovich Institute of Biomedical Chemistry, Russian Academy of Medical Sciences, ul. Pogodinskaya 10, 119121 Moscow, Russia

[⊥]Department of Chemistry, Lomonosov Moscow State University, Leninskie Gory 1-3, 119991 Moscow, Russia

Supporting Information

ABSTRACT: A method to enumerate labile hydrogens in all constituents of molecular ensemble of natural organic matter (NOM) based on our previously developed simple hydrogen/deuterium (H/D) exchange (electrospray ionization (ESI) ion source (Kostyukevich et al. *Anal. Chem.* **2013**, *85*, 5330) and ultra-high-resolution Fourier transform ion cyclotron resonance mass spectrometry is presented. The method was applied for analysis of Suwannee River fulvic acid (SRFA), which is an International Humic Substances Society standard, as well as Siberian crude oil; and lignosulfonate. We found that SRFA and lignosulfonate molecules contain 2−5 labile hydrogens, and their number increases with the number of oxygens in the molecule. Also, we observed that compounds of Siberian crude oil ionizing in positive-ESI mode do not have labile hydrogens, while compounds ionizing in negative-ESI mode have one labile hydrogen that detaches during ESI ionization.



Natural organic matter (NOM) presents a complex mixture of degraded natural organic compounds that plays an important role in carbon cycling. However, molecular structures of NOM remain largely unknown. This is because the extreme complexity of NOM renders impossible their separation into individual compounds by conventional techniques such as liquid chromatography, electrophoresis, or isoelectric focusing.[1] The same is true for structural studies of NOM by powerful physical methods that work perfectly for monomolecular samples (e.g., NMR spectroscopy, soft X-ray spectroscopy, fluorescence polarization) but are not capable of revealing the individual structures of NOM despite many efforts.[2,3]

Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) proved to be an important and useful tool for investigation of NOM on the molecular level, making it possible to determine accurate masses and elemental compositions of all compounds,[4,5] perform fragmentation studies of isolated molecules,[6] and implement gas-phase reactions inside the FTICR cell with previously separated compounds.[7,8]

In mass spectrometric studies, one of the most important methods of obtaining chemical and structural information about a molecule (from metabolites to proteins)[9,10] is based on the exchange of labile hydrogens for deuteriums. Labile hydrogens belonging to functional groups such as amide, carboxyl, or hydroxyl can be easily replaced by deuterium in solution or in the gas phase. At the same time, hydrogens attached to the carbon backbone may be exchanged only under chemical ionization conditions.[11]

As a consequence, the number of hydrogens exchanged by deuterium in a molecule under soft experimental conditions might serve as an equivalent to the number of the corresponding functional groups containing labile hydrogens in this molecule. Thus, simultaneous determination of labile hydrogens in all compounds of NOM might yield chemical and structural information about different molecules of a complex mixture.

Labile hydrogens in the large class of NOM belong to carboxylic and hydroxylic groups, and their determination by conventional techniques based on exchange in liquid phase is impossible due to a fast back-exchange in the ionization source.[9,10,12] Gas-phase exchange based on the infusion of deuterated gaseous phase (such as D_2O vapor or ND_3) in the FTICR cell is difficult to implement on the arbitrary FTICR and requires long-lasting postexperimental cleaning.

Here we report a use of the simple in-source H/D exchange method, previously developed in our group, combined with ultra-high-resolution FTICR mass spectrometry[12] for simultaneous

Received: August 16, 2013
Accepted: October 7, 2013
Published: October 7, 2013

   dx.doi.org/10.1021/ac402609x | *Anal. Chem.* 2013, 85, 11007−11013

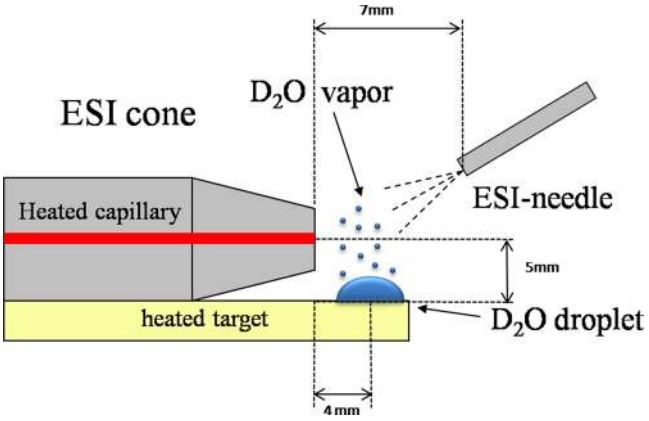


Figure 1. Design of the ESI source modified for H/D exchange experiments.

determination of labile hydrogens in all compounds composing NOM. We investigated Suwannee River fulvic acid (SRFA), which is an International Humic Substances Society standard, as well as Siberian crude oil and lignosulfonate. Those substances correspond to different degrees of NOM transformation in the environment.

METHODS

Sample Preparation. SRFA was dissolved in methanol (normal or deuterated) and diluted to a concentration of 1 g/L. Siberian oil was first dissolved in toluene and then diluted in methanol (MeOH or MeOD). Final concentration of oil was 1 g/L. Solvent composition was 30% toluene and 70% methanol. Lignosulfonate was dissolved in water (normal or deuterated) and diluted to a concentration of 1 g/L. Solvent composition was 50% water and 50% methanol.

Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. All experiments were performed on LTQ FT Ultra (Thermo Electron Corp., Bremen, Germany) equipped with a 7 T superconducting magnet. The obtained resolving

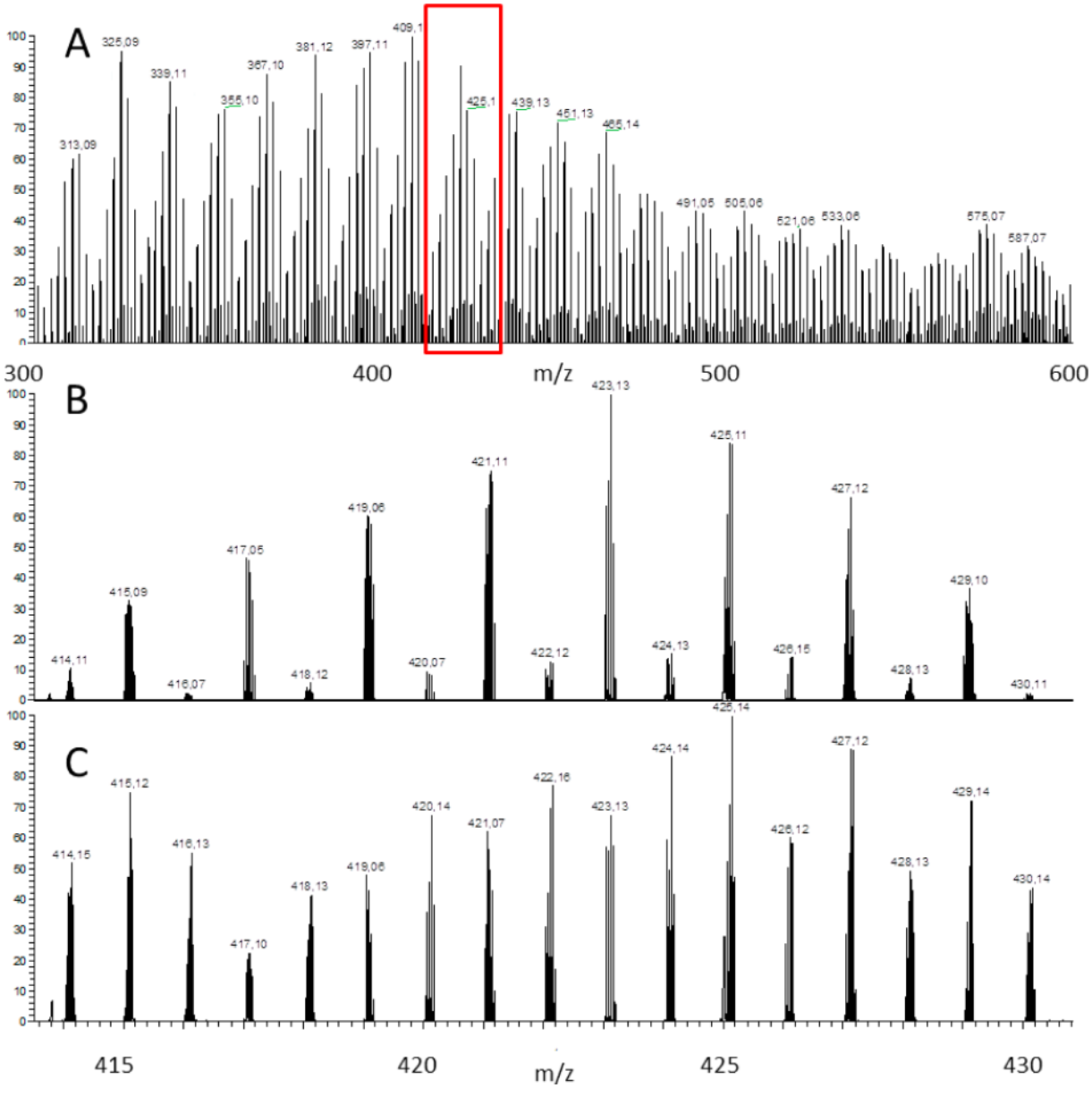


Figure 2. (A) FTICR mass spectrum of SRFA. (B) Magnified portion of FTICR mass spectrum of SRFA. (C) Magnified portion of FTICR mass spectrum of SRFA after H/D exchange.

dx.doi.org/10.1021/ac402609x | *Anal. Chem.* 2013, 85, 11007−11013

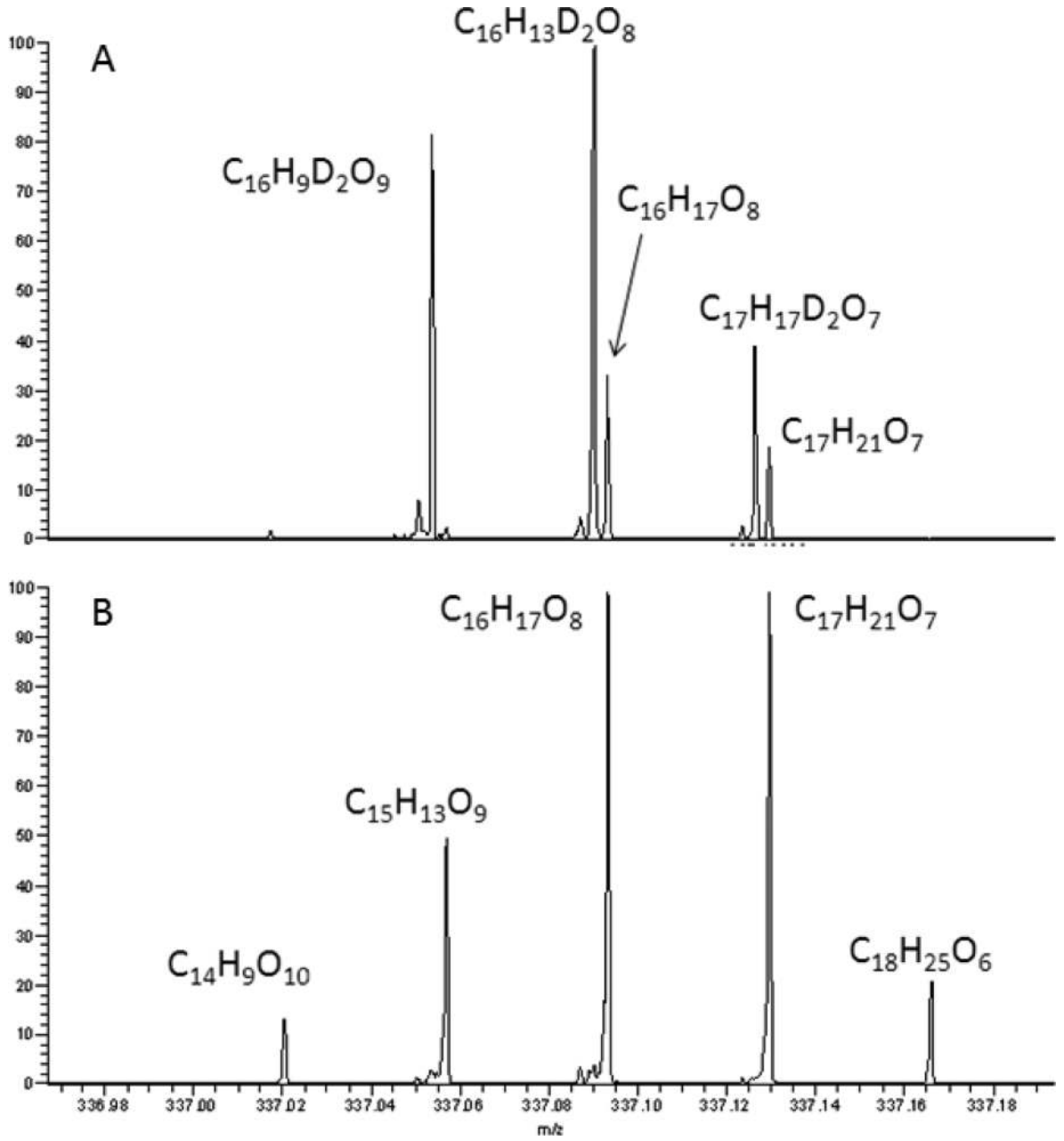


Figure 3. Fragments of mass spectra of (B) nonexchanged SRFA and (A) H/D-exchanged SRFA.

power was 400 000 at m/z = 400. Each spectrum was a sum of 400 consecutive scans. Ions were generated by IonMax Electrospray ion source (Thermo Electron Corp., Bremen, Germany) in positive and negative electrospray ionization (ESI) mode.

H/D Exchange Reaction. The design of the modified ESI source for H/D exchange experiments is presented in Figure 1. A copper plate was installed just beneath the ESI cone and a droplet of D_2O was placed on it. The desolvating capillary was maintained at a temperature of 250 °C, and the needle voltage was 2400 V. Due to evaporation of the droplet, an atmosphere of D_2O vapor in the region between the ESI needle and the MS entrance capillary is created. D_2O molecules can penetrate ESI droplets or participate in ion−molecular reactions inside the desolvating capillary or in the vacuum capillary skimmer region. As a result of these processes, H/D exchange takes place. The relative intensity of the peak corresponding to n exchanges with depth of exchange P equals

$$h(n) = C_N{}^n P^n (1 - P)^{N-n} \quad (1)$$

Here N is the total number of labile hydrogens. Mass difference between peaks in the series formed by incomplete H/D exchange reaction of the same parent ion equals 1.006 277 Da.

RESULTS AND DISCUSSION

Suwannee River Fulvic Acid. The mass spectrum of SRFA is presented in Figure 2A. It can be seen that the spectrum (just as of any other NOM) is very complicated and contains thousands of peaks. Elemental compositions of SRFA molecules that are ionized in negative-ESI mode can be presented as $C_cH_hO_o$. A magnified portion of the SRFA mass spectrum is presented in Figure 2B. Major peaks correspond to monoisotopic compounds, and minor peaks correspond to ^{13}C isotopologue peaks.

The observed changes in mass spectra of the nonexchanged sample and of deuterated SRFA are represented in Figure 2 B,C

dx.doi.org/10.1021/ac402609x | Anal. Chem. 2013, 85, 11007−11013

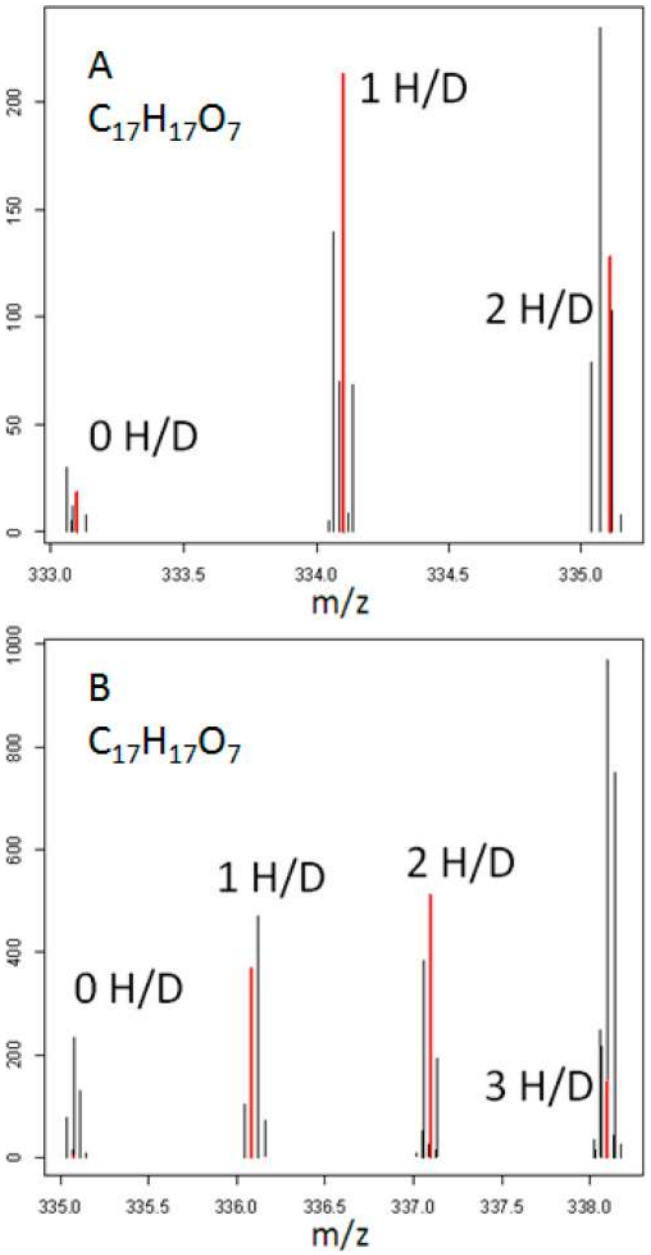


Figure 4. Distribution of H/D exchange peaks for SRFA compounds: (A) $C_{17}H_{17}O_7$ and (B) $C_{16}H_{16}O_8$.

and in Figure 3. It can be seen that, by use of ultra-high-resolution FTICR-MS, it is possible to unambiguously determine peaks corresponding to the H/D exchange reaction of a certain parent ion and quantitate those peaks.

H/D exchange of fast exchangeable hydrogens is always incomplete because of the interaction with traces of atmosphere water. As a consequence, the mass spectrum of H/D-exchanged SRFA becomes much more complicated as compared to the original one. We observed a factor of 3−4 increase in the number of peaks that can be reliably identified.

Filtration and extraction of peaks corresponding to H/D exchange reaction of the same parent ion is a challenging problem due to the shifting of peaks and disappearance of small peaks in the mass spectrum. To enumerate labile hydrogens in SRFA, we used the following procedure. First we observed that the parent (nondeuterated ion) is not always present in the H/D exchange mass spectrum, but the peak corresponding to one H/D exchange can be identified clearly. So for known elements in SRFA, we identified peaks corresponding to one H/D exchange, and for those peaks we extracted peaks with mass differences close to the mass difference of the H/D exchange reaction. Then those series were manually analyzed (see Supporting Information) to identify the number of labile hydrogens in each parent ion.

The peaks corresponding to the H/D exchange reaction of arbitrarily chosen compounds of SRFA are shown in Figure 4. It can be seen that isotopic distribution obeys binomial distribution. Relative intensities of peaks corresponding to the H/D exchange reaction of several compounds are presented in Table 1. We calculated the depth of H/D exchange reaction by use of the following formula:

$$P = \frac{1}{N}\frac{\sum_{i=0}^{N} i h_i}{\sum_{i=0}^{N} h_i} \quad (2)$$

Table 1. Number of Labile Hydrogens Observed in Different Compounds of SRFA

brutto formula[a]	D0[b]	D1	D2	D3	D4	d^c	P^d
$C_{16}H_{15}O_7$	2	27	10	0	0	3	0.6
$C_{16}H_{17}O_7$	5	81	37	0	0	6	0.63
$C_{16}H_{19}O_7$	17	180	75	0	0	3	0.6
$C_{17}H_{17}O_7$	3	46	29	0	0	3	0.66
$C_{17}H_{19}O_7$	10	113	50	0	0	4	0.61
$C_{18}H_{19}O_7$	6	41	16	0	0	5	0.58
$C_{15}H_{13}O_8$	0	26	78	36	0	4	0.69
$C_{14}H_{15}O_9$	0	83	256	71	0	1	0.65
$C_{16}H_{13}O_8$	0	22	65	18	0	3	0.66
$C_{15}H_{15}O_9$	3.3	155	232	82	0	3	0.61
$C_{16}H_{13}O_9$	0	16	67	44	0	6	0.74
$C_{14}H_{11}O_9$	0	13	60	93	21	15	0.66
$C_{14}H_{11}O_{10}$	0	10	53	73	50	9	0.72

[a]Elemental composition. [b]Intensity of peak corresponding to H/D exchange (D1, one exchange; D2, two exchanges, etc.). [c]Maximum mass difference between neighbor peaks and mass difference corresponding to H/D exchange (10^{-5} amu). [d]Depth of exchange.

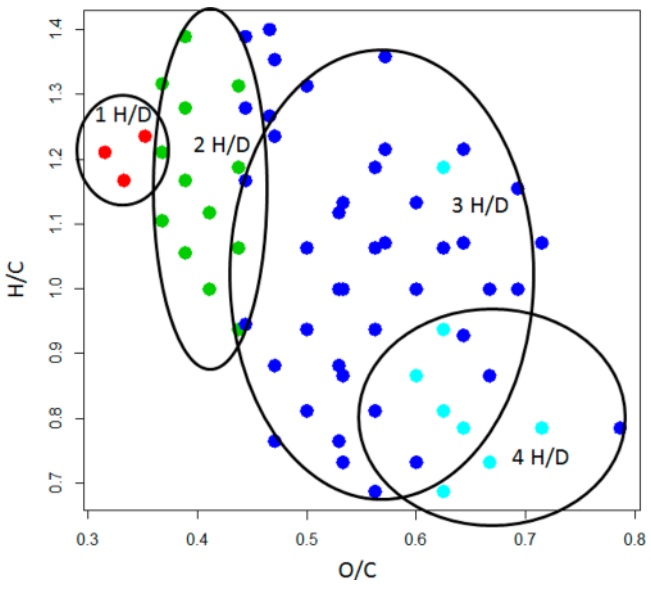


Figure 5. Van Krevelen diagram for reliably investigated SRFA compounds: (red) one H/D exchange, (green) two H/D exchanges, (dark blue) three H/D exchanges, (light blue) four H/D exchanges. The number of labile hydrogens in the molecule is bigger by 1 because ions were generated in negative mode and one hydrogen detached during ionization.

dx.doi.org/10.1021/ac402609x | Anal. Chem. 2013, 85, 11007−11013

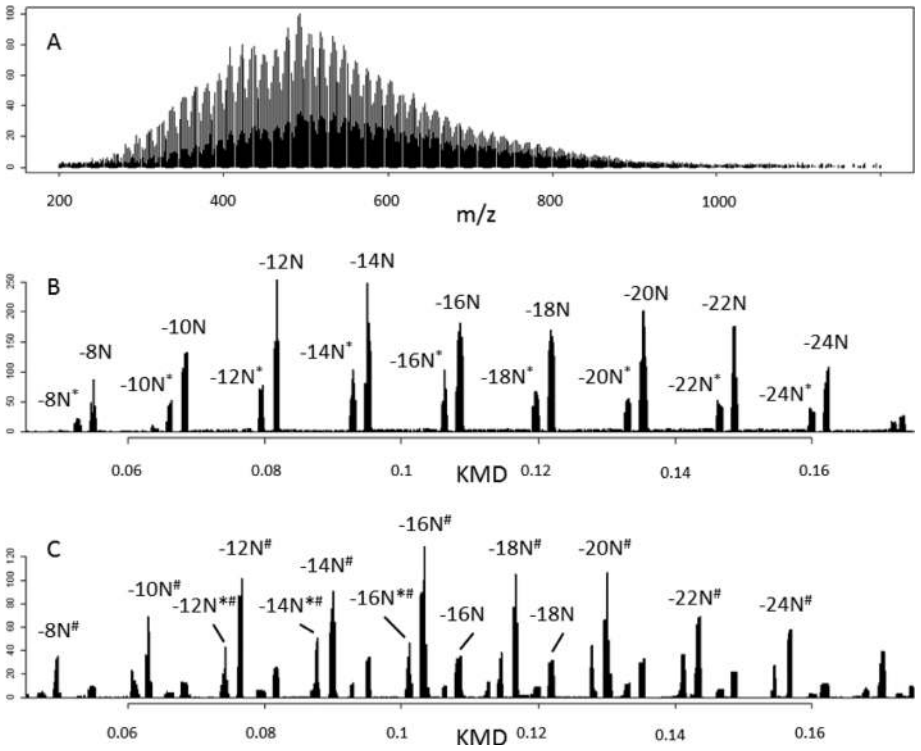


Figure 6. (A) Positive ESI FT ICR mass spectrum of Siberian crude oil. (B, C) Weighted Kendrick mass defect histograms for (B) normal and (C) H/D exchange conditions. (*) $^{12}C_{c-1}{}^{13}C_1$ species; (#) compounds with one H/D exchange that belong to the same homologous series.

Here h_i is the intensity of the peak corresponding to the ith H/D exchange and N is the total number of labile hydrogens.

In Figure 5 is presented the Van Krevelen diagram for reliably investigated parent ions. We can see the trend that the number of labile hydrogens increases with the number of oxygens in the molecule.

The latter trend and the fact that only hydrogens attached to carboxyl and hydroxyl groups are labile under soft H/D experimental conditions (in ESI source)[12] allow us to surmise that we can obtain information about types of oxygen in the molecule by comparing the number of labile hydrogens with the total number of oxygens. For example, the compound $C_{17}H_{17}O_7$ has three labile hydrogens. Even if all these hydrogens were from carboxyl groups (-COOH), there is still one oxygen left and it must stem from other functional groups, such as carbonyl or ether group or others.

Crude Oil. Crude oil is another example of very complex natural organic matter (Figure 6A).[13] Unlike dissolved organic matter, crude oil is composed of long homologous series that make it possible to implement a compact visual analysis method based on Kendrick mass defect approach.[14−16] The elemental composition of a crude oil molecule can be represented as $C_cH_{2c+Z}N_nO_o$, and then a homologous series formed by C_cH_{2c} can be referred to as ZN_nO_o. It is convenient to introduce a Kendrick mass scale by setting the mass of C_cH_{2c} fragment (14.015 65) to equal 14:

$$\text{Kendrick mass} = \frac{(\text{IUPAC mass})(14)}{14.01565} \quad (3)$$

Compounds with the same constitution of heteroatoms will have identical Kendrick mass defect (KMD):

$$\text{KMD} = \text{nominal Kendrick mass} - \text{exact Kendrick mass} \quad (4)$$

If one plots KMD versus mass, then compounds belonging to the same homologous series forms a straight horizontal line on the plot. This approach works perfectly for general analysis of crude oil, but for H/D exchange experiments it is more convenient to introduce a weighted Kendrick mass defect histogram. To create a weighted Kendrick mass defect histogram, one must first calculate Kendrick mass defect for all compounds and then plot a histogram where the number of entries of each KMD is proportional to the intensity of the corresponding peak.

In Figure 6B,C are presented weighted Kendrick mass defect histograms for crude oil under normal and H/D exchange conditions. Ions were generated in positive-ESI mode. We see that major homologous series are compactly represented, and for each series we observe a series corresponding to the isotope exchange $^{12}C \rightarrow {}^{13}C$. Under H/D exchange conditions we see that all series exchanged one hydrogen for deuterium. In positive-ESI mode, the molecule is ionized by attaching a proton, and the fact that we observe only one H/D exchange for all molecules indicates that all molecules in the fraction ionizing in positive-ESI mode do not have labile hydrogens. Only the ionizing proton exchanges.

We did not observe any differences in negative-ESI mass spectra of crude oil under normal and H/D exchange conditions. It proves that all molecules in this fraction have exactly one labile hydrogen and this hydrogen detaches during ionization.

Lignosulfonate. Previously investigated compounds (SRFA and crude oil) are products of the postmortem transformation of living organic matter. Lignosulfonate is a derivative of lignin, one of the most abundant organic polymers on Earth,[17] constituting a quarter to a third of the dry mass of wood. Lignosulfonate is also a complex mixture (see Figure 7A) containing many individual molecules. The elemental composition of the lignosulfonate molecule can be represented as $C_cH_hO_oS_s$. By the H/D exchange approach, it is possible to enumerate labile hydrogens in individual compounds. For example, we can see that compound $C_{10}H_{13}O_8S_2$ contains exactly three labile hydrogens.

dx.doi.org/10.1021/ac402609x | Anal. Chem. 2013, 85, 11007−11013

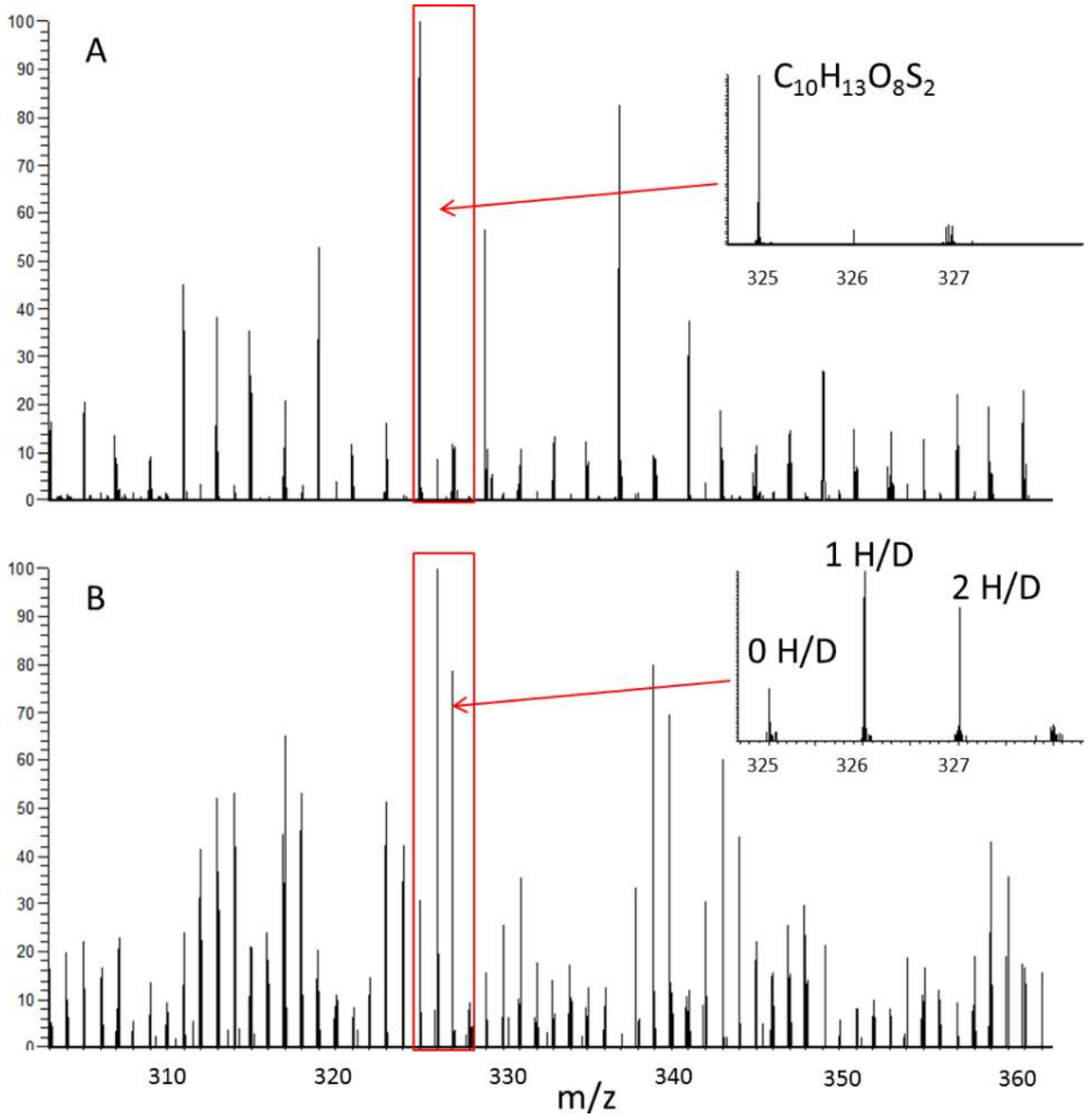


Figure 7. (A) FTICR mass spectra of lignosulfonate under (A) normal and (B) H/D exchange conditions (negative-ESI mode).

CONCLUSION

We demonstrate here the possibility to enumerate labile hydrogens simultaneously in all compounds of natural organic matter by using a simple experimental technique. Fulvic acids, crude oil, and lignosulfonate represents different degrees of transformation of living organic matter in nature. H/D exchange FTICR mass spectrometry is capable of bringing new insights into the investigation of carbon cycling in the Earth. Recent advances in FTICR, such as increased resolving power and dynamic range,[18,19] will be of great help for the further development of this technique.

ASSOCIATED CONTENT

Supporting Information

Manual analysis of mass spectral peaks as described in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail ennikolaev@rambler.ru.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was granted by Russian Foundation for Basic Research (Projects 12−08−33089-173 mol-a-ved, 13-04-40110-n komfi, 13-08-01445-a) and RFBR-CRDF project No 09-03-92500 and the Ministry of Science and Education (Grants 8149, 8153).

REFERENCES

(1) Perminova, I. V.; Konstantinov, A. I.; Kunenkov, E. V.; Gaspar, A.; Schmitt-Kopplin, P.; Hertkorn, N.; Kulikova, N. A.; Hatfield, K. In *Biophysico-Chemical Processes Involving Natural Nonliving Organic Matter in Environmental Systems*; Senesi, N., Xing, B., Huang, P. M., Eds.; Wiley−IUPAC Series in Biophysico-Chemical Processes in Environmental Systems; Wiley: Hoboken, NJ, 2009; Chapt. 13, pp 487−538.

(2) Abe, T.; Maie, N.; Watanabe, A. *Org. Geochem.* **2005**, *36*, 1490−1497.

(3) Cory, R. M.; McKnight, D. M. *Environ. Sci. Technol.* **2005**, *39*, 8142−8149.

(4) Stenson, A. C.; Marshall, A. G.; Cooper, W. T. *Anal. Chem.* **2003**, *75*, 1275−1284.

(5) Kunenkov, E. V.; Kononikhin, A. S.; Perminova, I. V.; Hertkorn, N.; Gaspar, A.; Schmitt-Kopplin, P.; Popov, I. A.; Garmash, A. V.; Nikolaev, E. N. *Anal. Chem.* **2009**, *81*, 10106−10115.

(6) Witt, M.; Fuchser, J.; Koch, B. P. *Anal. Chem.* **2009**, *81*, 2688−2694.

(7) Solouki, T.; Freitas, M. A.; Alomary, A. *Anal. Chem.* **1999**, *71*, 4719−4726.

(8) Alomary, A.; Solouki, T.; Patterson, H. H.; Cronan, C. S. *Environ. Sci. Technol.* **2000**, *34* (13), 2830−2838.

(9) Wales, T. E.; Engen, J. R. *Mass Spectrom. Rev.* **2006**, *25*, 158−170.

(10) Wolff, J.-C.; Laures, A. M.-F. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 3769−3779.

(11) Hunt, D. F.; Sethi, S. K. *J. Am. Chem. Soc.* **1980**, *102* (23), 6953−6963.

(12) Kostyukevich, Y.; Kononikhin, A.; Popov, I.; Nikolaev, E. *Anal. Chem.* **2013**, *85* (11), 5330−5334.

(13) Qian, K.; Rodgers, R. P.; Hendrickson, C. L.; Emmett, M. R.; Marshall, A. G. *Energy Fuels* **2001**, *15*, 492−498.

(14) Hughey, C. A.; Hendrickson, C. L.; Rodgers, R. P.; Marshall, A. G.; Qian, K. *Anal. Chem.* **2001**, *73*, 4676−4681.

(15) Hughey, C. A.; Rodgers, R. P.; Marshall, A. G. *Anal. Chem.* **2002**, *74*, 4145−4149.

(16) Marshall, A. G.; Rodgers, R. P. *Acc. Chem. Res.* **2004**, *37*, 53−59.

(17) Boerjan, W.; Ralph, J.; Baucher, M. *Annu. Rev. Plant Biol.* **2003**, *54*, 519−546.

(18) Kostyukevich, Y. I.; Vladimirov, G. N.; Nikolaev, E. N. *J. Am. Soc. Mass Spectrom.* **2012**, *23* (12), 2198−2207.

(19) Nikolaev, E. N.; Boldin, I. A.; Jertz, R.; Baykut, G. *J. Am. Soc. Mass Spectrom.* **2011**, *22* (7), 1125−1133.