Effective Algorithm of Charge State Determination in Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectra of Humic Substances

Erast V. Kunenkov[1], Andras Gaspar[2], Alexey S. Kononikhin[3], Philippe Schmitt-Kopplin[2], Irina V. Perminova[1], Andrey V. Garmash[1], Igor A. Popov[2], Eugeniy N. Nikolaev[2]

[1]Department of Chemistry, Lomonosov Moscow State University, Moscow, Russia, erast@mail.spunge.org

[2]HMGU – German Research Center for Environmental Health, Institute of Ecological Chemistry

[3]The Institute for Biochemical Physics Russian Academy of Sciences, Moscow, Russia

Humic substances (HS) are complex mixtures formed during decay of plant and animal materials. Ultrahigh resolution of Fourier Transform Ion Cyclotron Resonance mass spectrometry (FTICR MS) makes it the most powerful analytical tool for molecular level understanding of HS structure. However, only low molecular weight HS fraction is available for FTICR MS with electrospray (ESI) ion source. ESI FTICR mass spectrum of HS contains about ten thousands of peaks in mass range 250–1200 Da (the most intensive peaks are usually located near 400 Da) with tenths of peaks, appeared at each nominal mass with high regularity. This unique peak density provides a significant challenge for data interpretation.

ESI source is known to produce ions with different charge states. The common way of charge-state determination in high-resolution mass spectrometry is based on the fact that the organic species containing one or more ^{13}C atoms (M + 1 ions) will appear 1/z above the m/z of the corresponding monoisotopic (e.g., all ^{12}C) ions. This approach shows the most intensive peaks in HS ESI FTICR mass spectra are singly charged (1). However, it is not necessary applicable to all peaks in the spectrum, because this approach can't be directly applied to all peaks in the spectrum.

At first, the abundance ratio between species with one ^{13}C atom and all ^{12}C is 0.0111n, where n is a total number of carbon atoms in the molecule. This means ^{13}C peak is expected to have lower intensity for species containing lesser than 90 carbon atoms. Most of the peaks in HS ESI FTICR mass spectrum have low signal-to-noise ratios. Thus, correspondent ^{13}C peak may be just absent in the spectrum making impossible to determine charge state by isotopologue mass. High peak density also increases difficulty of higher charge states determination because the expected location of isotopologues

correspondent to high charge states is crossed with the location of monoisotopic peaks with higher fractional parts of their mass.

Recently, formation of doubly-charged ions from HS in ESI source was proved using ion mobility mass spectrometry (2). The goal of this work was to develop an effective algorithm of charge state charge state determination in HS ESI FTICR mass spectra, capable to determine charge states for monoisotopic peaks without ^{13}C isotopologues.

FT ICR mass spectra were acquired using 12 Tesla Apex Qe mass spectrometer with Apollo II electrospray ion source (Bruker Daltonics, Bremen, Germany) and 7 Tesla hybrid LTQ FT mass spectrometer with IonMax electrospray ion source (Finnigan, Bremen, Germany). Polymethacrylic acid (PAA) with M_w=3290 Da (Polymer Standard Service, Germany) was used as model compound for testing of the developed method. IHSS standard sample of Suwannee River fulvic acid (SRFA) was used as HS sample. The algorithm was implemented as a part of home-built FIRAN software developed for complex mixtures FTICR MS data treatment.

The developed algorithm is based on recently introduced Total Mass Difference Statistics (TMDS) approach (3, 4) for finding repetitive patterns in complex mixtures. This method finds a set of characteristic mass differences, which are frequently appearing between peaks in the mass spectrum, and corresponds to some repetitive structural units of the analyzed mixture. There are some series of peaks, which are differing one from each other by a number of a certain structural unit (e.g., a number of $-CH_2-$ groups in hydrocarbon skeleton). In this case, masses of these peaks will be differing one from each other by d/z, where d is mass of the structural unit, and z is the charge state of all peaks in series. The flowchart of the algorithm is presented on the Figure 1.

This algorithm was tested on model polymer compound (PAA, M_w=3290 Da). The major structural unit found using TMDS method is $C_4H_6O_2$ (PAA monomer unit). Charge states from –1 to –7 of 1901 peaks in mass range 197.1183...4025.6625 were determined successfully. The algorithm was also applied to FTICR mass spectra of SRFA sample acquired on different instruments. The structural units found in SRFA mass spectra were CH_2, CO, C_2H_2O, H_2, CH_2O, C_2H_4O and $C_3H_4O_2$. Peak series with more than 50 ions was found. Most of the species appeared to ionize in form of singly-charged or doubly-charged ions (or in both forms). The comparison between the number of peaks with found charge states determined by ^{13}C isotopologue masses and new algorithm are given in the table 1.
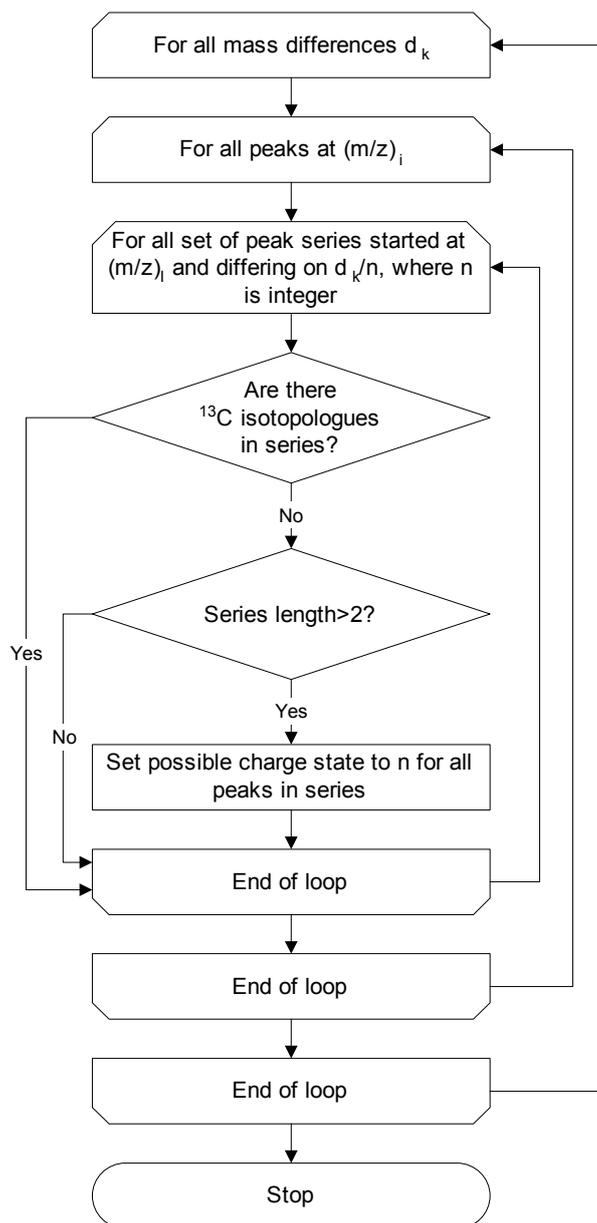
Figure 1. Flowchart of charge state detection algorithm based on
Total Mass Difference statistics.

Table 1. Comparison between efficiencies of the traditional charge state determination
algorithm based on ^{13}C isotopologue masses and new algorithm based on Total Mass
Difference statistics.

Instrument	Algorithm	Peaks with charge state detected			Estimated Mn
		1–	2–	Total	
12T Apex Qe	^{13}C	1324	740	2161	528
	New	3423	5169	9574	672
7T LTQ FT	^{13}C	808	–	808	639
	New	2876	140	3016	668

The obtained results shown the developed algorithm can be used effectively for treatment of ESI FTICR mass spectra of humic substances. It helps to improve the detection of charge states for both singly- and doubly-charged ions and to increase the wideness of molecular mass range available for analysis.

ACKNOWLEDGEMENTS

REFERENCES

1. Stenson, A. C.; Landing, W. M.; Marshall, A. G.; Cooper, W. T. Ionization and Fragmentation of Humic Substances in Electrospray Ionization Fourier Transform-Ion Cyclotron Resonance Mass Spectrometry // Anal. Chem. 2002, 74, 4397–4409.
2. Gaspar, A.; Kunenkov, E. V.; Lock, R.; Desor, V.; Perminova, I. V.; Schmitt-Kopplin, P. Combined utilization of Ion Mobility- and ultra high resolution-MS to identify multiple charged constituents in natural organic matter // Rapid Communications in Mass Spectrometry. 2008 (submitted).
3. Kunenkov E. V., Kononikhin A. S., Perminova I. V., Garmash A. V., Nikolaev E. N., Popov I. A. Analysis of FTICR-MS Data on Humic Substances and Synthetic Polyelectrolytes Using Different Data Processing Techniques. // Abstracts of the 1st International Symposium on Ultrahigh Resolution Mass Spectrometry for the Molecular level Analysis of Complex (BioGeo)Systems, 6-7 Nov. 2006, GSF, Oberschleissheim, Germany.
4. Kunenkov E. V., Kononikhin A. S., Perminova I. V., Gaspar A., Schmitt-Kopplin P., Hertkorn N., Popov I. A., Garmash A. V., Nikolaev E. N. Total Mass Difference Statistics Spectroscopy: A New Approach to Identification of High Mass Building Blocks in Electrospray Ionization Fourier Transform Ion Cyclotron Mass Spectrometry Data of Natural Organic Matter. // Anal. Chem. 2008 (submitted).