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Truly “exact” mass: Elemental composition can be determined uniquely from molecular mass measurement at ∼0.1 mDa accuracy for molecules up to ∼500 Da

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Abstract

At sufficiently high mass resolution, not all molecular masses are possible, even for arbitrary elemental composition. Here, we consider all possible elemental compositions, $C_cH_hN_nO_oS_s$, c unlimited, h unlimited, $0<n<5$, $0<o<10$, $0<s<3$, for even-electron ions $(M+H)^+$ and $(M-H)^-$, of molecular mass, 300–500 Da. Masses are then sorted into “bins” of width, 5, 1, 0.5, and 0.1 mDa, with inclusion (or not) of up to two ^{13}C’s. The number of different elemental compositions per bin varies from 0 to ∼20 for 5 mDa bins, dropping to 0–2 for 0.1 mDa bins (including one ^{13}C) and 0–1 for 0.1 mDa bins (not including ^{13}C). Thus, for molecules up to ∼500 Da in mass, mass resolution and accuracy of ∼0.1 mDa generally suffice to yield a unique elemental composition for molecules in even the most complex natural mixtures (namely, petroleum crude oil), and higher mass resolution and accuracy are not necessary. The required resolution and accuracy are now becoming available with high-field (9.4–14.5 T) Fourier transform ion cyclotron resonance mass spectrometry.

Keywords: Fourier transform mass spectrometry; Ion cyclotron resonance; ICR; Petroleum; Accurate mass

1. Introduction

This special issue of the *International Journal of Mass Spectrometry* celebrates Jürgen Kluge’s accomplishments, many of which feature ultraprecise determination of ions of a single mass or mass-to-charge ratio, *m*/*z* (analogous to weighing macroscopic objects on a single-pan balance). Recently, it has become possible to extend such measurements to the analog of a double-pan balance by trapping ions of two different *m*/*z* values simultaneously [1]. Although such measurements achieve extraordinary precision and accuracy predicted to approach 1 part in 10^{12}, the experiments are necessarily conducted at liquid helium temperature, for ions whose *m*/*z* values are nearly identical, so as to limit the frequency bandwidth (and thus noise) in the measurement. In this paper, we consider the analytical chemical problem of *broadband* accurate mass measurement, for complex organic mixtures whose mass spectral *m*/*z* values ranging over a factor of 2–10. Those experiments are typically conducted by Fourier transform ion cyclotron resonance mass spectrometry [2,3].

Accurate mass measurements offer a means to determine the chemical composition of complex organic mixtures such as petroleum [4,5], coal [6], humic/fulvic acids [7], and vegetable oils [8]; to identify proteins [9], and to assign amino acid compositions of peptides [10] and nucleic acid compositions in DNA and RNA [11]. For example, Spengler has shown how to apply accurate mass constraints to MS and MS/MS data, so as to improve the speed and accuracy of protein identification based on mass analysis. All of these methods basically rely on mass defects of various nuclides.

Every isotope of every chemical element has a different mass defect (i.e., difference in Daltons between its exact mass and the nearest integer mass) [12]. Moreover, because no two mass defects are integer multiples of each other, a molecule’s

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elemental composition, e.g., $C_cH_hN_nO_oS_s$, may be determined from sufficiently accurate measurement of its mass [13,14]. However, mass accuracy alone is not enough—it is necessary to *resolve* the masses of all components of a mixture in order to be sure that the measured mass corresponds to just one elemental composition.

Recently ultrahigh-resolution ($m/\Delta m_{50\%} > 300{,}000$, in which m is mass and $\Delta m_{50\%}$ is the mass spectral peak full width at half-maximum peak height) electrospray ionization (ESI) Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS (3)) has resolved more than 10,000 different elemental compositions in a single mass spectrum of petroleum crude oil [15] or coal [6], the most compositionally complex mixtures analyzed to date. Here, we establish the minimal mass resolution required to distinguish essentially all chemically possible (and chemically reasonable) elemental compositions for $C_cH_hN_nO_oS_s$ molecules of 300–500 Da, as encountered in fossil fuel mixtures. Because fossil fuel mixtures are the most compositionally complex, with tens of thousands of different elemental compositions spanning a relative abundance dynamic range of only ~10,000, they provide the most stringent test for mass resolution—all other organic mixtures will have fewer components and thus be easier to identify.

2. Methods

2.1. Computation methods

Possible elemental compositions within a stated mass range were calculated by use of a program compiled with Lab Windows/CVI version 6.0 (National Instruments Co., USA). A calculated list of compositions was saved in comma separated value format (.csv format) and processed by use of the Origin 5.0 program (OriginLab Corp., USA).

2.2. Chemical constraints

Not all possible elemental compositions are chemically possible. For example, the valence rules of chemical bonding lead to the "nitrogen rule" [16]. For even-electron ions, such as $(M + n\mathrm{H})^{n+}$ or $(M - n\mathrm{H})^{n-}$ generated by electrospray ionisation:

Even-mass ions have odd number of nitrogen atoms (1a)

Odd-mass ions have even number of nitrogen atoms (1b)

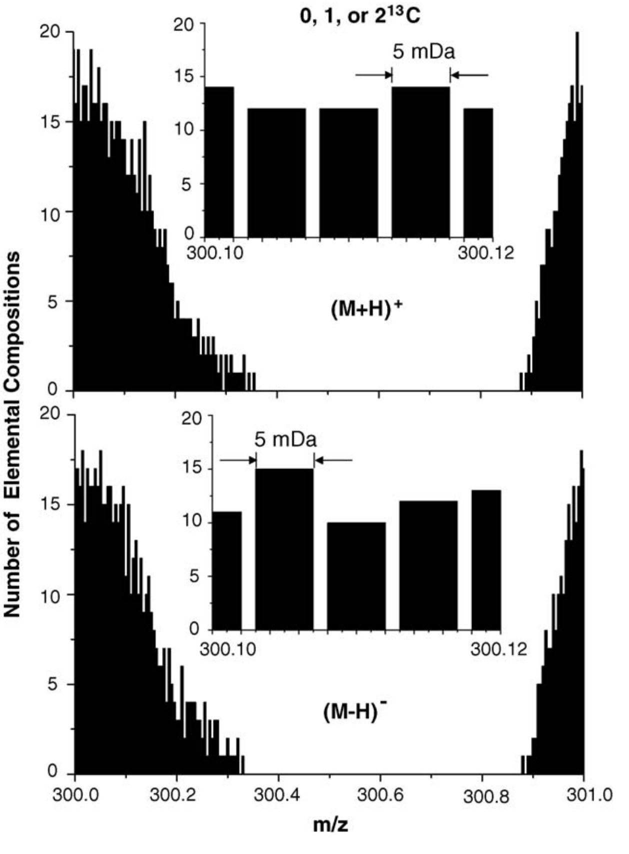

Fig. 1. Number of elemental compositions ($C_cH_hN_nO_oS_s$, c unlimited, h unlimited, $0 < n < 5$, $0 < o < 10$, $0 < s < 3$, including species containing up to two ^{13}C's) as a function of mass, in 5 mDa mass segments, for even-electron ions between 300 and 301 Da. *Top*: $(M + \mathrm{H})^+$ ions. *Bottom*: $(M - \mathrm{H})^-$ ions. *Insets*: mass scale expansion from 300.10 to 300.12.

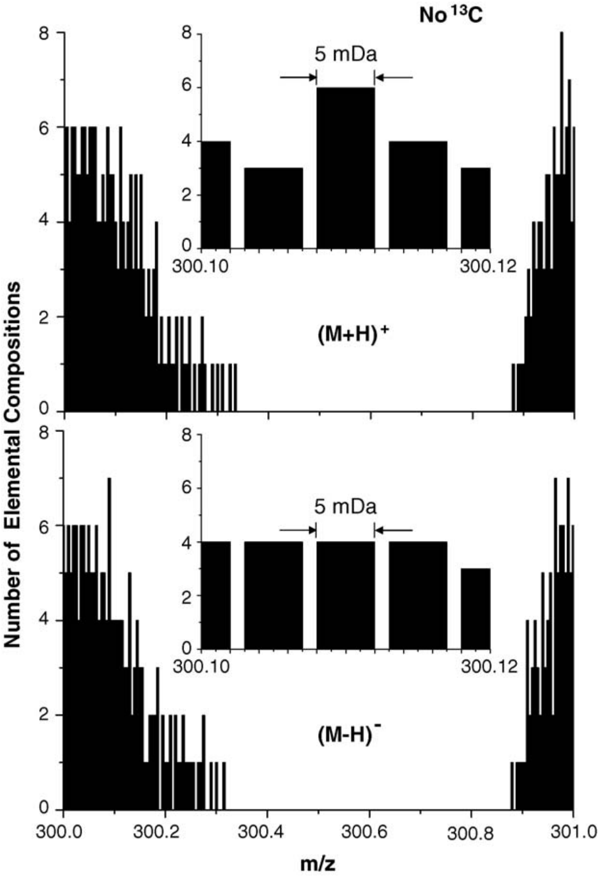

Fig. 2. Number of elemental compositions ($C_cH_hN_nO_oS_s$, c unlimited, h unlimited, $0 < n < 5$, $0 < o < 10$, $0 < s < 3$, excluding species containing ^{13}C) as a function of mass, in 5 mDa mass segments, for even-electron ions between 300 and 301 Da. *Top*: $(M + \mathrm{H})^+$ ions. *Bottom*: $(M - \mathrm{H})^-$ ions. *Insets*: mass scale expansion from 300.10 to 300.12.

whereas for odd-electron ions, such as $M^{+\bullet}$ radical cations generated by electron ionization or field desorption ionization,

Even-mass ions have even number of nitrogen atoms (1c)

Odd-mass ions have odd number of nitrogen atoms (1d)

Moreover, every additional ring or double bond reduces the number of hydrogen atoms by two. Thus, the "double bond equivalents" (DBE), namely, the number of rings plus double bonds, are related to the numbers of carbon, hydrogen, and nitrogen atoms according to [16]:

$$\mathrm{DBE} = c - \frac{h}{2} + \frac{n}{2} + 1 \tag{2}$$

Thus, for example, two molecules of the same nominal (nearest-integer) mass cannot differ in elemental composition by NH_2 versus O, because DBE must be an integer. We include these constraints in determining the possible masses for various $C_cH_hN_nO_oS_s$ compositions.

2.3. Other compositional constraints

Not all possible elemental compositions will be encountered in "real" mixtures. For example, a given molecule will not contain all nitrogens or all oxygens. For molecules of the types typically encountered in fossil fuels, we therefore additionally constrain the possible elemental compositions to $C_cH_hN_nO_oS_s$, with c unlimited, h unlimited, $0 < n < 5$, $0 < o < 10$, and $0 < s < 3$. Moreover, although a molecule with c carbon atoms may contain up to c ^{13}C's, ^{13}C is only 1% naturally abundant, so the relative abundance of molecules (of mass less than 1000 Da) containing more than two ^{13}C atoms is usually below the threshold (typically 5σ of baseline noise) in a mass spectrum. We therefore consider molecules containing at most two ^{13}C atoms.

Moreover, the assignment of an experimentally determined mass to an elemental composition containing ^{13}C atoms can readily be tested by looking for the mass spectral peak whose mass corresponds to the same elemental composition with all ^{12}C atoms. If such a peak is not found, then the assignment to a ^{13}C-containing species may be excluded. Thus, we shall consider molecules containing 0 or 0, 1, or 2 ^{13}C atoms. Thus, we consider two sets of compositional constraints: namely, molecules containing either no ^{13}C atoms or up to two ^{13}C atoms, because both types of searches are in use.

2.4. Mass bins

The smallest mass difference commonly encountered in broadband mass spectra of fossil fuels [4,5,17] or peptides [18] is 3.4 mDa, corresponding to elemental compositions differing by $^{12}C_3$ versus $^{32}SH_4$. The closest mass doublet resolved and identified to date in such spectra, 1.1 mDa, is $^{12}C_4$ versus $^{13}CH_3{}^{32}S$ [19].

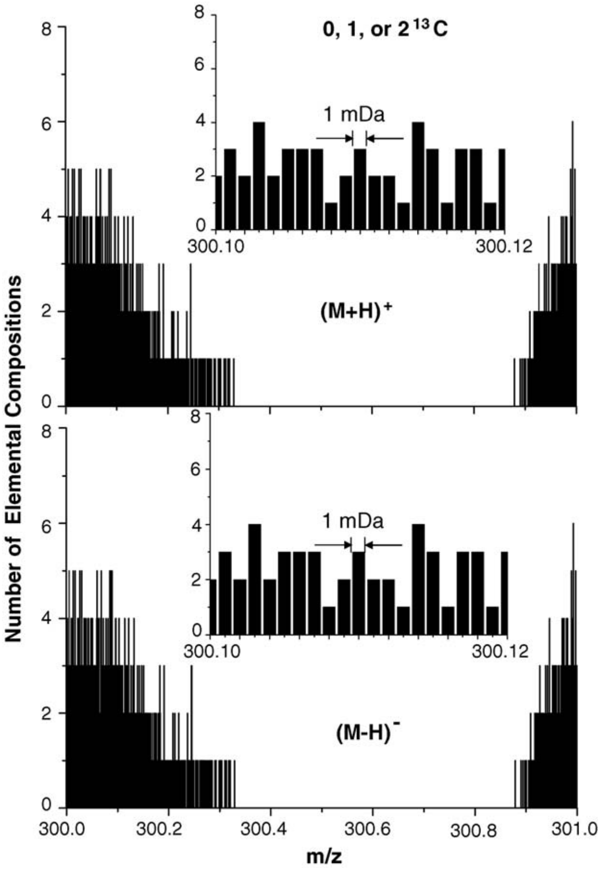

Fig. 3. As for Fig. 1, but for 1 mDa mass segments.

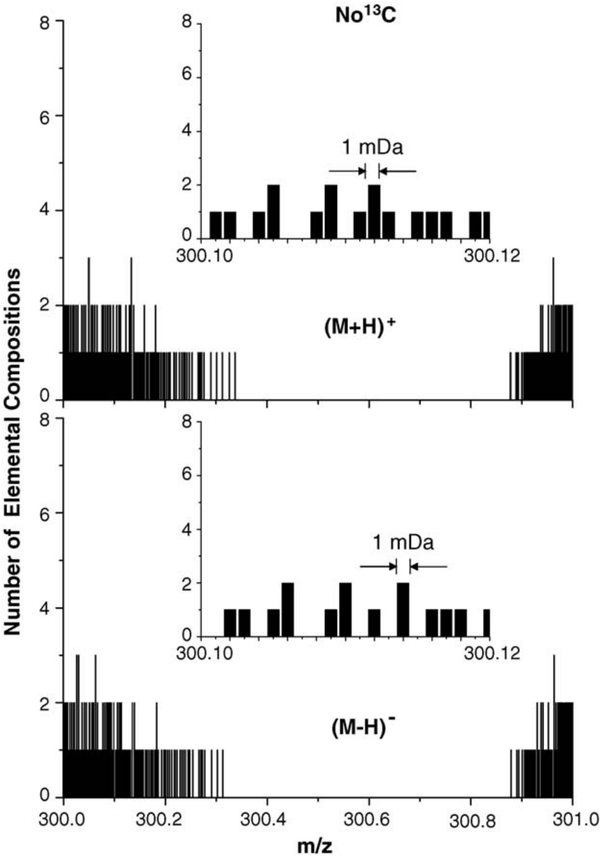

Fig. 4. As for Fig. 2, but for 1 mDa mass segments.

3. Results and discussion

3.1. Sorting elemental compositions according to mass

We begin by pooling all of the chemically possible (and chemically reasonable, as noted above) elemental compositions, $C_cH_hN_nO_oS_s$, c unlimited, h unlimited, $0 < n < 5$, $0 < o < 10$, and $0 < s < 3$, for even-electron ions $(M + \mathrm{H})^+$ and $(M - \mathrm{H})^-$, of molecular mass, 300–500 Da, into mass "bins" of various defined width. The width of a "bin" may be thought of as the smallest mass difference that is experimentally resolvable for two mass spectral peaks of equal height and width: alternatively, the mass "bin" width may be defined as the width of a given mass spectral peak at half-maximum peak height.

Fig. 1 shows a distribution of number of elemental compositions as a function of mass, sorted into mass "bins" each 5 mDa wide, for positive (top) and negative (bottom) even-electron ions containing up to two ^{13}C's. Note the complete absence of elemental compositions spanning ~0.5 Da between ~300.35 and 300.85 Da—a similar result obtains for species between 301 and 302 Da, except for differences in species containing nitrogens according to (Eqs. (1a) and (1b)). Moroever, the blank window shifts in position, but remains ~0.5 Da wide, as mass increases to ~500 Da. Over the ~0.5 Da range within which most elemental compositions fall, the number of possible elemental compositions per bin averages ~15, with a high of 37, and essentially no empty bins. Fig. 2 shows a similar distribution, but without including species containing ^{13}C. The shape of the distribution is essentially the same, but the height is lower because of the absence of species with ^{13}C.

Narrowing the mass bin width from 5 to 1 mDa (Figs. 3 and 4) reduces the average number of elemental compositions per bin to ~1 (without ^{13}C) to 2–3 (including up to two ^{13}C's). Further narrowing of the mass bin width to 0.5 mDa (Figs. 5 and 6) reduces the number of elemental compositions per bin to an average of 1 (without ^{13}C) to ~2 (including up to two ^{13}C's). However, adjacent bins are in general occupied, so that actual mass resolution would need to be smaller than the stated bin width in order to ensure confident assignment.

By now the trend is clear. Narrowing the bin width to 0.1 mDa (Figs. 7 and 8) achieves unique compositional assignment per bin in most cases, often with empty bins on either side (for even more confidence in elemental compositional assignment). Finally, although only even-electron species are considered here, the distributions of elemental compositions versus mass are essentially similar for odd-electron ions.

3.2. Prospects for experimental realization of unique elemental composition assignment

Recent electron ionization (EI) [19], electrospray ionization (ESI) [20], field desorption ionization (FDI) [21], and atmospheric pressure photoionization (APPI) [22] FT-ICR mass

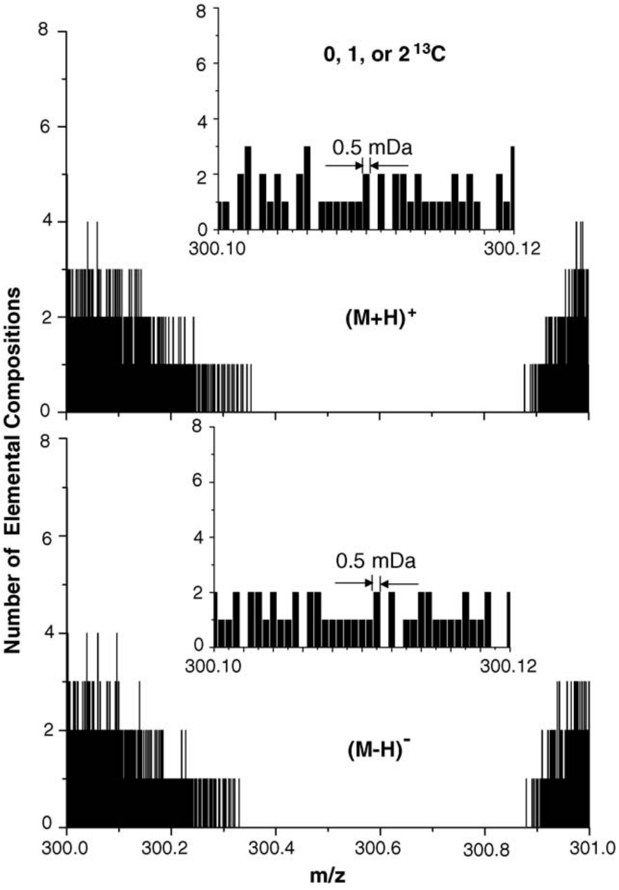

Fig. 5. As for Fig. 1, but for 0.5 mDa mass segments.

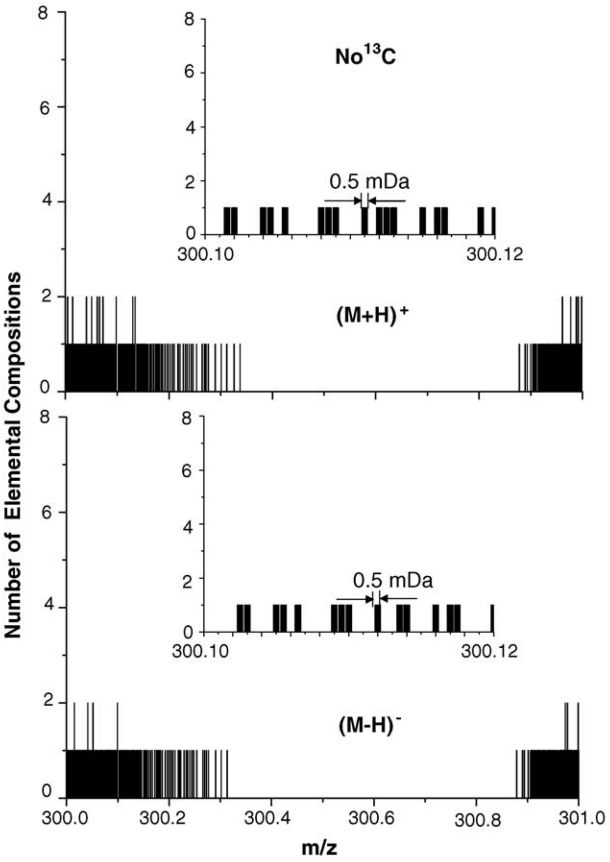

Fig. 6. As for Fig. 2, but for 0.5 mDa mass segments.

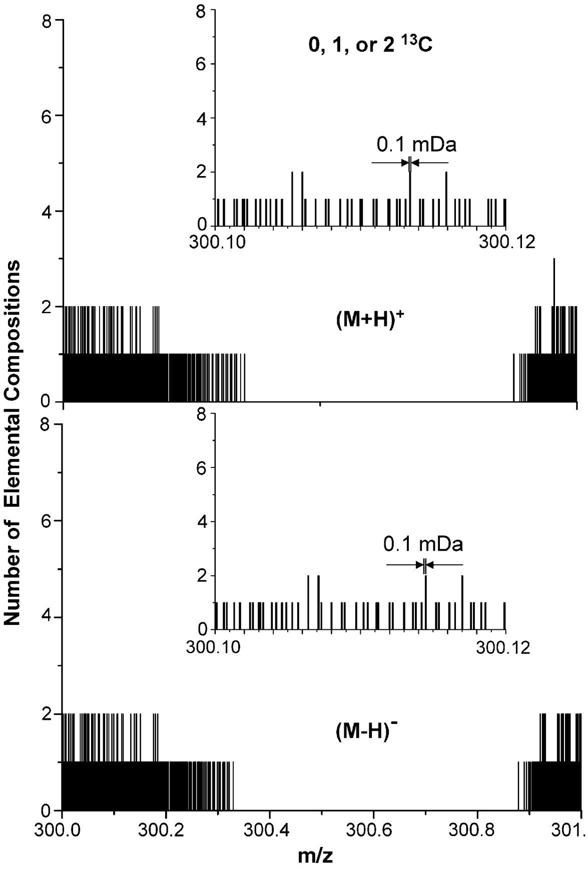

Fig. 7. As for Fig. 1, but for 0.1 mDa mass segments.

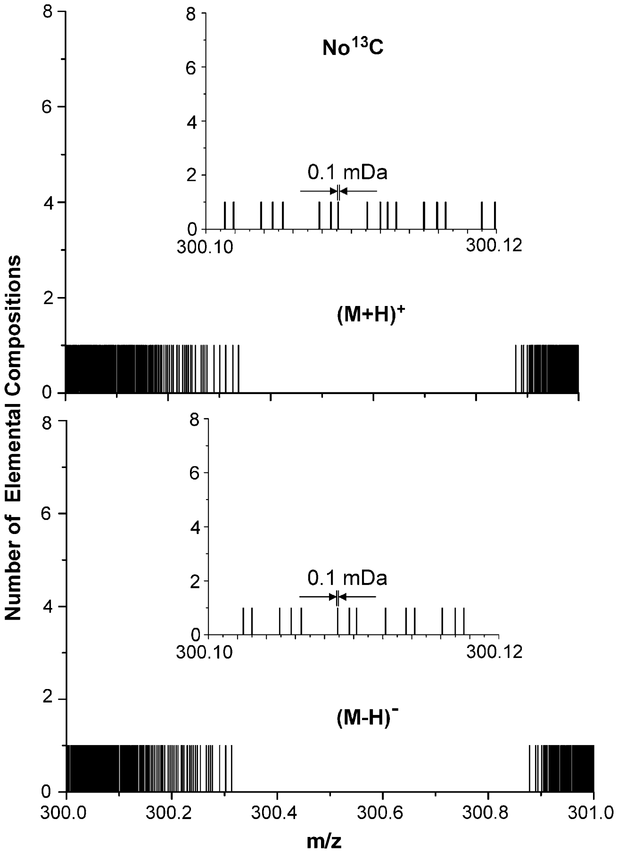

Fig. 8. As for Fig. 2, but for 0.1 mDa mass segments.

spectrometry at mass resolving power, $m/\Delta m_{50\%} > 400{,}000$, have achieved mass measurement accuracy of ∼200 ppb (e.g., 1 mDa at 500 Da). However, it is important to recognize that mass measurement *precision*, $m/\sigma(m)$, in which $\sigma(m)$ is the standard deviation from many repeated measurements, is related to peak height-to-noise ratio, S/N, and the number of data points per peak width of a single spectrum according to [23]:

$$\frac{m}{\sigma(m)} = K\left(\frac{\mathrm{S}}{\mathrm{N}}\right)\sqrt{\text{data points per peak width}} \tag{3}$$

in which K is a peak shape-dependent factor of order unity. Thus, for sufficiently high S/N and number of data points per peak width, it is possible to measure mass with imprecision much less than the mass spectral peak width. Nevertheless, Eq. (3) applies only if the observed mass spectral peak derives from a *single* elemental composition. Mass *resolution* (namely, the mass difference between two barely resolved mass spectral peaks) must still be able to distinguish between compositions of closely spaced mass, before Eq. (3) may be applied. In any case, we shall show that current FT-ICR MS performance, approaching 0.1 mDa resolution, can be expected to achieve unique elemental composition assignment up to ∼500 Da (and higher, if homologous series of compounds can be identified based on their identical Kendrick mass defects [24]).

Two recent developments promise to improve FT-ICR broadband mass measurement accuracy significantly. First, simultaneous excitation and detection enable deconvolution-based spectral phase correction to yield absorption-mode rather than magnitude-mode spectral display [25], with a corresponding improvement in mass resolving power by a factor ranging from $\sqrt{3}$ to 2 [26]. Second, mass resolving power increases linearly with increasing magnetic field strength [27]. Thus, a broadband FT-ICR mass spectrum of petroleum acquired at 3.0 T had to be generated by stitching together many individual spectral segments [17], whereas FT-ICR mass spectra at 7 T [28] or 9.4 T [15] may be acquired in a single spectral band. There is thus good reason to expect improvement in proceeding from 9.4 T (the highest field at which the most complex organic mixtures have yet been analyzed), to the recently installed 14.5 T system at the National High Magnetic Field Laboratory in Tallahassee, FL. Moreover, a 110 mm diameter bore 21 T superconducting magnet for FT-ICR MS is currently under development at NHMFL.

4. Conclusion

The present analysis will suffice for elemental compositions of any five elements, because virtually all other elements are heavier than C, H, N, and O, so that fewer combinations of those elements (and thus fewer possible masses to resolve at each nominal mass) are possible at a molecular mass below 500 Da. Also, in this paper, we considered C, H, N, O, and S compositions because they are the most common for high-resolution mass spectra derived from many common ionization methods (electron ionization, electrospray, field desorption, APPI) applied to environmental organic matter and fossil fuels. Adding, e.g., a few Na or P will not significantly affect the main conclusion (i.e., that 0.1 mDa resolution is enough to ensure unique mass assignment for virtually all chemically reasonable elemental compositions in the stated mass range).

It is reasonable to wonder how much higher resolution would be necessary to resolve mass differences if more elements were included in the possible elemental compositions. The answer obviously depends on the choice of the sixth (or additional) element(s). With 80+ other elements (many with several isotopes of comparable abundance) to choose from, one has to limit the analysis somewhere, and we chose here to cut off at five elements (i.e., enough to handle 98% of the elemental compositions encountered in the most complex mixtures analyzed to date (i.e., fossil fuels and their products; humic/fulvic acids, dissolved organic matter, etc.). Also, other tricks can be invoked (e.g., matching isotopic distributions for species containing halogens, transition metals, etc.), so that one need not rely on mass alone.

Our own extensive library of FT-ICR mass spectra at 9.4 T [29,30] demonstrates that mass accuracy (which has been shown to be approximately the same as mass precision [31] of 200 ppb over a range from $300 < m/z < 800$ is routinely achievable (i.e., ~0.1 mDa at 500 Da, as in Figs. 7 and 8). Preliminary data from our 14.5 T instrument suggest that ±50 ppb may be achievable there. To realize that performance, double-precision arithmetic is necessary to provide the requisite digital accuracy. Mass resolution of ~0.5 mDa is sufficient to resolve virtually all of the close mass doublets encountered in proteomics [18] and petroleomics [4]: e.g., $^{12}C_3$ versus $^{32}S_1H4$, 3.4 mDa; $^{1}H_3{}^{32}S^{13}C$ versus $^{12}C_4$, 1.1 mDa).

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