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Comparison and interconversion of the two most common frequency-to-mass calibration functions for Fourier transform ion cyclotron resonance mass spectrometry

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Abstract

In a perfect three-dimensional axial quadrupolar electrostatic potential field, Ledford et al. showed that the frequency-to-mass calibration relation $m/z = A_L/\nu + B_L/\nu^2$ is valid for ions of any mass-to-charge ratio, $m/z < (m/z)_{\text{critical}} = e\,B_0^2 a^2/4V_{\text{trap}}\alpha$, in which ν is the "reduced" (observed) ion cyclotron frequency, e is the electronic (elementary) charge, z is the number of elementary charges per ion, B_0 is magnetic field induction, a is a characteristic trap dimension, v_{trap} is the potential applied to each trap endcap, α is a constant determined by the trap geometrical configuration, and A_L and B_L are constants that are determined by fitting experimental ion cyclotron resonance (ICR) frequencies for ions of at least two known masses in a Fourier transform ICR (FT-ICR) mass spectrum. In the further limit that $m/z \ll (m/z)_{\text{critical}}$, Francl et al. obtained a different frequency-to-mass relation $m/z = A_F/(B_F + \nu)$. Here, we rederive both frequency-to-mass relations to derive a simple conversion between A_L and B_L, versus A_F and B_F (e.g. for comparing calibrated FT-ICR mass spectral data from different vendors). For accurate mass measurement, the conversion introduces a small error (a few parts per billion) that can usually be neglected. More important, by applying both calibration equations to the same experimental time-domain data, we find that mass accuracy resulting from the two calibration functions (or their interconversion) is indistinguishable, because Ledford et al.'s validity criterion, $m/z < 0.001\,(m/z)_{\text{critical}}$, is generally satisfied for modern high-field instruments with optimized cell geometry. Interestingly, a small difference may result when different forms of the same calibration function are employed, presumably due to different roundoff errors in the calculation. (Int J Mass Spectrom 195/196 (2000) 591–598) © 2000 Elsevier Science B.V.

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1. Introduction

Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry (MS) [1] offers by far the highest mass resolving power and mass accuracy of any broadband mass analysis technique. For example, it is now possible to determine simultaneously (and routinely) the masses of hundreds of species over a

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Dedicated to Bob Squires for his many seminal contributions to mass spectrometry and ion chemistry.

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mass range of 100–300 Da with sub-parts-per-million (ppm) mass accuracy [2]. At higher mass, FT-ICR MS has resolved isotopic peaks (i.e. species whose nominal masses differ by 1 Da) for a protein of 112 kDa [3], and has resolved isotopic “fine structure” (i.e. species of the same nominal mass but different elemental composition) for proteins up to 16 kDa, at a mass resolving power, $m/\Delta m_{50\%} \sim 8\,000\,000$ [4], in which $\Delta m_{50\%}$ is the mass spectral peak width at half-maximum peak height.

High mass resolving power and mass accuracy occur for several reasons. First, mass is determined by measurement of *frequency*, a parameter that is more precisely measurable than any other. Second, the time stability of the measurement depends ultimately on the time stability of the magnetic field that defines the ion cyclotron frequency, and superconducting magnets routinely achieve a time stability of a few parts per billion per hour (ppb/h). ICR mass precision is thus inherently higher than that from a Paul (quadrupole) ion trap, whose time stability ultimately depends on the time stability (typically 100 ppb/h) of the rf voltage that confines the ions. Third, in a spatially uniform (typically a few ppm over a few centimeters diameter spherical volume) magnetic field, ICR frequency does not depend on ion speed: as the ion linear speed increases, the cyclotron radius increases proportionately, so that the angular (cyclotron) frequency is unchanged. Fourth, unlike ion-beam-based mass measurements, ICR does not require the use of narrow slits (with resultant loss in signal-to-noise ratio) to achieve high mass precision. Fifth, the behavior of ions near the center of a Penning trap [5] (i.e. an axial static magnetic field and an electrostatic potential generated from opposed endcap electrodes in an orthorhombic, cylindrical, or hyperbolic configuration) is very accurately described by a three-dimensional axial quadrupolar potential [6]. Moreover, the electric potential field from an alternating voltage to a pair of opposed side electrodes of a Penning trap is very accurately represented by an electric dipolar potential field, and subsequent detection of an alternating induced charge on the same (or an orthogonal) pair of side electrodes is essentially independent of ion axial position in the trap [6]. Thus, unlike a Paul trap, for which the electrodes must be machined very accurately to produce the correct electric potential field shape, a Penning trap produces sufficiently accurate dc and rf electric potentials from less accurately machined surfaces of ordinary shape (e.g. flat, circular).

One might wonder why mass calibration in FT-ICR MS works as well as it does. After all, the effects of spatial nonideality in the magnetic, electrostatic, and alternating electric fields have been explored in great detail, and include the introduction of sidebands [7,8], cyclotron frequency shift and drift [9], mass-dependent axial ejection [10,11], space charge [12], and coalescence of closely spaced resonances [13]. Remarkably, however, ultrahigh mass accuracy (to sub-ppm) may nevertheless be produced from a frequency-to-mass conversion formula derived for spatially uniform axial magnetic field and three-dimensional axial quadrupolar electrostatic trapping potential. First, even at large radial or axial separations from the center of a Penning trap, the rapid periodic cyclotron and axial motions of an ion effectively time average (and thus eliminate) spatial nonidealities [14] in much the same way that physical spinning of a nuclear magnetic resonance (NMR) sample averages the effect of spatial inhomogeneity in an NMR experiment [15]. Thus, although it is possible to “shim” the dc and rf electric potential fields by cutting a Penning trap into smaller segments and applying appropriate voltages to the various segments [16], it isn’t necessary in practice for most applications. Second, the frequency-to-mass conversion formula is readily adapted to an actual Penning trap by insertion of calculable “geometrical” factors that correct for the finite dimensions and nonideal shape of the various electrodes of the trap [6,17]. Because those factors do not change the functional form of the frequency-to-mass conversion formula, mass “calibration” (namely, the matching of experimental ICR frequencies to masses for “calibrant” ions of known elemental composition) may be carried out by a simple least-squares best fit, whose coefficients are generated directly from the data.

We begin by noting that the two most commonly used frequency-to-mass conversion formulas for FT-

ICR MS both originate from the same idealized model fields. Ledford et al. [18] derived a frequency-to-mass conversion that is exact for ions of any mass-to-charge ratio, $m/z < (m/z)_{\text{critical}}$ (see below) in a spatially uniform magnetic field and a three-dimensional axial quadrupolar electrostatic potential. Francl et al. [19] proposed a different formula based on the more restrictive condition $m/z \ll (m/z)_{\text{critical}}$. (Both frequency-to-mass conversion formulas require least-squares fit of experimental data to a two-parameter equation and thus require at least two mass calibrants of known elemental composition.) Thus, the Francl formula is inherently less accurate. We provide a direct comparison of the two formulas, both applied to the same time-domain data, to determine whether or not the Francl approximation reduces mass accuracy. Finally, because both methods are in common use, we show how to convert FT-ICR mass measurements produced from one formula to mass measurements produced by the other formula.

2. Periodic motions of an ion in a Penning trap

In a spatially uniform static magnetic field of induction B_0, an ion of mass m and charge q, undergoes "unperturbed" ion cyclotron rotation at angular frequency ω_c, in which

$$\omega_c = \frac{qB_0}{m} \tag{1}$$

Application of a dc voltage V_{trap} to each of the two endcap electrodes of a Penning trap confines ions in the axial direction (i.e. along or opposed to the magnetic field direction), by introducing a potential that varies approximately quadratically with axial z position. As a result, ions execute harmonic oscillator in the z direction, at an angular frequency ω_z [5]

$$\omega_z = \sqrt{\frac{2qV_{\text{trap}}\alpha}{ma^2}} \tag{2}$$

in which α, the trapping scale factor, ranges from 2 to 4, depending on the trap geometry (e.g. $\alpha = 2.77373$ for a cubic trap) [1]; and a is a characteristic dimension of the ICR cell (e.g. for a cubic trap, a is the length of one side).

By virtue of Laplace's equation, the quadratic variation in electrostatic potential as a function of z must be accompanied by a quadratic variation as a function of radial position r [1]. The resulting radially outward-directed force thus reduces (slightly) the effect of the radially inward-directed Lorentz force that produces cyclotron rotation. In a plane perpendicular to the magnetic field (r is the radial distance between the ion and the trap symmetry axis (z axis)), the force on an ion is

$$\text{Force} = m\omega^2 r = qB_0\omega r - \frac{qV_{\text{trap}}\alpha}{a^2}\, r \tag{3}$$

or

$$\omega^2 - \frac{qB_0\omega}{m} + \frac{qV_T\alpha}{ma^2} = 0 \tag{4}$$

The quadratic Eq. (4) yields two solutions for ion rotational frequency in a plane perpendicular to the magnetic field:

$$\omega_+ = \frac{\omega_c}{2} + \sqrt{\left(\frac{\omega_c}{2}\right)^2 - \frac{\omega_z^2}{2}}$$

$$\text{(Reduced ion cyclotron frequency)} \tag{5}$$

$$\omega_- = \frac{\omega_c}{2} - \sqrt{\left(\frac{\omega_c}{2}\right)^2 - \frac{\omega_z^2}{2}}$$

$$\text{(Magnetron frequency)} \tag{6}$$

One solution ω_+ is close in value to the unperturbed cyclotron frequency [Eq. (1)], and is called the "reduced" cyclotron frequency (because the frequency is slightly lower than the unperturbed value). The other solution ω_- represents a new "magnetron" rotation.

At this stage it is convenient to rearrange the magnetron frequency expression, Eq. (6), so that we may evaluate its behavior in the limit of low mass-to-charge ratio. We begin by moving the factor, $(\omega_c/2)^2$, outside the square root argument in Eq. (6).

$$\omega_- = \frac{\omega_c}{2} - \frac{\omega_c}{2}\sqrt{1 - \frac{2\omega_z^2}{\omega_c^2}} \tag{7}$$

Substituting for ω_z from Eq. (2), Eq. (7) becomes

$$\omega_- = \frac{\omega_c}{2} - \frac{\omega_c}{2}\sqrt{1 - \frac{4V_{\mathrm{trap}}\alpha m}{qB_0^2a^2}} \tag{8}$$

If we now define a "critical" mass-to-charge ratio, $(m/z)_{\mathrm{critical}}$,

$$\left(\frac{m}{z}\right)_{\mathrm{critical}} = \frac{eB_0^2a^2}{4V_{\mathrm{trap}}\alpha} \tag{9}$$

then Eq. (8) takes the form,

$$\omega_- = \frac{\omega_c}{2} - \frac{\omega_c}{2}\sqrt{1 - \frac{m/z}{(m/z)_{\mathrm{critical}}}} \tag{10}$$

In the limit,

$$m/z \ll m_{\mathrm{critical}}/z \tag{11}$$

we may apply a Taylor (or binomial) approximation,

$$(1 \pm x)^n \approx 1 \pm nx,\ x \ll 1 \tag{12}$$

to Eq. (8) to obtain

$$\omega_- \approx \frac{\omega_c}{2} - \frac{\omega_c}{2}\left(1 - \frac{2V_{\mathrm{trap}}\alpha m}{qB_0^2a^2}\right) \tag{13}$$

or simply (remember that $\omega_c = qB_0/m$)

$$\omega_- \approx \frac{V_{\mathrm{trap}}\alpha}{B_0a^2} \tag{14}$$

Adding Eqs. (5) and (6) reveals that

$$\omega_+ + \omega_- = \omega_c \tag{15}$$

or, in the limit $m/z \ll (m/z)_{\mathrm{critical}}$, substituting Eq. (14) into Eq. (15) gives

$$\omega_+ = \omega_c - \omega_- \approx \omega_c - \frac{V_{\mathrm{trap}}\alpha}{B_0a^2} \tag{16}$$

Thus, the observed cyclotron frequency ω_+ is "reduced" from the unperturbed cyclotron frequency ω_c by the (approximately *m/z*-independent) magnetron frequency ω_-.

3. Mass calibration

In the absence of an electrostatic potential field, ion cyclotron frequency and mass are related by Eq. (1), and mass calibration would simply require measurement of the ICR frequency for ions of a single mass-to-charge ratio. That approximation is still reasonably accurate over a very narrow *m/q* range. However, because the experimentally observed cyclotron frequency is, in fact, the "reduced" cyclotron frequency of Eq. (5), it is necessary to manipulate Eq. (5) to provide an appropriate frequency-to-mass conversion formula that will be valid over a wide mass-to-charge ratio range. We begin by rearranging Eq. (5) to the form

$$\omega_+ - \frac{\omega_c}{2} = \sqrt{\left(\frac{\omega_c}{2}\right)^2 - \frac{\omega_z^2}{2}} \tag{17}$$

After squaring both sides, we obtain the quadratic expression,

$$\omega_+^2 - \omega_c\omega_+ + \frac{\omega_z^2}{2} = 0 \tag{18}$$

Substituting for ω_c from Eq. (1) and ω_z from Eq. (2) gives

$$\omega_+^2 - \frac{qB_0\omega_+}{m} + \frac{qV_{\mathrm{trap}}\alpha}{ma^2} = 0 \tag{19}$$

Multiplying by m/ω_+^2 gives the frequency-to-mass conversion relation of Ledford et al. [18], in which we have made use of the relation $q = ze$, in which z is the number of elementary charges per ion, and e is the elementary charge.

$$m/z = \frac{eB_0}{\omega_+} - \frac{eV_{\mathrm{trap}}\alpha}{a^2\omega_+^2} \tag{20}$$

or

$$\frac{m}{z} = \frac{A_{\mathrm{Ledford}}}{\omega_+} + \frac{B_{\mathrm{Ledford}}}{\omega_+^2} \tag{21a}$$

in which

$$A_{\mathrm{Ledford}} = eB_0 \tag{21b}$$

$$B_{\mathrm{Ledford}} = \frac{eV_{\mathrm{trap}}\alpha}{a^2} \tag{21c}$$

The Ledford frequency-to-mass conversion formula, Eqs. (21a)–(21c), is valid whenever $m/q < m_{\mathrm{critical}}/q$, i.e., whenever cyclotron orbital motion is stable [1]. In practice, it is usual to assign absolute values to ω_+, e, z, q, and V_{trap}, so that B_{Ledford} is negative for either positive or negative ions.

In the more restrictive limit $m/q \ll m_{\mathrm{critical}}/q$, a different frequency-to-mass relation may be derived starting from Eq. (16).

$$\omega_+ \approx \frac{qB_0}{m} - \frac{V_{\mathrm{trap}}\alpha}{B_0 a^2} \tag{22}$$

Multiplying by m, and grouping terms in m, we obtain an expression originally proposed by Francl et al. [19] (again note that $q = ze$)

$$\frac{m}{z} \approx \frac{eB_0}{\omega_+ + (V_{\mathrm{trap}}\alpha/B_0 a^2)} \tag{23}$$

or

$$\frac{m}{z} \approx \frac{A_{\mathrm{Francl}}}{\omega_+ + B_{\mathrm{Francl}}} \tag{24a}$$

in which

$$A_{\mathrm{Francl}} = eB_0 \tag{24b}$$

$$B_{\mathrm{Francl}} = \frac{V_{\mathrm{trap}}\alpha}{B_0 a^2} \tag{24c}$$

Note that, at the same level of approximation as Eq. (14), namely, $m/z \ll (m/z)_{\mathrm{critical}}$,

$$B_{\mathrm{Francl}} \approx \omega_- \tag{25}$$

Comparison of Eqs. (21b) and (21c) and (24b) and (24c) provides simple relations between the Ledford and Francl coefficients

$$A_{\mathrm{Ledford}} = A_{\mathrm{Francl}} \tag{26a}$$

$$B_{\mathrm{Ledford}} = A_{\mathrm{Francl}} B_{\mathrm{Francl}} \tag{26b}$$

It is interesting to note that the concept of an m/z-independent frequency shift, which is the basis of the Francl calibration function, was first suggested for a sideband calibration technique [7]. However, because the sideband magnitude is typically very small (<5% of the centerband magnitude), sideband calibration is not used for analytical applications.

FT-ICR mass calibration consists of fitting either Eqs. (21) (Ledford) or Eqs. (24) (Francl) to the ICR frequencies of ions of two or more known elemental compositions (and thus known masses) to yield either A_{Ledford} and B_{Ledford} or A_{Francl} and B_{Francl}. The mass-to-charge ratios of other ions in the mass spectrum may then be computed from their experimental ICR frequencies by use of Eq. (21) [or Eq. (24)]. We shall now proceed to test the experimental accuracy of the Ledford formula [Eq. (21)], the Francl formula [Eq. (24)], and their interconversion [Eq. (26)].

4. Experimental

A 5.6 T FT-ICR frequency-domain spectrum for an electron-ionized complex mixture of hydrocarbons was provided by R.P. Rodgers et al. [2]. ICR experiments were performed in a 5.08-cm i.d. enlongated closed cylindrical cell with solid circular disk endcaps and an aspect ratio of 1.5. A (low) trapping potential of 0.4 V was applied for better mass accuracy. An electrospray-ionized FT-ICR frequency-domain spectrum of a polymer sample, poly(ethylene glycol) bis(carboxymethyl) ether (PEG BCME), number average molecular weight, $M_n = 600$ (Aldrich, Milwaukee, WI), was obtained at 9.4 T [20,21]. That instrument is equipped with a 10.16-cm i.d. open cylindrical cell (aspect ratio 1.0 for each segment). A standard trapping potential of 2 V was applied to each cylindrical endcap electrode. Calibration calculations were performed in a Microsoft (Redmond, WA) Excel spreadsheet.

The Ledford function [18] may be expressed in either of the following two forms for linear least squares fitting ($y = a \cdot x + b$). (It is useful to compare two different expressions of the same for-

Table 1
Mass calibrations obtained from each of two forms of the Francl and Ledford frequency-to-mass functions, and conversion from Francl-based to Ledford-based mass spectra. The experimental FT-ICR frequency-domain data (5.6 T) are for an electron-ionized hydrocarbon mixture in positive ion mode

Composition	Theoretical *m/z*	Ledford 1 Error (ppm)	Francl 1 Error (ppm)	Conversion 1 Error (ppm)	Ledford 2 Error (ppm)	Francl 2 Error (ppm)	Conversion 2 Error (ppm)
C_8H_{13}	109.101177	−0.126	−0.126	−0.126	−0.102	−0.102	−0.102
C_9H_{15}	123.116827	0.105	0.105	0.104	0.125	0.125	0.124
$C_{10}H_{18}$	138.140302	0.074	0.074	0.073	0.089	0.089	0.088
$C_{11}H_{17}$	149.132477	0.134	0.134	0.133	0.146	0.146	0.145
$C_{12}H_{16}$	160.124652	0.050	0.050	0.049	0.059	0.059	0.057
$C_{12}H_{21}$	165.163777	0.063	0.063	0.061	0.070	0.070	0.068
$C_{13}H_{22}$	178.171602	−0.206	−0.206	−0.208	−0.203	−0.203	−0.205
$C_{13}H_{25}$	181.195077	−0.065	−0.065	−0.067	−0.062	−0.062	−0.064
$C_{15}H_{22}$	202.171602	−0.021	−0.021	−0.023	−0.025	−0.025	−0.027
$C_{16}H_{30}$	222.234203	−0.041	−0.041	−0.044	−0.051	−0.051	−0.054
$C_{18}H_{26}$	242.202902	0.080	0.080	0.076	0.064	0.064	0.060
$C_{19}H_{24}$	252.187252	0.122	0.123	0.119	0.104	0.103	0.100
$C_{19}H_{39}$	267.304628	−0.811	−0.811	−0.816	−0.835	−0.835	−0.839
$C_{22}H_{35}$	299.273328	0.655	0.655	0.650	0.622	0.622	0.617
	RMS	0.2945	0.2945	0.2944	0.2940	0.2940	0.2940

mula to test for possible difference arising from digital roundoff errors.) Experimental ICR frequency ν is measured in Hertz, and the best-fit Ledford coefficients are A_L and B_L. The forms are obtained by multiplying Eq. (21) by ν^2 or ν.

$$\frac{m}{z}\,\nu^2 = A_L\nu + B_L \tag{31a}$$

$$\frac{m}{z}\,\nu = A_L + \frac{B_L}{\nu} \tag{31b}$$

Similarly, for the Francl function, we compare two different forms by linear least-squares fitting. The first is obtained by taking the reciprocal of each side of Eq. (24). And second is obtained by multiplying Eq. (24) by $(\nu + B_F)$.

$$\frac{1}{m/z} = \frac{1}{A_F}\,\nu + \frac{B_F}{A_F} \tag{32a}$$

$$\frac{m}{z}\,\nu = A_F - B_F\,\frac{m}{z} \tag{32b}$$

5. Results and discussion

5.1. Comparison of the Francl and Ledford frequency-to-mass conversion formulas

The Ledford formula is the basis for mass calibration in our own data system [22] and ThermoQuest (Bremen, Germany) commercial FT-ICR instruments. Mass calibration in IonSpec (Irvine, CA) and Bruker (Billerica, MA) FT-ICR instruments is based on the Francl formula. As noted above, the Ledford formula is derived under the general condition $m/z < (m/z)_{\text{critical}}$, which is required for stable ion cyclotron orbital motion, whereas the Francl formula is derived under the more limiting condition $m/z \ll (m/z)_{\text{critical}}$. Thus, we might expect the Francl formula to fail at sufficiently high mass-to-charge ratio, small trap dimensions, and/or high trapping potential. We therefore calibrated two different FT-ICR frequency-domain spectra according to the Francl and Ledford formulas. Two forms of each calibration function were tested. The mass accuracies for the calibrated peaks are shown in Tables 1 and 2. The masses in

Table 2
Mass calibrations obtained from each of two forms of the Francl and Ledford frequency-to-mass functions, and conversion from Francl-based to Ledford-based mass spectra. The experimental FT-ICR frequency-domain data (9.4 T) are for an electrospray-ionized polymer sample, poly(ethylene glycol) bis(carboxymethyl) ether (PEG BCME, number average molecular weight, $M_n = 600$) in negative-ion mode.

Composition	Theoretical *m/z*	Ledford 1 Error (ppm)	Francl 1 Error (ppm)	Conversion 1 Error (ppm)	Ledford 2 Error (ppm)	Francl 2 Error (ppm)	Conversion 2 Error (ppm)
$C_{18}H_{33}O_{12}$	441.197750	−1.285	−1.285	−1.286	−0.890	−0.889	−0.890
$C_{20}H_{37}O_{13}$	485.223965	−0.021	−0.020	−0.021	0.324	0.325	0.324
$C_{22}H_{41}O_{14}$	529.250180	2.319	2.319	2.318	2.614	2.614	2.613
$C_{24}H_{45}O_{15}$	573.276395	2.588	2.588	2.587	2.832	2.832	2.831
$C_{26}H_{49}O_{16}$	617.302609	1.828	1.828	1.827	2.022	2.022	2.021
$C_{28}H_{53}O_{17}$	661.328824	−4.233	−4.233	−4.235	−4.089	−4.089	−4.091
$C_{30}H_{57}O_{18}$	705.355039	1.977	1.977	1.974	2.070	2.070	2.068
$C_{32}H_{61}O_{19}$	749.381254	1.141	1.141	1.138	1.184	1.184	1.181
$C_{34}H_{65}O_{20}$	793.407468	−7.278	−7.279	−7.281	−7.286	−7.286	−7.289
$C_{36}H_{69}O_{21}$	837.433683	−4.474	−4.474	−4.477	−4.532	−4.532	−4.535
$C_{38}H_{73}O_{22}$	881.459898	−2.356	−2.356	−2.360	−2.464	−2.464	−2.468
$C_{40}H_{77}O_{23}$	925.486113	2.098	2.098	2.095	1.940	1.940	1.936
$C_{42}H_{81}O_{24}$	969.512328	3.915	3.915	3.911	3.707	3.707	3.703
$C_{44}H_{85}O_{25}$	1013.538542	0.659	0.659	0.655	0.400	0.400	0.395
$C_{46}H_{89}O_{26}$	1057.564757	1.383	1.383	1.378	1.074	1.074	1.069
$C_{48}H_{93}O_{27}$	1101.590972	0.331	0.331	0.326	−0.029	−0.029	−0.034
$C_{50}H_{97}O_{28}$	1145.617187	3.787	3.788	3.782	3.377	3.378	3.372
$C_{52}H_{101}O_{29}$	1189.643401	−1.795	−1.795	−1.801	−2.256	−2.255	−2.261
	RMS	2.9674	2.9675	2.9672	2.9557	2.9558	2.9557

columns labeled "Ledford 1" and "Ledford 2" were generated from Eqs. (31a) and (31b); whereas the masses in columns labeled "Francl 1" and "Francl 2" were generated from Eqs. (32a) and (32b).

For the two different linear fits based on the Ledford calibration, the results based on Eq. (31a) (column "Ledford 1" in Tables 1 and 2) gave slightly different calibration results than those based on Eq. (31b) (column "Ledford 2" in Tables 1 and 2). Although the overall calibration error [root mean square (rms) value of the errors] is comparable, the results derived from Eq. (31b) do yield a slightly smaller rms error. A similar slight difference is observed for the two forms of the Francl calibration function, with Eq. (32b) as the close winner. We attribute these (very small) differences between forms of the same calibration function to roundoff errors during the linear least-squares fitting process. Such roundoff error is usually encountered when data points with smaller slope are fitted: for example, the slope in Eq. (31a) is A_L, whereas the slope in Eq. (31b) is B_L. Because B_L is usually larger than A_L, Eq. (31a) may give a better fit than Eq. (31b).

The calibration constants obtained for the data in column "Francl 1" were converted [Eqs. (26)] to the equivalent Ledford equation constants to yield the mass accuracies shown in column "Conversion 1." Similarly, "Francl 2" constants were converted to give the corresponding Ledford equation mass accuracies shown in column "Conversion 2." There is little difference in accuracy between direct calibration with Ledford (Francl) formula versus converting Francl-calibrated data [by Eq. (26)] to Ledford calibration (or vice versa).

Interestingly, the more accurate form of each of the two calibration functions [Eqs. (31b) and (32b)] gave results identical to within 1 ppb. The other two forms [Eqs. (31a) and (32a)] also agree with each other to within 1 ppb, suggesting that under typical FT-ICR conditions and within the usual *m/z* range, the two calibration functions are interchangeable in practice.

Such a result is actually not surprising. The error introduced by the approximation used in the Francl calibration equation increases monotonically with increasing $(m/z)/(m/z)_{\text{critical}}$. For $(m/z)/(m/z)_{\text{critical}} < 0.001$, the Francl calibration mass accuracy is ~0.1

ppm [18]. Thus, for a 3 T instrument with a 2.54 cm (1 inch) cubic cell and 1 V trapping potential, the critical *m/z* is ~50 kDa, and the Francl calibration mass accuracy reaches 0.1 ppm only for ions <50 Da in mass. However, at higher magnetic field and optimized cell geometry, the critical *m/z* is much higher (see below), so that the $(m/z)/(m/z)_{\text{critical}} < 0.001$ criterion is easily met.

For example, 9.4 T ESI instrument built at National High Magnetic Field Laboratory (NHMFL), Tallahassee, FL [20] operates with a large-bore (10.16-cm i.d.) open cylindrical cell. The critical *m/z* at 1 V trapping potential is 5.6 MDa, so that ions <5600 Da are accurately calibrated by the Francl equation. Even at a higher trapping potential of 2 V, for which the critical *m/z* drops to 2.8 MDa, ions up to *m/z* 2800 are still $< 0.001\ (m/z)_{\text{critical}}$. The actual $(m/z)/(m/z)_{\text{critical}}$ ratio for 9.4 T experiments was $< 4 \times 10^{-4}$.

Even at lower magnetic field, an enlongated cell [6] can dramatically increase the critical *m/z*. For example, our 5.6 T instrument [2] equipped with an 5.08-cm i.d. enlongated closed cylindrical cell (aspect ratio 1.5) has a critical *m/z* of 4.4 MDa at 1 V trapping potential. The calibration performance of that instrument is therefore comparable to that of the 9.4 T instrument described above, even at lower field and a smaller magnet bore diameter (89 versus 220 mm). For the present 5.6 T experiments, the critical *m/z* was further increased to 11 MDa by reducing the trapping potential to 0.4 V. The actual $(m/z)/(m/z)_{\text{critical}}$ ratio for 5.6 T experiments was $< 3 \times 10^{-5}$. For $(m/z)/(m/z)_{\text{critical}} < 10^{-4}$, the difference between Ledford and Francl calibration should be <1 ppb, consistent with our experimental observations.

Finally, because there is a larger difference in accuracy between two forms of the same calibration function than between the two calibration functions, one should be careful to choose consistently one form over the other. We therefore choose to replace the standard form [Eq. (31b)] with the slightly better form [Eq. (31a)] for reduced rms error. The mass difference caused by this choice is <0.5 ppb maximum error in the data set (see Tables 1 and 2) and may be neglected in most cases.

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